Micronutrients & HIV Infection

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Contributors from any bona fide area of nutrition, including the controversial, are welcome.

We welcome this important and timely contribution to this series. This book will be useful to a broad spectrum of nutritionists and life scientists of all walks.

Ira Wolinsky, Ph.D.
University of Houston
Series Editor
To date, 22 million people have died from AIDS and 36 million are currently living with HIV infection. The full impact of the HIV pandemic is, therefore, yet to be felt. In the meantime, HIV continues to spread, with more than 5 million newly infected during 2000. While neither a cure nor a vaccine exists, drugs have been developed that allow patients to live with the infection for a longer period. However, among the 1% of the world’s population fortunate enough to have access to these drugs, side-effects and the development of drug resistance are major concerns. For the vast majority of those living with HIV infection, antiretroviral drugs are not available. Given the magnitude and complexity of the HIV scourge, there is no simple solution to combat it. Rather, there is a need for a multifaceted response with an aim to prevent primary HIV infection, to halt its progression and prevent its transmission to partners and children, and to provide care to those affected.

Before the antibiotic era, it was common knowledge that proper nutrition was essential to a host’s resistance to infections, as well as an important part of the therapy. While this knowledge base is still viable among laymen and in veterinary medicine, it seems to have vanished from clinical medicine. However, the past 3 decades have seen a renaissance of the interest in the role of nutrition in infectious diseases. In recent years, randomized, controlled trials have confirmed that improved micronutrient intake effectively reduces morbidity and mortality from a range of specific infections.

Micronutrient deficiencies are widespread in developing countries, and even in developed countries among the underprivileged. Also, micronutrient deficiencies develop during early asymptomatic HIV infection. Evidence suggests that improved micronutrient intake may reduce HIV transmission and progression, as well as the morbidity from common and opportunistic infections. This book was written to help translate current knowledge on the role of micronutrients in HIV and other infections into better case management and public health interventions for the benefit of people living with or exposed to HIV.
Henrik Friis, M.D., Ph.D., a native of Aarhus, Denmark, graduated as a medical doctor from the University of Aarhus and received clinical training at Gentofte University Hospital. He next conducted research in Zimbabwe as a research fellow at the Danish Bilharziasis Laboratory, and obtained his Ph.D. from the University of Copenhagen in 1994. That same year he joined the Research Department of Human Nutrition at the Royal Veterinary and Agricultural University in Denmark, where he is currently an Associate Professor teaching nutritional epidemiology. He also is affiliated with the Danish Bilharziasis Laboratory. He is supported by the Danish Council for Medical Research.

Dr. Friis’ research in the field of nutrition relates to growth and body composition, reproduction, geophagy, and infectious diseases. The malnutrition-infection complex is his main research interest, particularly the roles of iron, zinc, and other micronutrients in morbidity from infectious diseases. He has done research in that field in Denmark, Botswana, Sudan, Kenya, Tanzania, Zambia, Guinea-Bissau, and Zimbabwe, and is presently supervising seven Ph.D. students in four African countries.

The role of micronutrients in HIV infection, in particular mother-to-child transmission, has become a major topic of concern. Between 1995 and 1999, Dr. Friis was a member of UNAIDS’ Informal Working Group on Prevention of Mother-to-Child HIV Transmission.

Dr. Friis has long been interested in the role of micronutrients in relation to the blood fluke schistosomiasis and infections with intestinal helminths. He has studied this in controlled trials in endemic areas, as well as in large laboratory animal studies, in collaboration with the Danish Bilharziasis Laboratory and Center for Experimental Parasitology. He also is involved in research on micronutrients in relation to malaria and lymphatic filariasis, and has recently begun studies on the effects of zinc and other micronutrients in pulmonary tuberculosis.
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MICRONUTRIENTS AND INFECTIONS: AN INTRODUCTION

Henrik Friis

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MICRONUTRIENTS AND INFECTIONS

Background

It is conventional wisdom that infections and undernutrition are causally associated and that many infections, especially if generalized or severe, may lead to weight loss. It is also understood that good nutritional status can prevent infectious and other diseases, as expressed in the saying, “An apple a day keeps the doctor away.” Nevertheless, since the antibiotic era, the importance of good nutrition as a fundamental principle in preventive and curative medicine seems to have lost hold, especially among health professionals.

In the monograph *Interactions of Nutrition and Infection* published by the World Health Organization (WHO) in 1968, Scrimshaw, Taylor, and Gordon reviewed, based on an earlier review, the considerable available literature on the effect of nutritional deficiencies on specific infections, and the effect of specific infections on nutritional status. From the perspective of today’s standard of research methodology, many of the reviewed studies obviously had flaws such as inappropriate animal models and inadequate study design. Furthermore, in experimental animal studies, the severe single-nutrient deficiencies were usually of little relevance to human nutrition, while in human studies the assessment of nutrient status was often inadequate. Nevertheless, Scrimshaw, Taylor, and Gordon found that most infections may aggravate nutritional status, while single-nutrient deficiencies, in turn, may lead to increased frequency or severity of a specific infection. Such a relationship was called synergistic, as it may constitute a vicious circle. However, single-nutrient deficiencies may occasionally result in reduced frequency or severity of infections, in which case the relationship was called antagonistic.

In 1989, the WHO published a monograph, *Malnutrition and Infections*, in which Tomkins and Watson critically reviewed more recent human studies. As Scrimshaw noted in his retrospective comment, the intervening years had not changed the conclusions, but had led to a greater understanding of the mechanisms involved, in particular the key importance of cell-mediated immunity.

Looking back, the monograph *Interactions of Nutrition and Infection* from 1968 served to put nutrition back on the infectious disease research and control agenda. The 1970s saw nutritional immunology being established as an important research discipline. In the early 1980s, observational studies were conducted to assess the relationship between single-nutrient deficiencies and morbidity and mortality (e.g., the studies by Sommer on vitamin A*). In the late 1980s and the 1990s, randomized, controlled trials to assess
and estimate the effects of nutritional interventions (e.g., vitamin A, iron, zinc, multivitamins) on infectious disease morbidity and mortality became established as the gold standard. All along, methods for assessment of status were refined for some micronutrients (e.g., iron, vitamin A), and new micronutrients were found to be of importance (e.g., zinc, selenium, vitamin E). Similarly, new functional properties of some of these micronutrients were discovered (e.g., antioxidant and prooxidant properties as reviewed by Allard in Chapter 3 on oxidative stress and infections) and the understanding of their role in host defense, growth/replication of the infectious agent, and pathogenesis was improved, although it was still inadequate.

Following is a general overview of the mechanisms responsible for the relationship between nutritional deficiencies and infections, and its public health implications.

**Effects of Infections on Nutrition**

Infections may affect the nutritional status of the host. Most generalized infections may lead to reduced food intake and absorption of nutrients, and increased utilization and loss of nutrients. This may reduce the nutritional status of the host depending on its prior nutritional status and dietary intake during convalescence. These effects are mediated by the acute phase response as well as superimposed local manifestation of the infection. The acute phase response is a stereotypic host response to infections, inflammation, surgical and accidental trauma, burns, etc., due to the release of pro-inflammatory cytokines (tumor necrosis factor and interleukins 1, 6, and 8) from activated macrophages. Through the effects of a network of cytokines on target cells and organs, the infection gives rise to fever, headache, myalgia, anorexia, and malaise, in addition to a large number of endocrine, biochemical, and physiological responses. The pattern and time sequence of these responses are basically the same, irrespective of the infectious agent or type of injury. The acute phase response comprises both catabolic and anabolic processes. Muscle tissue is catabolized with the release of amino acids that are taken up by the liver. In the hepatocytes, the amino acids are used for the synthesis of acute phase proteins, enzymes, complement, and antibodies, and for production of glucose, the fuel needed during the hypermetabolism. This is accompanied by negative balances of not only nitrogen, but also of magnesium, phosphate and potassium, and sulphur and zinc.

As part of the acute phase response, the serum concentrations of iron and zinc decline dramatically and within a few hours, primarily due to dislocation within the body. Sequestration of iron in mononuclear phagocytes in the liver, spleen, and intestine is mediated by the iron-binding transferrin and lactoferrin released from activated neutrophils. Zinc is taken up by
the hepatic acute phase protein metallothionein and sequestered in the liver, thymus, and marrow. This serves a dual purpose: to make the minerals available to the organs and cells where they are needed for synthesis (of antibodies, complement, acute phase proteins, and for tissue repair), and to withhold the minerals from the pathogens that require them for growth and replication. Serum concentrations of vitamin A also decline during the acute phase, but then rebound during convalescence despite the fact that the infection is reducing the stores of vitamin A in the liver. It has been suggested that these changes are purposeful and part of the host's defense.

While the acute phase response is obviously beneficial in terms of overcoming the precipitating infection, it also has serious nutritional costs. If the infection is severe, prolonged, or recurrent, it may result in infection-induced malnutrition, as the requirements are increased yet the intake and absorption may be profoundly reduced. Infection-induced malnutrition is best exemplified by weight loss and xerophthalmia. Weight loss accompanies most severe, generalized infections, and is a cardinal symptom in infections like tuberculosis and HIV due to loss of lean and fat body mass. Xerophthalmia is the specific eye symptom of vitamin A deficiency, and is often precipitated by measles and other serious infections. This is mediated by reduced synthesis of retinol-binding protein, excretion of vitamin A in urine, and probably a flux into the extra-vascular space. In addition to these conspicuous signs of malnutrition, the status of most micronutrients will also be impaired.

Furthermore, the infection may be accompanied or complicated by local manifestations that further impair nutritional status. For example, painful ulcers in the mouth and esophagus may reduce food intake, and diarrhea may reduce absorption and increase the loss of nutrients. Disturbances of taste and smell due to an infection or the medication prescribed may also contribute.

It is worth noting that infection in an individual, in addition to the direct metabolic effects, may have effects at the family and community levels. For example, river blindness — onchocerciasis caused by the filarial nematode *Onchocerca volvulus* — in adults leads to reduced work capacity and, hence, food insecurity of households and communities. Similarly, in Tanzania, wives of HIV/AIDS patients spend less than half the time on agriculture as they would do otherwise. Even in developed countries, frequent or chronic infections may lead to considerable medical expenditures or the loss of jobs or job opportunities, with possible consequences for household food security.
Effects of Nutrition on Infections

The nutritional status of an individual may affect exposure to infectious agents, susceptibility to infection, and, in particular, the severity and duration of the infection. It may even affect the virulence of an infectious agent.

Host exposure – Although evidence is scarce, it is conceivable that the host’s nutritional status may affect behavior related to exposure to infectious agents. For example, it has been shown that iron deficiency is strongly, probably causally, associated with soil-eating,20,21 a widespread practice in developing countries.22,23 Furthermore, eating soil is a strong risk factor for infections with geohelminths such as roundworm (*Ascaris lumbricoides*) and whipworm (*Trichuris trichiura*),22 infections affecting 1300 and 750 million people, respectively.24 Iron deficiency may thus exemplify a specific nutritional deficiency that changes human behavior and thereby increases exposure to infectious agents. In contrast, low intake of zinc has been shown to reduce spontaneous physical activity.25 On the one hand, this is likely to result in reduced recreational or domestic fresh water contact and, hence, reduced exposure to infective larvae of the *Schistosome* spp., which infects approximately 200 million individuals in the world.24 This constitutes an example of a single nutritional deficiency that leads to reduced exposure and, therefore, infection with a specific pathogen. On the other hand, reduced physical activity also could give rise to crowding in unsanitary conditions with increased exposure to other infectious agents, such as those causing respiratory and intestinal tract infections. Likewise, specific nutritional deficiencies could affect human libido or other factors associated with the risk of sexual behavior and exposure to sexually transmitted infections. It seems that little attention has been devoted to establishing these relationships, in comparison to extensive research on the role of nutritional deficiencies in susceptibility to infections if exposed. From a disease control perspective, however, behavioral aspects seem equally important.

Host immune functions – Most micronutrients are essential to various specific and non-specific immune functions, and deficiencies may thus impair resistance to infections and lead to increased frequency or severity. Deficiencies of antioxidant vitamins and minerals, and administration of the prooxidant iron, also may lead to oxidative stress and result in increased apoptosis of immune cells and increased morbidity. There is even evidence to suggest that deficiencies in pregnancy could have an effect on immune functions of the offspring. This has been demonstrated in the rat, where zinc deficiency during pregnancy had effects on immune functions in the second generation offspring.26 Furthermore, recent data from The Gambia showed that individuals born in the hunger season had higher infectious disease mortality in adulthood when compared to individuals born in the
harvest season. Although the responsible correlates of the hunger season remain unidentified, nutritional deficiencies of the fetus or young infant resulting in premature aging of the immune system (immunosenescence) was suggested. However, it is important to note that high intake of various nutrients is not only toxic, but may even be immunosuppressive.

Effects on the pathogen – The direct effects of micronutrient intake and status on the pathogen seem to be more complex and responsible for the occasional antagonistic relationship between malnutrition and infection. Various micronutrients are required for the growth or replication of the pathogen. In fact, reductions in serum iron and zinc during the acute phase response are probably important in the host’s defense. Accordingly, administration of iron and possibly other micronutrients during latent or uncontrolled infections may be hazardous, as it could benefit the pathogen rather than the host’s immune system. Such detrimental effects of iron are well established for bacterial infections in neonates, and also have been a concern with respect to malaria. Apparently, the determinant is the availability of iron for the pathogen and not iron status per se. For example, reasonable oral doses do not cause any problems with respect to malaria, as recently expressed in the Consensus Statement on Safety of Iron Supplementation Programs in Malaria-Endemic Regions by the International Nutritional Anemia Consultative Group. Parenteral administration of iron, however, results in more malarial morbidity in nonimmune individuals such as young children and pregnant women, and may result in increased morbidity from other infections. Intramuscular or intraperitoneal administration, previously a common practice in developing countries and recommended in major medical textbooks, should now be considered obsolete. Even oral administration of iron may be harmful if given to infected, undernourished individuals with low serum concentrations of transferrin to take up the iron. For example, during treatment of kwashiorkor, a condition caused by infections and oxidative stress in severely undernourished children, iron should not be given in the first week. By virtue of its prooxidant properties, iron also may be detrimental in viral infections, in that oxidative stress directly stimulates viral replication. In contrast to what was the case for bacterial and malarial infections, iron status per se may be of importance in viral infections. Accordingly, deficiencies of antioxidant vitamins and minerals may have similar effects, and their status may modify the effect of iron.

A hitherto unknown aspect of the relationship between malnutrition and infection was recently revealed when Beck and Levander, in a mouse model, demonstrated that deficiencies of antioxidant micronutrients such as selenium and vitamin E — or even administration of the prooxidant iron — could make an avirulent pathogen (coxsackievirus) virulent through changes in the genome. This finding may be of tremendous importance because it may not only explain the emergence of new epidemics, but
even allow speculations that improved nutritional status could play a role in controlling development of drug resistance. The possible interactions between malnutrition and infections, and the mechanisms involved, are shown in Figure 1.1.

**Public Health Implications**

Of the 13 to 14 million children dying each year in developing countries, 70% die from infectious diseases, and most of them die malnourished. Understanding the specific role of malnutrition in child mortality is important in terms of developing rational control strategies and health policies. Previously, malnutrition was thought to be an independent cause of child mortality, so that if the severity of malnutrition exceeded a certain threshold, it would contribute additively to child mortality. It has since been shown epidemiologically that child mortality increases exponentially with increasing weight-for-age deficits. The relative mortality risks of mildly, moderately, and severely malnourished children were 2, 3, and 11, respectively. These estimates were consistent across populations. Based on data from 53 developing countries, it was estimated that
56% of child deaths were due to the potentiating effect of malnutrition on infectious diseases, and mild-moderate malnutrition, being more common than severe malnutrition, accounted for 83% of these. Thus, the epidemiological evidence that the relationship between malnutrition and infection is indeed multiplicative is in accordance with Scrimshaw's observation that these relationships are predominantly synergistic in nature.

Antagonistic relationships — where micronutrient deficiency reduces and repletion increases susceptibility for an infectious disease — are indeed more than academic curiosities. Although most convincingly demonstrated with iron and malarial and bacterial infections, iron seems to play a particularly important role in HIV and other viral infections. That such antagonistic relationships may exist and constitute serious public health dilemmas is exemplified by the results of a recent randomized, controlled trial in western Kenya among adults with low hemoglobin concentration. The study demonstrated that iron supplementation considerably reduced reinfection with helminths. However, although the iron status was improved, the hemoglobin concentration, in fact, declined. The HIV status of the study participants could not be determined, but the HIV prevalence was probably around 50%. The authors suspected that the negative effect of iron supplementation on hemoglobin concentration was due to increased progression of HIV infection with suppression of the bone marrow.

Determining the effect of micronutrient deficiencies on specific infections should be a research priority because the preventive potential of such knowledge is enormous. When a specific infection leads to a micronutrient deficiency, which again increases the frequency or severity of an infection, controlling either will ideally break the vicious circle and serve to control both the deficiency and the infection. In contrast, when a specific deficiency reduces — or repletion increases — the frequency or severity of a specific infection, caution should be exercised to avoid exacerbating the infection in the individual patient, or increasing the infectious disease burden in the population. However, this does not mean that the nutritional deficiency should be accepted, but that the nutritional deficiency and the specific infection should be fought simultaneously. Whether the relationship is synergistic or antagonistic, any rational strategy or health policy should address both micronutrient deficiencies and infections.

It is now increasingly recognized that isolated protein-energy-malnutrition does not exist except under experimental conditions, as it is invariably associated with multiple micronutrient deficiencies. Although deficiencies of protein clearly impair a number of immune responses, micronutrient deficiencies — the “hidden hunger” — are probably responsible for the large contribution of mild-moderate malnutrition to child mortality. However, in contrast to the macronutrients protein, fat, and carbohydrates, it is operationally feasible to improve the micronutrient status among certain high-risk
groups through the primary healthcare system, or among the general population through food diversification, modification, and fortification.

**MICRONUTRIENT REQUIREMENTS**

**Recommended Dietary Allowances**

Recommendations on daily intakes of micronutrients have been developed by committees in the United States, Canada, and European countries. For example, the Recommended Dietary Allowances (RDAs), first published in 1943 by the Food and Nutrition Board of the United States National Research Council, are defined as “The levels of intake of essential nutrients that, on the basis of scientific knowledge, are judged by the Food and Nutrition Board to be adequate to meet the known nutrient needs of practically all healthy persons.” The RDAs, and the large number of similar terms and concepts — often with similar acronyms — developed by committees in the United States and other countries, should be interpreted and used with caution. The values are statistical terms for use when planning adequate nutrition for populations, not guides for the individual. Also, the scientific knowledge and the methods to determine the requirements are inadequate, and the recommendations are based on what intakes are necessary to avoid clinical and biochemical signs of deficiencies rather than what brings optimal health. Finally, the values are meant for healthy people and are not necessarily adequate for people with infections or other diseases, or convalescents from such diseases. It should be stressed that the intake of these micronutrients should be based on a variety of foods in order to also provide adequate amounts of nutrients for which requirements are not defined, as well as other beneficial bioactive food components.

Over the past couple of decades, evidence has emerged that intakes above the recommendations may be beneficial in the prevention of chronic diseases, such as cardiovascular diseases and cancers. The recommendations are currently being revised with an aim to develop estimates of intakes that should not only prevent nutritional deficiencies, but also diseases. For example, the recommended daily intake of vitamin C for adults has varied between 40 and 80 mg/d in different countries. Nonetheless, intake of foods with vitamin C above current recommendations seems to play a role in the prevention of various diseases such as cancers, tuberculosis, cataract, etc., and it has recently been suggested that the recommendations should be increased to between 100 and 200 mg of vitamin C per day. Finally, the recommended intakes are not necessarily optimal for people exposed to pathogens because higher levels of intake may make us
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<td>75</td>
<td>15</td>
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<td>1.2</td>
<td>2.4</td>
<td>400</td>
<td>65</td>
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<td>19–50 y</td>
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<td>1.1</td>
<td>1.1</td>
<td>14</td>
<td>1.3</td>
<td>2.4</td>
<td>400</td>
<td>75</td>
<td>15</td>
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<tr>
<td>51+ y</td>
<td>800</td>
<td>1.1</td>
<td>1.1</td>
<td>14</td>
<td>1.5</td>
<td>2.4</td>
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<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>18+ y</td>
<td>800</td>
<td>1.4</td>
<td>1.4</td>
<td>17</td>
<td>1.9</td>
<td>2.6</td>
<td>600</td>
<td>85</td>
<td>15</td>
<td>30</td>
<td>15</td>
<td>60</td>
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<td>1300</td>
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<td>1.6</td>
<td>17</td>
<td>2</td>
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<td>500</td>
<td>120</td>
<td>19</td>
<td>15</td>
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</tbody>
</table>

<sup>a</sup> RDAs for vitamin A, iron, and zinc are based on age categories 4–6, 7–10 (y) for children, and 11–14, 15–18 (y) for males and females.<sup>47</sup> RDAs for the B vitamins, and vitamins C and E, and selenium are based on recent revisions.<sup>52,53</sup>

<sup>b</sup> Retinol equivalents. 1 retinol equivalent (RE) = 1 mg retinol or 6 mg β-carotene. RDA for children 4–6 y: 500 RE; 7–10 y: 700 RE; 11–14 y, males: 1000 RE; 11–14 y, females: 800 RE. RDA for lactating women beyond 6 months is only 1200 RE.

<sup>c</sup> Niacin equivalents. 1 niacin equivalent = 1 mg niacin or 60 mg dietary tryptophan.

<sup>d</sup> As dietary folate equivalents (DFE). 1 DFE = 1 mg food folate = 0.6 mg of folic acid from fortified food or as a supplement.
RDA for vitamin C for pregnant women <18 y is 80 mg/d, and for lactating women <18 y is 115 mg/d.

α-Tocopherol equivalents. 1 α-tocopherol equivalent = 1 mg d-α tocopherol.

RDA for iron in children 4–6 and 7–10 y: 10 mg/d.

RDA for zinc in children 4–6 and 7–10 y: 10 mg/d. RDA for lactating women beyond 6 months is only 16 mg.

Adequate intake, given when RDAs cannot be determined.

less susceptible to infections. The most recent RDAs for the micronutrients covered in this book are presented in Table 1.1.47,52,53

**An Evolutionary Perspective**

An evolutionary perspective on human nutrition may be useful in order to achieve an understanding of human nutritional requirements. Throughout millions of years of evolution in Africa, man genetically adapted to his environment, lifestyle, and diet. However, since the agricultural revolution only 9000 years ago,54 and later during the industrial revolution, our dietary intake has changed dramatically. During this, from an evolutionary perspective, short period of time, virtually no genetic adaptations have taken place; today’s humans are considered genetically identical to our late paleolithic or preagricultural ancestors.55 The paleolithic dietary pattern and intake are, therefore, of considerable interest, as they could be considered a paradigm of the nutritional requirements of modern man.56

In the absence of data on dietary intake of our preagricultural ancestors, the dietary intake of recent or modern-day hunter-gatherers has been suggested as a model of paleolithic nutrition.56 Based on data from 58 hunter-gatherer groups studied in this century, the ratio of vegetable and animal food intake was 65:35 on a weight basis.57 Similarly, although the approach and conclusions have been challenged,58,59 more recent studies conclude that most hunter-gatherers derive around 60% of their energy intake from animal foods.60 Eaton and Konner have collected dietary intake and food composition data from a large number of hunter-gatherer communities, and have established a database of 236 wild plants and 85 animal foods.61 It appeared that the diet of hunter-gatherers — and thus preagricultural man — comprised a large number of different vegetable and animal food items. Although specific plant foods were often consumed in bulk when in season, the number of vegetable food items or species eaten over a year often exceeded 100. The wide range of vegetable foods included fruits, nuts, green leaves, legumes, tubers, pulps, roots, etc., which were often consumed unprocessed shortly after being gathered. Interestingly, although wild cereal grains were found, they were probably not part of the paleolithic diet, as they were too time-consuming to process. The animal food items comprised not only game meat and organs, but also insects, mollusks, fish, and other seafood items.61,62

Given the high content of micronutrients in most of these foods, and the practical absence of cereal grains, the diet was remarkably micronutrient dense. Assuming a vegetable:animal subsistence pattern of 65:35 and a 3000 kcal (12,558 kJ) energy intake per day, the daily intake of most vitamins and minerals was several times higher than current recommendations (Table 1.2).61
Table 1.2  Estimated Daily Paleolithic Intake of Selected Nutrients Compared to U.S. Recommended Dietary Allowances (RDA) for Adult Men

<table>
<thead>
<tr>
<th>Paleolithic Intake</th>
<th>RDA</th>
<th>Paleolithic:RDA Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Vitamins (mg/d)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A (RE/d)</td>
<td>3797</td>
<td>1000</td>
</tr>
<tr>
<td>B1</td>
<td>3.9</td>
<td>1.2</td>
</tr>
<tr>
<td>B2</td>
<td>6.5</td>
<td>1.3</td>
</tr>
<tr>
<td>Folate</td>
<td>0.4</td>
<td>0.4</td>
</tr>
<tr>
<td>C</td>
<td>604</td>
<td>90</td>
</tr>
<tr>
<td>E</td>
<td>32.8</td>
<td>15</td>
</tr>
<tr>
<td><strong>Minerals (mg/d)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Iron</td>
<td>87.4</td>
<td>10</td>
</tr>
<tr>
<td>Zinc</td>
<td>43.4</td>
<td>15</td>
</tr>
</tbody>
</table>

- RDA for adult men 19 to 24 years.
- Provitamin A carotenoids and pre-formed vitamin A combined.

In addition to the high intake of most micronutrients in the paleolithic diet, there is evidence to suggest that paleolithic man supplemented his diet with various minerals. From the European archaeological record of animal bone remains from the mesolithic period, there seems to be evidence that animal bones were fragmented, burned, ground, and pulverized, most likely for the purpose of consumption as a supplement. Similarly, geophagy, or soil-eating, is a practice considered to be a vestige of paleonutrition. Geophagy was practiced daily among 73% of school-aged children and 56% of pregnant women in Kenya. In both cases, geophagy was associated with iron deficiency and anemia. Indeed, the median intake of 28 and 42 g soil per day, respectively, provided 4.7 and 4.3 mg of iron per day. It has been postulated, but not proven, that the association between iron deficiency and geophagy is causal, in that deficiency of iron leads to a craving for soil or another type of pica.

Interestingly, soil from termite mounds is often preferred due to its fine texture, but probably also due to very high contents of iron and other minerals. The giant termite mounds (termite genus *Macrotermes*) found over most of southern Africa from Natal to Uganda, where man evolved, is a common source of soil for man and animals. The mounds may be as old as 700 years, reaching a diameter of 100 feet and a height of 20 feet. Over the years, colony after colony of termites has carried vegetable matter from the surroundings to the heap, eventually creating a gradient of mineral concentration. For example, the concentration of iron and zinc in the mound may be 3 to 4 times higher than that of surrounding soils, whereas it
may be 50 to 100 times or more for manganese, magnesium, and phosphorus. Daily intakes of 40 to 100 g of termite soil — a common practice — would provide pregnant women with 30 to 80 mg iron. As such, the mounds can be seen as giant mineral supplement factories.

Since geophagy is practiced by most mammals and nonhuman primates, it seems likely that man may have eaten soil from giant termite mounds and other insect soils throughout evolution, and that the estimated daily paleolithic intake of iron and other minerals may even be underestimated.

Some nutritional paradoxes may be explained by the paleonutrition paradigm. For example, the RDA for iron during pregnancy is 30 mg/d. Nonetheless, it is generally acknowledged that, even in the most affluent countries, such requirements cannot be met through the diet. But with a paleolithic diet providing an estimated 80 mg/d through food, and even more through supplements of termite soil, these requirements were comfortably met by our ancestors. Similarly, the RDA for vitamin E for adults is around 10 mg/d, yet it is well established that various immune functions increase with intakes several times the RDA. This phenomenon is usually referred to as a pharmacological effect, but in fact may partially reflect that we need more for optimal immune functions and health than what is currently recommended.

**GLOBAL NUTRITIONAL SITUATION**

The evolutionary perspective on contemporary diets highlights fundamental nutritional problems, as most of the energy intake in developed as well as developing countries stems from foods that were not part of the paleolithic diet.

**Developed Countries**

In developed countries, infectious diseases are no longer the scourges they were in the past. This is primarily attributable to improvement in socio-economic conditions, and also to effective immunization coverage and antibiotic treatment. Because overt micronutrient deficiencies — with the exception of iron — are rare in the general population, the contribution of suboptimal micronutrient status to the incidences of infectious diseases in developed countries is probably modest. Nevertheless, social inequality also exists within developed countries, where incidences of obesity and noncommunicable diseases increase while there are pockets of undernutrition. Micronutrient deficiencies may be common in subgroups such as the poor, drug users, pregnant women, the elderly, and in hospitalized and otherwise institutionalized individuals, where they could be important risk
factors of infectious disease morbidity. For example, low micronutrient status has been documented in injecting drug-users, and could be a determinant of susceptibility to, and outcome from, HIV infection and viral hepatitis B and C. Similarly, elderly people, even in affluent countries, are at risk of nutritional deficiencies. A daily supplement with multimicronutrients to the elderly in Canada resulted in improved immune functions and reduced incidences of illness due to infections.

Developing Countries

In developing countries, the staple food is a cereal such as rice, maize, wheat, sorghum, or millet, or tubers or legumes, comprising 70% of the energy intake. In addition, small amounts of vegetables and fruits are part of the diet, while animal products are only rarely affordable. The diet is therefore bulky and has a low energy and nutrient density. In addition, the bioavailability of some vitamins and minerals is low. For example, 90% of vitamin A intake is as the less efficient provitamin A carotenoids, while most of the iron is the poorly bioavailable non-heme iron. Further, cereals have a high content of phytates that binds non-heme iron, zinc, and other minerals in the intestinal tract, thus preventing their absorption. The bioavailability of zinc in a typical cereal-based diet may be less than 15%, whereas it is around 40% in a refined animal protein-based diet. Given the dietary pattern in developing countries, the intakes required to meet the demands are above what is recommended in developed countries. Further, people in developing countries frequently acquire diarrhea or respiratory tract infections, and commonly harbor several parasites simultaneously throughout their lives. For example, hookworm, roundworm, and whipworm live in the intestinal tract; blood flukes (Schistosoma spp.) live in the mesenteric and vesical vessels; malaria parasites live in the liver and blood cells. These infections may further increase the requirements for various micronutrients.

Data on global micronutrient malnutrition were published in the 3rd and 4th Reports on The World Nutrition Situation. Iodine deficiency is a public health problem in at least 130 countries, but through salt iodization schemes its elimination may be a realistic goal. Vitamin A deficiency is considered a public health problem in more than 60 countries. It is estimated to affect more than 250 million children worldwide, and to be the underlying cause of several million cases of child deaths each year. Iron deficiency is probably the greatest nutritional problem in the world. Using anemia as a proxy of iron deficiency, more than 3.5 billion people — particularly children and women of reproductive age — are estimated to be iron deficient. Only recently has zinc deficiency been recognized as widespread in developing countries, and as an underlying cause of stunting, as
well as of child and maternal mortality.\textsuperscript{73,78} In developing countries, these deficiencies often coexist, and also may coexist with deficiencies of other micronutrients, although data are lacking.

In 2000, it is estimated that of the children under 5 years of age in developing countries, 32.5% are stunted and 26.7% are underweight.\textsuperscript{74} The prevalence, and to a lesser extent the absolute number, of stunted and underweight children has declined since 1980. However, there are large regional differences. In eastern Africa, for example, the prevalence has actually increased over the last 20 years and is now approaching 50%.

Increasing cereal grain production to keep pace with the estimated 50 to 100% increase in the world's population during the next 25 to 50 years seems difficult, but it may be possible with modern agricultural technologies.\textsuperscript{79} Eradicating micronutrient deficiencies in the world's population under this scenario is a major challenge, especially when considering that previous plant-breeding efforts focusing on grain production may have contributed to the reduction in dietary diversity.\textsuperscript{80} To succeed, unconditional commitment from not only health planners, but also from politicians is required to ensure multisectoral collaboration and the simultaneous use of several interventions such as plant breeding for higher micronutrient density, food diversification, food fortification, infectious disease control, and micronutrient supplementation.

**THE HIV PANDEMIC AND THE ROLE OF MICRONUTRIENTS**

The current estimates of the magnitude of the HIV pandemic have beaten all previous projections. According to UNAIDS' *AIDS Epidemic Update: December 2000*,\textsuperscript{81} as many as 5.3 million people were infected with HIV during 2000, bringing the total number of people living with HIV to 36.1 million. Sub-Saharan Africa is the worst afflicted region in the world; with only 10% of the world's population, this region harbors 70% — or 25.3 million — of the people with HIV worldwide. With 21.8 million AIDS deaths so far, and almost twice that number weakened by HIV infection, the epidemic has consequences that are already brutally felt in most families and communities in the hardest-hit countries, and has a devastating impact on social and economic development.

Due to the magnitude and the complexity of the pandemic, there is a need for a multifaceted, multisectoral response.\textsuperscript{82} It is well documented that micronutrients are important determinants of infectious disease morbidity. The role of micronutrients in HIV infections obviously should also be examined so that knowledge can be used as part of a range of interventions. In fact, in the early days of the AIDS pandemic — prior to the discovery of HIV — it was noted that the pattern of immune deficiencies was similar to that seen in severe malnutrition. Accordingly, it was suggested that AIDS was
caused by an opportunistic viral infection on the background of malnutrition-induced immune suppression. While this proved not to be the case, there is now ample evidence to suggest that micronutrient deficiencies — pre-existing or due to HIV infection — may be important co-factors in HIV progression and transmission. It should be a research priority to explore fully the nature of interrelationships between specific micronutrients and HIV infection so that the role of nutritional interventions in case management of people with HIV, as well as in public health interventions, can be defined.

REFERENCES

NUTRITIONALLY ACQUIRED IMMUNE DEFICIENCY SYNDROMES

William R. Beisel

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INTRODUCTION

In order to function at their full, optimal capacities, the immune system and other host defensive measures require a complete dietary array of essential vitamins, minerals, and unsaturated fatty acids, and an adequate intake of protein and energy.\textsuperscript{1,2} This same concept may be restated from an opposite point of view, i.e., any degree or variety of nutritional deficiency will likely impact adversely upon the body’s immunological defenses.\textsuperscript{3-6} Such adverse immunologic consequences of malnutrition have been termed “Nutritionally Acquired Immune Deficiency Syndromes,” or NAIDS.\textsuperscript{5}

NAIDS, in combination with various childhood infections (especially measles, and respiratory tract and diarrheal infections), cause the deaths of over 15 million children each year.\textsuperscript{7} NAIDS also lead to the deaths of additional thousands of elderly persons, and to the deaths of many patients debilitated by medical and surgical diseases. Thus, the combined consequences of NAIDS and various infectious diseases are by far the largest cause of human mortality. In comparison to the total overall worldwide mortality of AIDS caused by human immunodeficiency viruses (HIV, types 1 and 2), the consequences of NAIDS have been far more deadly. NAIDS-related deaths (over 15 million persons/year for decades upon decades) far exceed the several million deaths caused to date by AIDS.\textsuperscript{8} NAIDS also differ from AIDS in another important respect: NAIDS can be reversed by appropriate nutritional intervention. AIDS will eventually lead to death (unless a cure can be found), even though duration of life can be extended by now available antiretroviral drugs. Conversely, NAIDS responds promptly and generally completely to the restoration of appropriate nutritional balance by providing an adequate intake of all required dietary components.\textsuperscript{1-3,5}

Current attempts to treat micronutrient deficiencies among world children are markedly reducing the incidences of childhood NAIDS.\textsuperscript{5} This is especially true for public health programs that provide poorly nourished children with adequate amounts of vitamin A and zinc\textsuperscript{5} and, hopefully in the future, all other essential micronutrients. It thus seems possible that with the improving economic status of Third World countries, along with continued attempts to normalize the nutritional status of children living in afflicted lands, the worldwide impact of childhood NAIDS may be dramatically reduced.

Obviously, nutritional programs aimed at children will not reduce the incidence of NAIDS in elderly and diseased persons. Also, iron deficiency will undoubtedly persist in adult women worldwide.\textsuperscript{14} Nevertheless, it is possible that worldwide deaths due to AIDS will at some future date outnumber those associated with NAIDS.\textsuperscript{8} On the other hand, huge increases in the numbers of AIDS-related deaths in a Third World country might so impair its
social and economic status that an increased incidence of childhood malnutrition and NAIDS will become an inevitable secondary consequence.

It is important to understand the progressive, synergistic relationships that develop between AIDS and NAIDS whenever an asymptomatic HIV infection progresses into full-blown AIDS. Such a progression occurs whenever HIV retroviruses destroy sufficient numbers of T-helper (CD4+) lymphocytes to allow the development of secondary infections and/or malignancies. Loss of appetite, reduced food intake, falling body weight, and the nutrient losses associated with febrile hypermetabolism can all be caused by HIV infection per se, as well as by the secondary infections and malignancies in patients with full-blown AIDS. Because severe, AIDS-induced nutritional losses will eventually lead to the development of NAIDS, the immune systems of patients with advanced AIDS will almost certainly suffer from the synergistic effects of NAIDS as well. This synergism can lead to dysfunctions of immune system defenses throughout the body, as well as to those of the innate, antigenically nonspecific, generalized host-defense mechanisms.

**HOST DEFENSIVE MECHANISMS IMPAIRED BY NAIDS**

Various forms of NAIDS can impair immune system functions as well as innate, generalized host defenses (see Table 2.1). Generalized host defenses are numerous and diverse. They include (1) protective anatomic barriers (skin, mucosal surfaces, and their cilia) and the products these surfaces produce such as sweat, gastric acid, intestinal enzymes, and the large variety of other fluids synthesized by diverse mucosal cells; (2) phagocytic cells such as polymorphonuclear neutrophils (PMN) and fixed or mobile macrophages; and (3) circulating nonspecific mediators such as interleukins, tumor necrosis factor, complement, opsonins, cachectin, etc.

The immune system consists of antigenically responsive tissues such as the thymus, tonsils, and lymph nodes, and circulating individual lymphocytes, thymic hormones, and immunoglobulin antibodies. Functionally, the immune system is divided into a cellular system and a humoral system. The cellular system is composed of tissue-bound and circulating T-lymphocytes. These cells include many varieties, i.e., T-helper lymphocytes, T-suppressor lymphocytes, killer lymphocytes, etc. The humoral system includes B-lymphocytes, plasma cells, and the antigen-specific immunoglobulins they produce, including secretory IgA.

**VARIETIES OF NAIDS**

Because every individual micronutrient has its own unique role to play in contributing to the body’s normal biochemical and metabolic mechanisms,
Table 2.1  Effects of Nutritional Deficiencies on Host Defense

<table>
<thead>
<tr>
<th>Vitamin Deficiencies</th>
<th>Mineral Deficiencies</th>
<th>Other</th>
</tr>
</thead>
<tbody>
<tr>
<td>Host resistance</td>
<td>↓↓</td>
<td>↓↓</td>
</tr>
<tr>
<td>Infectious diseases</td>
<td>↑↑</td>
<td>↑↑</td>
</tr>
<tr>
<td>Mucocutaneous lesions</td>
<td>↑↑</td>
<td>↑↑</td>
</tr>
<tr>
<td>Lymphoid tissue atrophy</td>
<td>↑↑</td>
<td>↑↑</td>
</tr>
<tr>
<td>Blood lymphocyte counts</td>
<td>↓↓</td>
<td>↓↓</td>
</tr>
<tr>
<td>In vitro lymphocyte response</td>
<td>↓↓</td>
<td>↓↓</td>
</tr>
<tr>
<td>Splenic plaque cell</td>
<td>↓↓</td>
<td>↓</td>
</tr>
<tr>
<td>DDH†</td>
<td>↓↓</td>
<td>↓</td>
</tr>
<tr>
<td>Serum IgG concentration</td>
<td>↑↑</td>
<td>↓</td>
</tr>
<tr>
<td>IgG synthesis to new antigens</td>
<td>↓↓</td>
<td>↓</td>
</tr>
<tr>
<td>Antioxidant activity</td>
<td>↓↓</td>
<td>↓</td>
</tr>
<tr>
<td>Serum complement</td>
<td>↓↓</td>
<td>↓</td>
</tr>
<tr>
<td>PMN§ mobilization</td>
<td>↓↓</td>
<td>↓</td>
</tr>
<tr>
<td>PMN§ phagocytic activity</td>
<td>↓↓</td>
<td>↓</td>
</tr>
<tr>
<td>PMN§ bactericidal activity</td>
<td>↓↓</td>
<td>↓</td>
</tr>
<tr>
<td>PMN§ enzyme activity</td>
<td>↓↓</td>
<td>↓</td>
</tr>
<tr>
<td>Allograft survival</td>
<td>↑</td>
<td>↑</td>
</tr>
</tbody>
</table>

* B₁ = thiamin, B₂ = riboflavin, B₃ = niacin, B₅ = pantothenic acid, PEM = protein-energy malnutrition.  
  Fol = folate. PUFA = polyunsaturated fatty acids. AA = amino acids.  
  DDH = delayed dermal hypersensitivity. PMN = polymorphnuclear neutrophils. Blanks indicate lack of data.  
  Consider also that many data are old and poorly confirmed.

↑ increased, ↓ reduced. (Two arrows indicate human, and one arrow animal data.)
it is not surprising that they support and influence the immune system and nonspecific host defenses in many different ways.\textsuperscript{1–3}

The most devastating forms of NAIDS are associated with a severe atrophy of all lymphoid tissues. NAIDS-induced thymic gland atrophy was once termed “nutritional thymectomy.” Nutritionally induced atrophy of lymphoid tissues predominately affects the T-lymphocyte areas, while the B-lymphocyte areas tend to be spared. Some forms of NAIDS impair the production and function of T-lymphocytes, killer lymphocytes, and thymic hormones.

A detectable serologic response of B-lymphocytes to vaccine antigens generally occurs during NAIDS, but the magnitude and potency of resultant antibodies are usually reduced. The same holds true for the production, secretion, and potency of IgA surface antibodies.

NAIDS also impair the function of nonspecific host defense mechanisms by reducing the formation and mobilization of neutrophils and circulating monocytes, as well as by reducing the phagocytic and bacteriolytic capabilities of these cells. Dermal and mucosal defenses also become impaired.

As recently recognized, deficiencies of single micronutrients that possess antioxidant properties also act adversely on nonspecific host immunity. These micronutrients include vitamins C and E and the many provitamin A carotenoids, including α- and β-carotene, lycopene, lutein, and zeaxanthine. In addition, NAIDS can interfere with the production of cytokines, a unique group of antigenically nonspecific small proteins. Cytokines include interferons, interleukins, and tumor necrosis factor. These specialized proteins serve in a variety of individual ways as intercellular messengers. Cytokines are unique because they allow lymphocytes, phagocytes, and other body cells to communicate and interact.

Cellular release of “pro-inflammatory cytokines” (interleukins 1, 6, and 8, tumor necrosis factor, and γ-interferon) is a cardinal feature of many infectious diseases and other acute illnesses. Pro-inflammatory cytokines initiate acute phase reactions, which include fever, hypermetabolism, altered production of hepatic proteins, release of certain hormones, headache, nausea and vomiting, myalgia, and other symptoms.\textsuperscript{5} Pro-inflammatory cytokines also enhance the proliferation of HIV.\textsuperscript{5}

Cytokines thus contribute to the development of inflammatory processes and immune functions. Circulating in plasma and intercellular fluids, these endogenous messengers act in the manner of hormones. But, unlike hormones, they are not synthesized in specialized glands. Rather, cytokines are manufactured and released by a variety of cell types scattered throughout the body. The molecular structures and complex functions of individual cytokines are only now becoming fully appreciated, as are their releasing stimuli and target cells.
Protein-energy malnutrition (PEM) leads to the most common and most prominent form of NAIDS. The immunological consequences of PEM have been widely documented. However, PEM-induced NAIDS are rarely uncomplicated entities, for almost always PEM is accompanied and influenced by deficiencies of one or more micronutrients.

The possible coexistence of specific micronutrient deficiencies and PEM was rarely mentioned in earlier years. In fact, micronutrient deficiencies are seldom documented in patients with PEM. Clinically, however, corneal ulceration and other optic manifestations of vitamin A deficiency often accompany PEM. Conventional laboratory studies also have shown consistent hematological abnormalities, as well as changes in the frequently measured proteins and other components of plasma. Further, the great successes of widespread supplementation with micronutrients such as vitamin A and zinc demonstrate quite clearly that deficiencies of essential micronutrients exist in great numbers among Third World children, the most frequent victims of PEM.

As the overall nutritional status of a child worsens into full-blown PEM, there develops an extensive atrophy of body organs, lymphoid tissues, skeletal muscle, and other soft tissues. Eventually, a state of compensated marasmus (dry malnutrition) ensues, in which a child becomes severely emaciated. With severe PEM, an infant or child looks like a living skeleton, with loss of hair, sunken cheeks and eyes, and a prominence of ribs and other bony structures. The uncompensated state of PEM is termed “kwashiorkor,” or wet malnutrition. Kwashiorkor results from an acute deprivation of protein or, most frequently, from a severe infection in a child with preexisting NAIDS and low antioxidative capacity. Kwashiorkor is characterized by gross edema, an enlarged liver, extensive dermal lesions, and a reddish blond discoloration of the hair. Infection-induced deaths are the rule in children with either marasmus or kwashiorkor.

The immunological consequences of PEM are extensive and serve as a model for the immune system dysfunctions that result from isolated deficiencies of various essential micronutrients. Such PEM-induced immunological dysfunctions occur in patients of all ages, but are far more common in infants and children. For still unknown reasons, PEM-induced NAIDS have a much greater adverse effect on cell-mediated immunity than on humoral immunity.

Lymphoid tissue atrophy due to PEM is more prominent in the T-cell areas than in those of the B-cells, and is also reflected in circulating blood by larger declines in T-lymphocyte numbers than in those of B-cells. This difference may be influenced by declining concentrations of the thymic hormones in plasma that normally help to regulate the functions of peripheral
T-lymphocytes. Defective cell-mediated immunity during PEM is also shown by the disappearance of delayed dermal hypersensitivity skin responses that require functioning T-cells.

Sharp declines in concentrations of albumin and other individual plasma proteins are the rule during PEM. Paradoxically, the concentration of plasma IgG (formed by the B-lymphocytes and plasma cells) is consistently increased. This enigma may be due to the greater number of infections suffered by children with PEM, or perhaps by greater losses of T-suppressor lymphocytes in contrast to those of T-helper lymphocytes. As another example of continuing B-cell responsiveness in patients with PEM, inoculated vaccines generally induce the formation of antigen-specific immunoglobulins, although their IgG titers and activities tend to be depressed. The same reductions are true for the production and activities of secretory IgA antibodies in tears and intestinal fluids.

**NAIDS Due to Vitamin Deficiencies**

Most, if not all, vitamins play some role in supporting functions of the immune system or of generalized host defense measures. Conversely, vitamin deficiencies lead to various forms of NAIDS. Chief among these are deficiencies of vitamin A. In addition to its deleterious effects upon tissues of the eye, vitamin A deficiency induces broad immunological dysfunctions. These vitamin A-related dysfunctions mimic those caused by PEM, but are generally less severe. Deficiencies of vitamins B6 and C also contribute importantly to the development of NAIDS, while deficiencies of the other B-group vitamins usually have minimal, if any, immunological consequences. Vitamin E deficiency impairs a broad range of immune functions. Vitamin K has no known effect upon the immune system.

**Provitamin A Carotenoids**

Five hundred or more carotenoids are present in various fruits and vegetables, but little is known about the majority of them. The most widely studied, β-carotene, is cleaved within the body to form two molecules of vitamin A. Many other carotenoids are also vitamin A precursors. In addition, most carotenoids are antioxidants. They protect the body by destroying highly reactive, unstable free oxygen radicals that damage cells and their nuclear DNA. β-Carotene is absorbed into the body and can be found in fat depots and plasma. It is nontoxic and does not produce an excess of vitamin A. The effects of β-carotene upon the immune system are said to be similar to those of vitamin A. In addition, β-carotene is a potent antioxidant, with one molecule being able to neutralize up to 1000 free oxygen radicals.
Lycopene, the red pigment in tomatoes, is the most efficient known scavenger of single-oxygen radicals. Lycopene has another cancer-inhibiting property, i.e., the enhancement of gap-junction communications between cells. When cancer cells divide, they lose this form of gap-junction communication. The concentration of lycopene in plasma is greater than that of β-carotene. In addition, lycopene is especially abundant in the prostate, possibly protecting it from the eventual development of cancer. Lycopene is also thought to lower the incidence of many other forms of cancer. Lycopene appears to reduce the harmful effects of UV exposure on the skin. Protection from oxidized products of cholesterol and low-density lipoproteins, as afforded by lycopene, may reduce the formation of atherosclerotic plaques and the incidence of heart attacks. Although lycopene is available in tablet form, one daily serving of tomatoes (in any form) provides the body with adequate amounts. Daily tomato consumption is especially important in older individuals.5

Lutein and zeaxanthin are the principle carotenoids of the rods and cones of the optic retina, and both are potent antioxidants. Lutein is found throughout the retina and in the lens, while zeaxanthin concentrations are greatest around the macula. Both of these carotenoids protect against damage from UV and blue light. Adequate dietary amounts of lutein and zeaxanthin in spinach, kale, and collard greens appear protective against macular degeneration, while concentrated amounts in tablet form may even prevent the further development of this major cause of blindness in the elderly.5

Vitamin A

For many years after its discovery, vitamin A was known as the “anti-infective vitamin.” This alone should have suggested that vitamin A helped to protect host defenses and the immune system. But with the discovery of vitamin A’s importance to the eyes in the mid-1930s, all attention became focused on its optical functions. However, when Sommer’s studies involving vitamin A supplementation (of all children in Indonesian villages where eye lesions were numerous) revealed that mortality was greatly reduced, the anti-infective role of vitamin A was again brought into prominence.10 Now, vitamin A is properly recognized as one of the major micronutrients in terms of the broad immunological support it provides.5

Deficiency of vitamin A tends to produce the same pattern of NAIDS as seen in PEM. Both innate, generalized nonspecific immunity and the immune system itself are impaired, with dysfunctions of cell-mediated immunity being most prominent. Lymphoid tissues are often atrophic, especially in T-cell areas, and peripheral lymphocyte counts are depressed. Delayed dermal hypersensitivity reactions are generally reduced, and the
in vitro responses of lymphocytes to mitogens are often decreased.\textsuperscript{1-3,5} Experimentally induced vitamin A deficiency in animals is accompanied by a prolonged survival of tissue grafts. However, lymphoid tissue atrophy and cell-mediated immune dysfunctions in humans tend to be less severe during vitamin A deficiency than are the defects caused by PEM.

Vitamin A deficiency tends to reduce antibody formation after immunization. Splenic plaque-forming cell responses to immunization are also reduced in experimental animals. On the other hand, vitamin A supplements in children and animals have improved the success of immunizations.

In addition to its effects on the immune systems, a deficiency of vitamin A has broad effects on nonspecific host defenses. These are illustrated by the dysfunctions that occur in PMN and monocytes, which include depressed cellular enzyme functions as well as poor mobilization during inflammatory reactions. Vitamin A is also said to protect and strengthen the skin and mucosal membranes. Vitamin A deficiency leads to metaplasia and keratinization of mucocutaneous epithelium. Topical preparations containing vitamin A are claimed to eliminate or reduce skin wrinkles, but can cause damage if used in excess.\textsuperscript{1-3}

The administration of vitamin A to malnourished Third World children has saved thousands upon thousands of lives. If vitamin A is given orally or parenterally at the onset of measles, mortality is greatly reduced, so much so that the World Health Organization and the U.S. Academy of Pediatrics both recommend that vitamin A be given as a standard form of treatment for childhood measles.\textsuperscript{5} In addition to measles, other generalized febrile infectious illnesses reduce the body stores of vitamin A by increasing its metabolic degradation as well as its urinary losses. Concomitantly, nausea and vomiting impair the intestinal absorption of vitamin A, as do intestinal parasites.\textsuperscript{5} Febrile infections also depress the hepatic synthesis of vitamin A’s carrier proteins such as retinol-binding protein, transthrytin, and lipoproteins.

The abrupt declines of serum vitamin A values and those of its carrier proteins during acute illnesses now appear to represent newly identified components of acute phase reactions induced by pro-inflammatory cytokines. These abrupt declines have shown equally abrupt returns to normal once the infections are over. These infection-related falls in serum vitamin A concentrations appear analogous to similar abrupt declines in serum iron and zinc values during acute phase reactions.\textsuperscript{13} It remains unknown if the vitamin A changes are due solely to a reduced hepatic synthesis of its carrier proteins, or to a transient sequestration of vitamin A somewhere in the body. Administration of large excesses of vitamin A can cause toxic responses throughout the body (see Table 2.2). Smaller doses, however, can produce immunological benefits that involve both humoral and cell-mediated functions. In addition to its role as an essential
### Table 2.2  Effects of Micronutrient Intakes Larger than Recommended Dietary Allowances (RDA)\(^1,2,12,14\)

<table>
<thead>
<tr>
<th>Micronutrient</th>
<th>Immunological Effects</th>
<th>Toxic Effects</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vitamin A</td>
<td>Increased resistance to infection, improved and antibody synthesis, increased splenic plaque-forming cell numbers, supressed DDH(^a) and Arthus reactivity, and a more rapid rejection of skin grafts.</td>
<td>20–30 times RDA causes skin erythema and desquamation, hepatomegaly, abdominal pain, nausea, headache, and anorexia. 5× RDA β-carotene increase risk of lung cancer.</td>
</tr>
<tr>
<td>Pyridoxine</td>
<td></td>
<td>Huge doses may cause diarrhea.</td>
</tr>
<tr>
<td>Vitamin B(_6)</td>
<td>The most important of the B vitamins.  Deficiencies cause broad immunological deficits similar to PEM.</td>
<td>Large, chronic doses may cause nerve dysfunction, with loss of sensation, difficulty in walking, etc. Reduced milk production.</td>
</tr>
<tr>
<td>Vitamin B(_{12})</td>
<td></td>
<td>Allergic reactions may occur after parenteral administration.</td>
</tr>
<tr>
<td>Vitamin C</td>
<td>Megadoses do not prevent colds, but may reduce their severity and duration B-cell counts may fall, but (\text{in vitro}) lymphocyte transformations, PMN(^b) functions, vitamin C content, and complement concentrations may increase.</td>
<td>Megadose consumption appears safe. Urinary oxylate values increase.</td>
</tr>
<tr>
<td>Vitamin E</td>
<td>Intake 2–10 times RDA improves phagocytic capacity, humoral and cell-mediated immunity.</td>
<td>Bleeding, especially in elderly.</td>
</tr>
<tr>
<td>Iron</td>
<td>Susceptibility to infection increased, lactoferrin and transferrin stores saturated, bactericidal activity impaired</td>
<td>Hemochromatosis, with damage to liver, heart, etc., diabetes. Acute overload causes necrotizing gastroenteritis.</td>
</tr>
<tr>
<td>Zinc</td>
<td>Poor PMN functions, altered cell membrane fluidity.</td>
<td>Impaired absorption of copper.</td>
</tr>
<tr>
<td>Copper</td>
<td>May impair host resistance, antibody responses, and local inflammatory responses.</td>
<td>Wilson’s disease causes injury to liver and brain. Acute toxicity leads to hemolysis.</td>
</tr>
</tbody>
</table>

\(^a\)DDH = delayed dermal hypersensitivity. \(^b\)PMN = polymorphnuclear neutrophils. Blanks indicate lack of data. Lead, chromium, mercury, vanadium, gold, and silicon may show toxic effects at higher doses.
micronutrient, vitamin A can serve as an adjuvant if mixed with immunizing antigens before their inoculation.

**Vitamin B-Complex**

Deficiencies of several B vitamins produce clinically recognizable diseases such as beriberi, pellagra, and pernicious anemia. Most B vitamins function as enzyme cofactors; however, not all of them provide special support for the immune system or other generalized host-defensive mechanisms.

Thiamin (B₁) deficiency leads to beriberi. Only minor effects upon the human immune system accompany this disease, i.e., deficiencies in antibody production. Also, experimental thiamin deficiency reduces splenic plaque-forming responses to immunization in some laboratory animals. Riboflavin (B₂) deficiency may cause stomatitis, glossitis, seborrheic dermatitis, and/or anemia in humans, but otherwise it produces few signs or symptoms. Experimental riboflavin deficiencies in laboratory animals cause a depressed response to vaccines, but no evidence of defective cell-mediated immunity. Niacin (B₃) deficiency in humans causes the four Ds (dermatitis, diarrhea, dementia, and death) of pellagra. Accompanying dermal lesions (stomatitis, proctitis, vulvovaginitis, scrotal macerations, and infected lesions of the hands and arms) indicate that niacin affords protection against antigenically nonspecific lesions of the skin. However, niacin deficiency has little effect on cell-mediated immunity, but may impair antibody production. Pantothenic acid (B₅) deficiency in experimental animals causes depressed responses to vaccines, including those of antibody production and splenic plaque cell formation. Pyridoxine (B₆) is the B-group vitamin whose deficiency causes the most extensive dysfunctions within the immune system. As demonstrated in experimental animals, these dysfunctions involve both humoral and cell-mediated immunity. Lymphoid tissues become atrophic, and lymphocyte counts in blood are depressed. Delayed dermal hypersensitivity responses are often lacking, and allograft survival is prolonged. Immunizations are followed by impaired antibody production and splenic plaque cell formation. Inadequate inflammatory responses provide evidence that pyridoxine also affects nonspecific host defenses. Cobalamine (B₁₂) deficiency causes pernicious anemia, by far its most important consequence. Cobalamine deficiency also leads to a reduced magnitude of inflammatory responses and a lessening of *in vitro* lymphocyte responses in mixed cultures. Biotin deficiency in experimental animals impairs responses to vaccines. Folic acid deficiency, when produced experimentally in man, leads to a megaloblastic form of anemia. Folic acid deficiencies in laboratory animals cause atrophy of lymphoid tissue, lower blood lymphocyte counts, and depressed *in vitro* lymphocyte responses in mixed cultures. Antibody production and affinities after
immunizations are also reduced. Excess daily folic acid intake can mask a B$_{12}$ deficiency.

**Vitamin C**

Vitamin C (ascorbic acid) deficiency causes scurvy. Scorbatic guinea pigs may show decreased T-cell percentages in blood, delayed dermal hypersensitivity responses to injected antigens, and a prolonged survival of allografts. However, there is little evidence in humans that a lack of vitamin C has major effects on the immune system components of NAIDS. There are no reports of lymphoid tissue atrophy in scorbatic guinea pigs or humans. Scorbatic guinea pigs respond to vaccines as effectively as their controls.

On the other hand, the major effects of vitamin C deficiency in humans are on the nonspecific host defenses, particularly those involving phagocytic cells. Scurvy is accompanied by markedly impaired functions of phagocytes in blood and tissues, and by greatly reduced generalized host-defensive mechanisms that protect against infectious diseases. The random mobility of neutrophils and monocytes is depressed, their phagocytic capability is reduced, and their chemotactic ability to migrate into areas of inflammation becomes problematic. Reductions in chemotaxis may be due to an abnormality in contractile element functions, possibly involving an abnormal assembly of microtubules within these phagocytic cells. It must also be noted that infectious diseases reduce the content of vitamin C in phagocytic cells. A depressed content of vitamin C in polymorphnuclear cells is also a common finding in leukemias, lymphomas, other hematologic diseases, diabetes, scurvy, pregnancy, and in aged individuals. A reduced production of complement and interferon is observed during scurvy, and antioxidant values become depressed. Animal studies also indicate that ascorbic acid is required for the maintenance of cells that produce the thymic hormones known to support T-cell functions throughout the body. Despite the widespread craze to consume gram-sized quantities of vitamin C each day (accounting for an estimated $810 million in American sales in 1999), there is no confirmed evidence that megadose intakes will protect against the common cold, other infections, or cancer. With such large intakes, the body is forced to synthesize enzymes to destroy the excessive amounts of vitamin C, but no other harm seems to come from these doses. The antioxidant role of vitamin C may account for possible benefits associated with intakes of up to 500 mg/d. These benefits could include improved mobility, phagocytic activity, and bactericidal functions of phagocytes, and increased *in vitro* lymphocyte transformation functions.
Vitamin E

Vitamin E (α-tocopherol) is an antioxidant, scavenging free radicals and stabilizing cell membranes. It also has beneficial effects on fertility and aging.1 For these reasons, many individuals consume large excesses of it. Vitamin E deficiencies in animals and humans impair both humoral and cell-mediated immunity, and antioxidant activity as well.1,5 Administration of large daily doses to birds and animals increased their responsiveness to immunizations and caused an increased proliferation of splenic T-cells and an improvement in the phagocytic activities of neutrophils. Vitamin E appears to have synergistic effects with selenium. Vitamin E supplementation of 2 to 10 times higher than current recommendations significantly increases phagocytic capacity and humoral and cell-mediated immunity in man and animals.12 Although many have consumed doses of 800 units/d for long periods without apparent harm, supplemental doses of over 400 units may reduce the in vitro bactericidal activity of neutrophils and the responsiveness of lymphocytes to PHA. In addition, high doses of vitamin E have increased the incidence of hemorrhagic strokes in elderly patients.

NAIDS Due to Trace Element Deficiencies

A number of the trace elements contribute importantly to immune system functions as well as to nonspecific host-defensive measures. Chief among these are iron and zinc. On the other hand, most trace elements have few, if any, clearly defined effects on the immune system or other host-defensive mechanisms of human beings, and many can be toxic if consumed in excess.1,5,13

Iron

Either an excess or a deficiency of iron can impair host-defensive mechanisms. During cytokine-induced acute phase reactions associated with febrile infectious illnesses, the body sequesters plasma iron by binding it to transferrin or lactoferrin in storage depots. Iron also becomes bound to lactoferrin released by phagocytic cells in areas of localized inflammation. These processes can lower the concentrations of plasma iron to almost undetectable values.1 Serum ferritin concentration may double, mirroring its role as an acute phase reactant protein. Under normal states of health, serum ferritin values reflect the adequacy of tissue iron stores.

When infections become chronic, an “anemia of infection” may develop despite adequate stores of protein-bound iron in body tissues. Further, anemia of infection cannot be reversed by administering iron, either orally or parentally, but only by eliminating the infection.1,5 It is postulated that these
iron-sequestering mechanisms have the purpose of preventing iron uptake by invading microorganisms whose replication is dependent on the amounts of plasma iron that could become bound to bacterial siderophores and taken up by replicating bacteria. According to this postulate, and supported by considerable early clinical and experimental evidence, an excess of plasma iron could abet an infectious process. Much debate has revolved around this concept, for many believe that the value of administering iron to a malnourished, anemic child far outweighs its potential dangers. While clinical evidence has been amassed showing that iron can be given safely to anemic children who suffer other forms of severe malnutrition, many clinicians will not administer iron parenterally under those circumstances.

Iron deficiency is often a component of PEM in children and is of worldwide importance in many women during the menstrual years. Iron deficiency increases susceptibility to infectious illnesses by impairing both nonspecific host-defensive mechanisms and, to a lesser degree, those of the immune system. Iron deficiency causes vacuolar changes in lymphocyte mitochondria and may reduce blood lymphocyte counts, but iron deficiency does not alter plasma antibody values, salivary IgA production, or responses to vaccine antigens, and it does not reduce serum complement concentrations. The in vitro responsiveness to various mitogens of lymphocytes obtained from iron-deficient patients is controversial; investigators have reported differing results. Impaired killer T-lymphocyte activity in iron-deficient mice is evidence for a deficiency in cell-mediated immunity. The major reported abnormality in the nonspecific immunity of some iron-deficient patients is a reduction in the bactericidal effectiveness of phagocytic blood cells. This impaired killing of phagocytosed organisms may be related to a depressed activity of cellular myeloperoxidase, an iron dependent enzyme. Iron deficiency can also reduce the synthesis of other iron-containing enzymes in phagocytic cells. Iron deficits also have been associated with hypersegmentation of neutrophil nuclei, and lowered nitroblue tetrazolium (NBT) dye-reducing values in the neutrophils of some children.

**Zinc**

Zinc is the most important trace element in terms of its effects on immunity, largely because zinc is a component of more than 100 metalloenzymes. Further, the addition of plasma zinc to thymic hormones (after their release from the thymus) activates these hormones and allows them to stimulate peripheral T-cells throughout the body. Because there is no storage depot for zinc, deficiencies can be created rapidly in experimental animals. In humans, an inherited deficiency in the ability to absorb intestinal zinc, i.e., acrodermatitis enteropathica, demonstrates the
consequences of severe zinc deficiency. These include multiple immune dysfunctions, severe mucocutaneous lesions, and an increased susceptibility to infections. Fortunately, acrodermatitis enteropathica can be treated successfully by providing large amounts of orally administered zinc gluconate on a continuing basis. Zinc deficiency also can occur as a consequence of severe medical or surgical diseases.\textsuperscript{1,4,5,13}

Patients and laboratory animals with zinc deficiency have hypoplasia of all lymphoid tissues, which can progress to atrophy. Both T- and B-cell areas are involved, whereas in circulating fluids, dysfunctions of T-lymphocytes and killer cells predominate. Widespread administration of zinc to malnourished children in several Third World countries has reversed these problems, with reductions in infectious diseases, morbidity, and mortality. In such supplemented children, zinc lessens the severity of diarrheas and reduces the incidence of persistent diarrheas, dysenteries, and pneumonias.\textsuperscript{5} Defects in cell-mediated immunity outweigh those of humoral immunity during zinc deficiency. \textit{In vitro} T-cell responses to mitogens are severely depressed, as are delayed dermal hypersensitivity responses to injected antigens. Splenic plaque-forming cell responses become suppressed, but antibody responses to vaccines will still occur in zinc-deficient patients, although they may be attenuated. Nonspecific defenses also are impaired, as evidenced by depressed oxygen uptake, phagocytic activity, and bactericidal functions of neutrophils.\textsuperscript{1,5}

**Other Essential Nutrients and Immunity**

A sizable number of essential trace elements appear to have some effect upon immunity. These have generally been recognized as consequences of induced deficiencies in experimental animals.

Selenium deficiencies cause cardiomyopathy and retard growth development. They also lead to impaired antioxidant activity, poor immunological responses to foreign antigens, and to defective bactericidal activity of neutrophils. These phagocytic cells show low glutathione activities.\textsuperscript{1,15} In children with AIDS, low selenium values in plasma seem to correlate with depressed immunological functions.\textsuperscript{5} Selenium is also synergistic with vitamin E in its immunostimulatory and antioxidant actions. An administered small excess of selenium improves vaccine efficacy in animals, while large doses can be immunosuppressive.\textsuperscript{1,5}

Copper deficiency (Menkes’ disease) causes anemia, dermal depigmentation, kinky hair, and brain damage. There are also impaired lymphocyte and phagocytic cell functions. A copper deficiency in animals increases the severity of infections and reduces antibody responses to vaccines.\textsuperscript{1,5,13} A different congenital problem, Wilson’s disease, is associated with an accumulation of excessive body copper.\textsuperscript{13} Serum copper and ceruloplasmin
concentrations may increase by 20 to 30% during inflammatory states, at which time copper has value as an antioxidant. This infection-induced increase in plasma copper values has been explained by the increased hepatic synthesis of copper’s carrier protein, ceruloplasmin, which is one of the acute phase reactant proteins.

Iodine deficiency (hypothyroidism, goiter) may impair antibody production. Iodine is immunotoxic if given at high doses to experimental animals, but under normal conditions in humans it may function in microbicidal activities. Neutrophilic myeloperoxidase becomes active when combined with hydrogen peroxide and a halide co-factor, the most potent of which involves iodide’s conversion to iodine. Neutrophils obtain iodide by degrading thyroid hormones, a process that is accelerated when the cells engage in phagocytosis. Such activated neutrophils take up thyroid hormones from their surrounding body fluids.

Cobalt, after small doses, increases the movement and phagocytic activity of neutrophils. Manganese, after small doses, increases the movement of animal macrophages, as well as the oxygen uptake and phagocytic activity of granulocytes.

Magnesium and calcium each act in the manner of a micronutrient when they interact with host-defensive mechanisms. Magnesium ions are required for participation of properdin in the alternate complement pathway. Deficits of magnesium have no apparent effect on other human immune mechanisms, but create major problems in rodents. During experimental magnesium deficiency, rats and mice develop extremely high leukocytosis, with eosinophilia being most prominent. This leukocytosis can progress to fatal leukemias or lymphomas. Mast cells become degranulated and continually release large amounts of histamine. Curiously, the thymus becomes markedly hyperplastic instead of atrophic. Despite this, plasma immunoglobulin concentrations fall and there are only minimal immunologic responses to foreign antigens. The amazing difference between human and rodent responses to magnesium deficiency has never been explained. This difference remains an outstanding example of why data obtained in laboratory animals may have no relevance to human functions.

Calcium ions are needed to activate components of the complement and coagulation systems. They are also an absolute requirement for oxygen radical generation and degranulation in activated human neutrophils. Calcium ions must become bound to a small protein, calmodulin, to allow them to function in the production of the prostaglandins and other eicosanoids. Ionic calcium is thus involved in the response to the pro-inflammatory cytokines that stimulate the production of prostaglandins, leukotrienes, and other eicosanoids, and then, in turn, generalized, body-wide acute phase reactions.

Amino acids and several types of lipids also play a role in supporting host defenses. Although few reports are available, several individual
essential or lipotrophic amino acids appear to have effects on host immunity. In rodents, experimentally induced deficiencies of essential amino acids are accompanied by impaired immunoglobulin responses to foreign antigens. These essential amino acids include tryptophan, phenylalanine, valine, threonine, isoleucine, histidine, and lysine. None of these deficiencies, however, produce defects in cell-mediated immunity. In contrast to these deficiency states, an overload of leucine in protein-deficient rats caused reductions in immunoglobulin responses as well as in splenic plaque-forming and T-rosetting cell numbers. The lipotrophic amino acids, methionine and choline, also have effects on immune functions in rodents. Choline deficiency leads to thymic involution. With a combined deficiency of both choline and methionine, immunologic responses to foreign antigens are depressed. These dysfunctions can be passed on to offspring.

Severe obesity in humans is associated with an increased susceptibility to infections, a relative decrease in the size of lymphoid tissues, altered fluidity of lymphocyte surface membranes, and deficits in neutrophil functions. These include reductions in chemotaxis and in phagocytic and bactericidal activities. Excesses or deficiencies of individual lipids also can affect the immune system. The content of polyunsaturated fatty acids (PUFA) in cell surface membranes helps to determine their fluidity, with excesses causing greater fluidity. PUFA are the essential nutrients metabolized to form the eicosanoids (families of prostaglandins, leukotrienes, and thromboxanes) that initiate and regulate acute phase reactions. Individual eicosanoids may stimulate or suppress platelet aggregation, cause vasodilatation or contraction, cause bronchial contraction, and some can stimulate the release of the same pro-inflammatory cytokines that lead to the cellular production of the same eicosanoids. Thus, the pro-inflammatory cytokines do not initiate myriad acute phase signs and symptoms by their direct actions. Rather, they act as intercellular messengers, much in the manner of hormones, to stimulate target cells to synthesize eicosanoids from PUFA located in the cellular wall. It is the eicosanoids, in turn, that activate the acute phase phenomena.

The cytokine-to-eicosanoid progression is somewhat complicated. When pro-inflammatory cytokines make contact with cell wall receptors, they activate phospholipase A2 in the cell wall. This enzyme causes the conversion of omega-6 PUFA (from meat and most plants) to arachidonic acid, and the conversion of omega-3 PUFA (from fish and some green leaves) to eicosapentanoic acid. Arachidonic and eicosapentanoic acids then enter the cell interior, where other enzymes (cyclooxygenases or 5-lipoxygenases contained within different host cells) convert them into one of many species of eicosanoids.

Acute phase reactions stimulated by the eicosanoids include fever, anorexia, malaise and myalgias, hypermetabolic destruction of muscle
protein, hepatic synthesis of acute phase reactant enzymes and serum proteins, and an activation of the immune system. This reaction includes the release, from certain subsets of T-lymphocytes, of interleukins 2, 3, 4, and 5. All of these processes are markedly reduced by PUFA deficiencies. Generalized PUFA excesses, on the other hand, lead to an involution of lymphatic tissues and suppressed cell-mediated immunity, including depressed killer T-cell activity, prolonged allograft survival, and impaired T-cell responses to mitogens. On the other hand, PUFA deficits impair B-cell functions and phagocyte mobility. Major increases in body cholesterol also can lead to an increased susceptibility to infections. Impairment of cell-mediated immunity is suggested by depressed killer cell cytotoxicity, depressed T-lymphocyte responses to mitogens, and suppression of delayed dermal hypersensitivity reactions. Humoral immunity also may be impaired by hypercholesterolemia, as evidenced by decreases in antibody production and a foam cell appearance of macrophages.

**FUTURE STUDIES IN NUTRITIONAL IMMUNOLOGY**

During the last several decades, the concepts, methodologies, and investigative approaches employed in immunological research have undergone a logarithmic explosion, but our knowledge of nutritional immunology has not. Reasons for this are numerous. Few nutritionists are adept practitioners of immunology, and even fewer immunologists have an interest in nutrition. Human studies are difficult to prepare for, or conduct, for a number of reasons, and animal studies may be difficult to carry out. With the exception of iron deficiency, isolated deficiencies of single nutrients are rare in the free world. With the exception of congenital diseases such as acrodermatitis enteropathica or Menkes’ disease, deficiencies of single nutrients are generally difficult to recognize or to diagnose with certainty. And if such a diagnosis is made, few practitioners, or patients, would agree to delay replacement therapy while lengthy, time-consuming, and often invasive immunological studies are conducted.

Studies using volunteer subjects have been done successfully in the past, and are possible in the future. Such studies will require excellent experimental designs, modern methodologies, and the prior approval of human use committees. Modern studies concerning the immunological effects of iron deficiency would undoubtedly be the easiest to plan and conduct. In contrast, volunteer studies requiring the induction of other single-micronutrient deficiencies would likely be quite lengthy and expensive. Despite their shortcomings, animal studies continue to offer the most attractive way of advancing the field of nutritional immunology. Problems do remain, however. Animal data are not always applicable to humans. Some micronutrient deficiencies cannot be produced in all species (e.g.,
only the guinea pig develops scurvy), or may require long periods of time to develop. Great care must also be taken to avoid the development of an infectious disease that would immediately alter the immunological parameters under study. In fact, to be considered valid, reports of the immunological effects of a micronutrient deficiency should contain evidence that the animals were infection-free at the time of data collection.

Despite its overall importance to human health and well-being, the field of nutritional immunology involves a scientific subdiscipline that is still in its infancy. As illustrated in this chapter, most of the available data in the field are decades old, of questionable reliability, and grossly incomplete. As pointed out almost 2 decades ago, no single micronutrient deficiency has been evaluated for its effects on all available immunological parameters, in animals or in humans.1 The same is true today. Despite these inadequacies, available data do remain highly useful for planning therapeutic and preventive measures, and for designing public health strategies. This very book is an example of these latter points.

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OXIDATIVE STRESS AND INFECTIONS

Johane P. Allard

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INTRODUCTION

Oxidative stress reflects a shift in the prooxidant-antioxidant balance in favor of the former. Diverse biological processes such as infection, inflammation, carcinogenesis, aging, radiation, and photobiological effects can produce such an imbalance by increasing the production of prooxidants, also called reactive oxygen species (ROS), and/or by weakening the antioxidant defense system. It is now well established that increased production of ROS can lead to tissue damage and degeneration. However, ROS produced within the boundaries of the antioxidant defense system are part of the normal aerobic metabolism, and they play a significant role in some aspects of normal cell function. Because of the possible role ROS play in the pathogenesis of diseases, there has been a considerable interest in the use of antioxidant nutritional supplementation to counteract their detrimental effect.

This chapter reviews some of the concepts regarding ROS during normal metabolism and during infections, and will present evidence about their role and the use of antioxidants during the pathogenesis of viral infections.

GENERATION OF ROS

ROS are arrays of metabolites derived from molecular oxygen and are, to a large extent, molecules with unpaired electrons, also called “free radicals.” These metabolites are highly reactive and can damage DNA, proteins, carbohydrates, and lipids. ROS are numerous and include superoxide anion radical, hydrogen peroxide, hydroxyl radical, singlet molecular oxygen, peroxyl radical, nitric oxide, peroxynitrite, and others (Table 3.1).

Generation of ROS occurs normally during aerobic metabolism. For example, the production of ATP through electron transport is accompanied by the donation of an electron to oxygen molecules. In this normal reaction, a diatomic oxygen molecule \((O_2)\) accepts two electrons and two hydrogen ions and is reduced to water. However, leakage of electrons onto \(O_2\) produces superoxide anion radical \((O_2^-)\).

Another normal process is the respiratory burst by activated phagocytic cells such as neutrophils and macrophages. This produces superoxide anion radical through the NADPH oxidase enzyme system that serves a crucial role in killing several microorganisms. During infection, respiratory burst and
ROS production are stimulated in phagocytic cells by both bacteria and viruses.\textsuperscript{2,3}

**Table 3.1 Common Reactive Oxygen Species (ROS) or Precursors and Their Sources**

<table>
<thead>
<tr>
<th>ROS</th>
<th>Half-Life (seconds)</th>
<th>Sources</th>
</tr>
</thead>
<tbody>
<tr>
<td>$O_2^-$ superoxide anion radical</td>
<td>Enzymatic</td>
<td>Oxygen metabolism potentiated by hyperoxia, inflammation, radiation</td>
</tr>
<tr>
<td>OH$^+$ hydroxyl radical</td>
<td>$10^{-9}$</td>
<td>Oxygen metabolism potentiated by hyperoxia, inflammation, radiation</td>
</tr>
<tr>
<td>$H_2O_2$ hydrogen peroxide</td>
<td>Enzymatic</td>
<td>Oxygen metabolism potentiated by hyperoxia, inflammation, radiation</td>
</tr>
<tr>
<td>RO$^+$ alkoxy radical</td>
<td>$10^{-6}$</td>
<td>By-products of free radical propagation, lipid peroxidation, prostanoid metabolism</td>
</tr>
<tr>
<td>ROO$^+$ peroxyl radical</td>
<td>7</td>
<td>By-products of free radical propagation, lipid peroxidation, prostanoid metabolism</td>
</tr>
<tr>
<td>NO$^+$ nitric oxide radical</td>
<td>1–10</td>
<td>Phagocytes, respiratory burst</td>
</tr>
<tr>
<td>ONOO$^+$ peroxynitrite</td>
<td>0.05–1</td>
<td>Phagocytes, respiratory burst</td>
</tr>
<tr>
<td>HOCl hypochlorite radical</td>
<td>—</td>
<td>Inflammation, phagocytosis</td>
</tr>
<tr>
<td>Q$^-$ semiquinones</td>
<td>Days</td>
<td>Mitochondrial electron transport</td>
</tr>
<tr>
<td>$^{1}O_2$ singlet oxygen</td>
<td>$10^{-5}$</td>
<td>Photosensitization</td>
</tr>
<tr>
<td>Fe$^{2+}$, Cu$^{2+}$ divalent metals</td>
<td>—</td>
<td>Heme (iron) and other metal-containing proteins</td>
</tr>
</tbody>
</table>

**Generation of ROS during Infections**

Infection produces an oxidative stress because there is an increased production of ROS, mainly by activated neutrophils, macrophages, and monocytes. These cells are directed to sites of infections by chemoattractants such as leukotriene B$\textsubscript{4}$.\textsuperscript{4,5} Their interaction with opsonized particles is accompanied by degranulation with release of lysosomal enzymes, and a respiratory burst that is indispensable for the intraphagocytic killing of microorganisms (see Reaction (1)).

The immediate consequence of the respiratory burst is the generation, within seconds, of the superoxide anion radical that is the precursor of a series of microbicidal oxidants.\textsuperscript{2} Superoxide generation is mediated by a membrane-associated NADPH-oxidase system,\textsuperscript{6,8} which promotes the
transfer of a single electron from NADPH to molecular oxygen according to the reaction:

\[
\text{NADPH} + \text{O}_2 \rightarrow \text{NADP}^+ + \text{O}_2^\cdot + \text{H}^+ \quad (1)
\]

Superoxide anion (\(\text{O}_2^\cdot\)) radical is then dismutated spontaneously and rapidly to form hydrogen peroxide (\(\text{H}_2\text{O}_2\)):

\[
\text{O}_2^\cdot + \text{O}_2^\cdot + 2\text{H}^+ \xrightarrow{\text{superoxide dismutase}} \text{O}_2 + \text{H}_2\text{O}_2 \quad (2)
\]

In the cytosol, the dismutation of superoxide to hydrogen peroxide is catalyzed by superoxide dismutase.\(^9\) Hydrogen peroxide can then be reduced to yield the extremely reactive hydroxyl radical (\(\text{OH}^\cdot\)) via the Fenton-Haber-Weiss reaction.\(^{10}\) Electron donors may be semiquinones or reduced transition metal ions such as \(\text{Fe}^{2+}\) or \(\text{Cu}^{2+}\):

\[
\text{Fe}^{2+} + \text{H}_2\text{O}_2 \rightarrow \text{Fe}^{3+} + \text{OH}^- + \text{OH}^\cdot \quad (3)
\]

Under conditions where both superoxide and hydrogen peroxide exist, the hydroxyl radical can be generated as follows:

\[
\text{O}_2^\cdot + \text{H}_2\text{O}_2 \rightarrow \text{O}_2 + \text{OH}^- + \text{OH}^\cdot \quad (4)
\]

The hydroxyl radical has a very positive redox potential and an extremely short lifetime in biological media, therefore reacting closely to the site of its generation and producing oxidative damage. Hydroxyl radical is the major target of the antioxidative power of phenolics such as vitamin E, because, due to its thermodynamic and kinetic properties, it is not under the control of specific enzymes. Therefore, hydroxyl radical is an extremely reactive and potent oxidant and can be produced by stimulated neutrophils.\(^2\)

Singlet oxygen also can be produced by activated phagocytes via the myeloperoxidase (MPO)/\(\text{H}_2\text{O}_2/\text{halide}\) system, and by the Fenton-Haber-Weiss reaction:

\[
\text{O}_2^\cdot + \text{H}_2\text{O}_2 \xrightarrow{\text{iron salts}} '\text{O}_2 + \text{OH}^- + \text{OH}^\cdot \quad (5)
\]

Singlet oxygen ('\(\text{O}_2\)) is formed when energy absorption shifts a valence electron to an orbital of higher energy with an inversion spin.\(^{11}\) Singlet oxygen is extremely labile and reacts rapidly with most organic molecules, especially at double bonds of unsaturated fatty acids inducing lipid peroxidation, producing hydroperoxides that can then be activated by \(\text{Fe}^{2+}\) release from endogenous stores.\(^{12}\)
The oxidizing potential of hydrogen peroxide is also dramatically increased by the enzyme MPO, in combination with a halide like chloride (Cl\(^-\)) and bromide (Br\(^-\)). Catalyzed by MPO, halides are converted into hypohalous acids (HOCl, HOBr), which are strongly oxidizing or halogenating agents produced by activated leukocytes. These agents contribute to the degradation of ingested particles or to inflammation and tissue damage. Hypochlorous acid produced by the MPO/H\(_2\)O\(_2\)/Cl\(^-\) system also generates long-lived oxidants such as chloramines and chloramides.

Nitric oxide (NO\(^{•}\)) is another highly reactive radical produced by macrophages. Nitric oxide is important in mediating the bactericidal and tumoricidal actions of macrophages, and is synthetized from the amino acid arginine catalyzed by the enzyme nitric oxide synthase. Superoxide may react with nitric oxide by enzymatic reaction, or nonenzymatically by direct reduction from nitrite, to give peroxynitrite (ONOO\(^-\)). Peroxynitrite initiates lipid peroxidation, inhibits mitochondrial transport, and inactivates glyceraldehyde 3-phosphate dehydrogenase as well as Na/K-ATPases and membrane sodium channels. It is reported that large amounts of nitrates are excreted in urine during infections and are found to arise from macrophages. The ability of macrophages to kill bacteria and fungi depends upon external arginine. Stimuli such as interferon-\(\gamma\) and lipopolysaccharide from bacterial cell wall elicit new nitric oxide synthesis from macrophages, mediating the nitric oxide responses to inflammatory stimuli. Nitric oxide also can transfer electrons from NADPH to O\(_2\) and form superoxide anion and hydrogen peroxide.

**ROLE OF ROS**

ROS, when produced within the boundary of the antioxidant defense capacity, are involved in cell growth, cell death, and other normal cell functions. They play a role in inflammatory responses and blood vessel reactivity. Accumulating evidence shows that the redox state affects cell growth and functions via modulating or utilizing signaling pathways from cell surface receptors. In fact, several reports show that ROS, for example, produced by mitochondria or by lipoxygenase, are utilized as signaling messengers in a pathway from cytokine-receptor interactions to activation of transcription factors. One of these transcription factors is NF-\(\kappa\)B.

NF-\(\kappa\)B is an important immune regulator and an inducible transcription factor that can rapidly activate the expression of genes involved in inflammatory responses. NF-\(\kappa\)B is found in B- and T-lymphocytes, macrophages, monocytes, and other cells. It is sequestered as an inactive, cytoplasmic complex by binding to I-\(\kappa\)B, an inhibitory subunit. Exposure of cells to a wide variety of pathological stimuli such as viral or bacterial infections, inflammatory cytokines, or UV irradiation leads to activation of NF-\(\kappa\)B
through the phosphorylation of I-κB. The common mechanism used by these different agents has been proposed to be the synthesis of ROS. These then serve as messengers to mediate the stimulation of NF-κB. The mechanism is as follows: an inhibitory factor, I-κB, functions to retain NF-κB in the cytoplasm. ROS release I-κB from NF-κB and consequently, the free NF-κB translocates into the nucleus, where it will bind to the DNA. Following translocation to the nucleus, NF-κB activates transcription of a large variety of genes, including those of cytokines like TNF-α and IL-1, hematopoietic growth factors, cell adhesion molecules and promoter regulatory regions of human viruses such as HIV-1 and HIV-2, human T-cell leukemia virus type-1, hepatitis B virus, herpes simplex virus type-1, and influenza virus. The characteristic rapidity of NF-κB induction also can be utilized by viruses as a strategic tool to initiate self-replication, or to place their life cycles under the control of the host cell. NF-κB also can be a survival signal, and can even be recruited by certain viruses to suppress programmed cell death in infected cells. For example, in hepatitis C virus (HCV)-infected liver tissues and in HCV core-transfected cells, NF-κB activation conferred resistance to TNF-α-induced apoptosis.

This effect is again related to ROS, because inhibition of NF-κB activation by the antioxidant dithiocarbamate sensitized the cells to TNF-α-induced apoptosis. These findings suggest that HCV infection may cause antiapoptosis by activation of NF-κB and evading the host’s immune surveillance, leading to viral persistence, and possibly to hepatocarcinogenesis. Therefore, although the detailed molecular mechanism of these interactions has not been completely elucidated, there is growing evidence that ROS are used as signaling molecules.

**Role of ROS during Infections**

During infection, phagocyte-derived ROS can damage and kill microorganisms by various mechanisms. Superoxide anion, hydroxyl radical, hydrogen peroxide, and singlet oxygen promote lipid peroxidation, which is implicated in microbicidal activity. Superoxide anion may be involved as an intermediate in lipid peroxidation, while hydroxyl radical can abstract hydrogen atoms from unsaturated lipids to yield hydroperoxides. Singlet oxygen reacts directly with unsaturated fatty acids to generate hydroperoxides. These same ROS can damage microbial DNA and can be mutagenic. Exposure of bacteria to hypochlorous acid causes rapid irreversible oxidation of cytochromes, resulting in cessation of energy-linked mitochondrial respiration due to oxidation of essential components of electron transport chains. Hypochlorous acid also oxidizes essential sulphhydryl groups, and chloramines and chloramides cause peptide and other amide bond cleavage, leading to microbial death. Nitric oxide has bactericidal
activity that can be blocked pharmacologically or by deleting arginine from macrophage cultures. Recent studies indicate that besides killing bacteria, nitric oxide can inhibit viral replication.\textsuperscript{40,41} Transfection of nitric oxide into cell culture lowers viral titers.\textsuperscript{40} In rats, nitric oxide inhibitors elevate coxsackie viral titers and increase mortality from the viral infection.\textsuperscript{41} On the other hand, low levels of nitric oxide and superoxide may also facilitate viral replication because of their mitogenic effects on cells.\textsuperscript{42}

During infection, activated phagocytes release cytokines such as TNF-\(\alpha\) and IL-1. These cytokines also interreact with ROS. TNF can act on host cell mitochondria, producing a prooxidant effect.\textsuperscript{43} This effect can be inhibited by antioxidants such as vitamin E. TNF also acts to release NF-\(\kappa\)B from the cytoplasmic inhibitor protein I-\(\kappa\)B.\textsuperscript{44} This is mediated by ROS, and NF-\(\kappa\)B-induced gene transcription can be inhibited by certain antioxidants.\textsuperscript{44} Activated monocytes also release IL-1 that stimulates neutrophils to release lysosomal proteins, including lactoferrin.\textsuperscript{45} Lactoferrin rapidly binds iron and accumulates in the reticuloendothelial system. If the accumulated iron exceeds cellular iron-binding capacity, unbound prooxidant iron could interact with superoxide, according to the Fenton-Haber-Weiss reaction, to produce highly reactive hydroxyl radical.

**DAMAGE FROM EXCESSIVE PRODUCTION OF ROS**

Antimicrobial ROS generated by activated phagocytes are not restricted to the intracellular milieu, and extracellular release of ROS occurs,\textsuperscript{2} influencing the pathogenesis of diseases. Phagocytosis minimizes oxygen toxicity by removal of microorganisms from the tissues, thereby promoting selective intraphagocytic exposure of microbial pathogens to ROS. However, extracellular release of ROS still occurs during this process. These ROS are toxic to a wide variety of eukaryotic cells.\textsuperscript{46,47} If not promptly neutralized by antioxidant defense mechanisms, ROS will damage surrounding cells and tissues by disrupting vital cellular processes such as membrane transport and mitochondrial respiration.\textsuperscript{48} Nitric oxide production also has been implicated in pathologic conditions such as septic shock.\textsuperscript{49,50}

**Lipid Peroxidation**

ROS involved in cell dysfunction are products of hydrogen peroxide/superoxide interactions, or of the myeloperoxidase/hydrogen peroxide/halide system.\textsuperscript{46,47} Hydrogen peroxide can easily penetrate cell membranes and produce hydroxyl radical. Hydroxyl radical is likely directly responsible for most of the oxidative damage \textit{in vivo}, most frequently due to lipid peroxidation, a process whereby lipids containing polyunsaturated fatty acids and their esters can be readily oxidized by molecular oxygen. Lipid peroxidation
is usually a deleterious process and is involved in a variety of pathological events. Such lipid oxidation proceeds by a free radical chain mechanism as follows:51

Initiation:

\[
\begin{align*}
\text{OH}^+ + \text{LH} & \rightarrow \text{L}^+ + \text{H}_2\text{O} \\
\text{O}_2 + \text{L}^+ & \rightarrow \text{LOO}^-
\end{align*}
\]

Propagation:

\[
\text{LOO}^- + \text{LH} \rightarrow \text{LOOH} + \text{L}^+
\]

Termination:

\[
\begin{align*}
\text{Antioxidant} + \text{LOO}^- & \rightarrow \text{Radical intermediate} \\
\text{Radical intermediate} + \text{LOO}^- & \rightarrow \text{Nonradical product}
\end{align*}
\]

In the initiation step, the reaction of an initiating radical (e.g., hydroxyl radical OH+) with a polyunsaturated fatty acid (LH) yields a lipid radical (L+) (6). The lipid radical reacts with oxygen rapidly to form lipid peroxyl radical, LOO^- (7). In the propagation phase, the peroxyl radical (LOO^-) then abstracts a hydrogen atom from another polyunsaturated fatty acid (LH) to generate a lipid hydroperoxide (LOOH) and another lipid radical, which continue the chain reaction (8). Thus, many molecules of lipids may be oxidized to lipid hydroperoxides for every initiation event. Transition metals, particularly iron, play an important role in peroxyl radical generation by catalyzing chain-branching reactions. Vitamin E and other phenolic antioxidants inhibit lipid peroxidation, primarily by inhibiting reaction (8). The antioxidant traps a peroxy radical to form a transient radical intermediate (9), which then reacts further to produce nonradical product(s) (10).

### Carcinogenic Effects

Activated phagocytes produce ROS that are potentially carcinogens. Highly reactive forms of molecular oxygen such as superoxide anion, hydroperoxyl radical, singlet oxygen, hydroxyl radical, and hydrogen peroxide are potent inducers of DNA strand breaks and chromosomal aberrations.52-55 This may have some relevance in viral infections. For example, based on studies using transgenic animal models for hepatitis B virus, ROS, which can be produced by hepatic Kupffer cells, may play a role in hepatocarcinogenesis through oxidative DNA damage.56,57 Oxidant-mediated immune dysfunction as a
consequence of chronic phagocyte activation also may predispose to an increased risk of cancer.

**Apoptosis**

Apoptosis is a physiological mechanism that preserves homoeostasis in the turnover of normal tissue. Many agents that induce apoptosis are either oxidants or activators of cellular oxidative metabolism, suggesting that generation of ROS plays a significant role in inducing apoptosis. Conversely, many inhibitors of apoptosis have antioxidant activities. ROS-related apoptosis plays a significant role during infection. In HIV, apoptosis of T-lymphocytes has been documented as linked to ROS production, and might contribute to the depletion of lymphocytes and the pathogenesis of the disease. It was also reported that HIV gene expression enhances T-cell susceptibility to hydrogen peroxide-induced apoptosis. Induction of apoptosis was reported to be blocked by antioxidants glutathione, N-acetylcysteine, and catalase.

In the brains of AIDS patients with dementia, apoptosis of neurons and nonneuronal cells has also been demonstrated. It was shown that TNF-α-induced neuronal apoptosis by HIV-1 Tat via a mechanism that also involves increased oxidative stress and antioxidants inhibits the induction of neuronal apoptosis. Another mechanism for mental alteration in viral infection of the brain also implicates ROS, whereby nitric oxide produced by inflammatory cells of the central nervous system may interfere with its normal role as a second messenger in neurons.

Other viruses, including influenza A and B viruses, also produce apoptotic cytotoxicity, possibly a natural phenomenon that evolved to contain viral infections. On the other hand, some proteins of DNA viruses may actually exert an antioxidant effect that may be linked to the oncogenic potential of the viruses. The latent membrane protein1 of Epstein-Barr virus and the 19-kD adenovirus E1B-transforming protein inhibit apoptosis, thereby promoting transformation.

**Proteolytic Effects**

ROS can produce tissue damage by uncontrolled proteolysis. α-1-Protease inhibitor (API) is the major blood and tissue protease inhibitor. API is crucial to the biological defense of tissues from protease enzymes, primarily by protecting against phagocyte-derived elastase. However, API is particularly vulnerable to oxidative inactivation with consequent loss of elastase-inhibitory capacity. Exposure of API to activated neutrophils or to a cell-free myeloperoxidase/hydrogen peroxide/halide system leads to loss of the elastase-inhibitory capacity of the API.
Chronic phagocyte activation may therefore be accompanied by a tissue protease/protease inhibitor imbalance potentiating the activity of phagocyte-derived elastase and leading to uncontrolled tissue proteolysis. This is a probable cause of tissue damage in chronic infection and inflammation.

**Immune Dysfunction**

Phagocyte-derived ROS released extracellularly are also autotoxic to surrounding phagocytes, causing inhibition of chemotaxis, phagocytosis, and antimicrobial activity.\(^ {73-75} \) They have also been reported to inhibit the proliferation of T- and B-lymphocytes, as well as the cytotoxic activity of natural killer cells.\(^ {76-78} \) Superoxide and hydrogen peroxide inhibit immune reactivity\(^ {75} \) and oxidants generated by the myeloperoxidase/hydrogen peroxide/halide system are particularly potent immunosuppressive agents.\(^ {76-78} \)

**ANTIOXIDANT DEFENSE SYSTEM**

ROS have vast differences in their half-lives (see Table 3.1) — from nanoseconds for the hydroxyl radical, to seconds for peroxyl radical, nitric oxide, and peroxynitrite — requiring several biological lines of defense (Table 3.2). To protect themselves from the harmful effects of these ROS, the cells had to develop complex defense mechanisms that involve enzymatic and nonenzymatic antioxidants. Antioxidants are any substances that significantly delay or inhibit oxidation of a substrate. There are different and overlapping strategies of defense against oxidative stress, and many compounds of endogenous nature exhibit antioxidant functions.

Intracellular protection of cytoplasmic components from oxidant-inflicted damage is probably mediated, for the most part, by the antioxidant enzymes superoxide dismutase, catalase, and glutathione peroxidase.\(^ {46} \)

Extracellular neutralization of phagocyte-derived oxidants is largely attributable to nonenzymatic antioxidant mechanisms. Ceruloplasmin, low-molecular-weight agents such as ascorbate, and protein and nonprotein sulphydryls are important extracellular scavengers of reactive oxidants.\(^ {79} \) In human blood, urate, plasma proteins, ascorbate, and \( \alpha \)-tocopherol account for 35–65%, 10–50%, 0–20%, and 5–10%, respectively, of the total peroxyl radical-trapping antioxidant capacity.\(^ {80} \) The lipid-soluble, low-molecular-weight antioxidants \( \alpha \)-tocopherol and \( \beta \)-carotene function as oxidant scavengers, predominantly in the hydrophobic cell-membrane compartment. These biological antioxidant defense mechanisms are indispensable for the maintenance of cell and tissue integrity. However, during excessive and chronic phagocyte activation, antioxidant systems may be overwhelmed, leading to uncontrolled activity of reactive oxidants.
This review mainly discusses antioxidants related to micronutrients. They are divided in two categories: chain-breaking and preventing antioxidants.51

### Chain-Breaking Antioxidants

The chain-breaking antioxidants scavenge free radicals and suppress the free radical chain oxidation by molecular oxygen. There are both water-soluble and lipid-soluble chain-breaking antioxidants in vivo, and they function at different sites. Water-soluble chain-breaking antioxidants are, for example, ascorbic acid and uric acid. They scavenge oxygen-centered radicals and suppress lipid peroxidation. As for the lipid-soluble chain-breaking antioxidants, the most potent and most significant in vivo is vitamin E.
**Vitamin C**

Vitamin C (L-ascorbic acid), present as ascorbate in most biological settings, is considered the most important antioxidant in extracellular fluids. Vitamin C has been shown to efficiently scavenge superoxide, hypochlorite, hydrogen peroxide, hydroxyl radical, peroxy radicals, and singlet oxygen.\(^{48,81-83}\) In studies with human plasma lipids, it was shown that ascorbate was far more effective in inhibiting lipid peroxidation initiated by a peroxy radical initiator than other plasma components such as protein thiols, urate, bilirubin, and \(\alpha\)-tocopherol.\(^{84}\) When the radicals are generated initially in the aqueous region and attack the membranes from outside, vitamin C and other water-soluble antioxidants can scavenge the radicals in the aqueous phase before the radicals attack the membranes. Hence, vitamin C in the membrane may not be consumed initially, but will trap radicals after most of the antioxidants in the aqueous phase are used up and the radicals reach the membranes. This effect of vitamins C and E is then additive.\(^{51}\) Another mechanism by which ascorbate can protect membranes against peroxidation is by restoring the radical scavenging activity of vitamin E, located in the lipid membrane.

**Vitamin E**

Vitamin E refers to one or more of the structurally related phenolic compounds called tocopherols and tocotrienols. Among them, \(\alpha\)-tocopherol has the highest biological activity. Vitamin E scavenges the chain-carrying peroxy radicals rapidly and interrupts the chain propagation. \(\alpha\)-Tocopherol is incorporated into liposomal membranes where it suppresses lipid peroxidation.

The vitamin E radical formed may undergo a few competing reactions: It may react with another peroxy radical to give a stable adduct, react with another vitamin E radical to give a dimer, or react with vitamin C to regenerate vitamin E.\(^{51}\) Vitamin C regenerates vitamin E by reducing tocopheroxyl radical.\(^{85}\) The tocopheroxyl radical that forms in membranes reacts with ascorbate to yield tocopherol and the ascorbyl radical. This reaction leads to the maintenance of the radical-scavenging potential within the membrane by regenerating tocopherol, and to the transfer of the oxidative challenge to the aqueous phase. This interaction between vitamins E and C is called “synergistic inhibition” and it is presumably performed at the surface of the membranes.\(^{51}\)
Other Antioxidants

β-Carotene, in addition to being an effective quencher of singlet oxygen, also has been found to be a radical-trapping antioxidant. Other carotenoids also have been shown capable of inhibiting free radical reactions. Bilirubin can function as an antioxidant. Estrogens, such as estrone, estradiol, and 2-hydroxyestradiol, inhibit lipid peroxidation.

There are also several examples of interplay among antioxidants. In addition to ascorbic acid, glutathione and cysteine are capable of reducing α-tocopheroxyl radical to give α-tocopherol. There is also coupling to the general cellular redox potential of the NADH or NADPH systems for ascorbate, dihydrolipoate, and glutathione. Therefore, the relative importance of a given antioxidant may not rely only on its reactivity and concentration in a given compartment, but also on its capacity to interact with regenerating systems. Many such interactions are carried out enzymatically by preventive antioxidants.

Preventive Antioxidants

Preventive antioxidants deactivate the active species and possibly precursors of free radicals, thereby suppressing the generation of free radicals and reducing the rate of chain initiation. These are the antioxidant enzymatic systems located intracellularly, like glutathione peroxidase and catalase, that decompose hydroperoxides and hydrogen peroxide, respectively.51

Catalase and peroxidases can be considered free radical scavengers, even though hydrogen peroxide is not a radical species. They help lower the steady-state concentrations of hydrogen peroxide, which is a precursor of more potent radical species such as hydroxyl radical. Thus, the cytotoxic potential of hydrogen peroxide is in large part a function of intracellular catalase and peroxidase activities that scavenge hydrogen peroxide, and the concentration of transition metals that can induce hydrogen peroxide to hydroxyl radical.

Superoxide dismutases are metalloproteins that function to dismute superoxide to hydrogen peroxide. Examples of superoxide dismutases are Zn, Cu superoxide dismutase (Cu, Zn-SOD) and Mn superoxide dismutase (Mn-SOD). These enzymes have been reported to be located in mitochondria, but some of these activities are probably cytoplasmic.86 Glutathione, a cysteine-containing tripeptide, acts as a co-factor in enzymatic reactions and maintains protein sulphydryl redox status. It is also another important line of defense against intracellular oxidants.87 The tripeptide glutathione (GSH), in concert with its reductant NADPH and enzymatic catalysts, can reduce hydrogen peroxide, lipid peroxides, disulfides, ascorbate, and free radicals. A class of enzymes termed glutathione peroxidases, which are selenium dependent, catalyzes peroxide reduction. The product of the
reaction is glutathione disulfide (GSSG) or a GSH adduct of lipid or protein. Glutathione reductase will reduce disulfides (GSSG) back to GSH using NADPH as a co-factor. Another enzyme, cellular transhydrogenase, then serves to maintain NADH and NADPH in equilibrium. Therefore, cellular oxidative stress may deplete the NADPH needed for GSSG reduction and may affect countless cellular integrated metabolic processes.

Other examples of preventive antioxidants are transferrin and albumin that bind to iron and copper, chelating metal ions and preventing initiation of lipid peroxidation. β-Carotene is also a preventive antioxidant as a singlet-oxygen quencher, preventing singlet oxygen from oxidizing double-bonds that would lead to lipid peroxidation and the production of lipid hydroperoxides. Among carotenoids, lycopene is the most efficient singlet-oxygen quencher.

Thioredoxin (TRX) is also a system that has been recently reported to contribute to the cellular redox system in addition to glutathione. TRX is a multifunctional protein with a redox-active disulfide/dithiol in the active site; it operates together with NADPH and TRX reductase as a general protein disulfide-reducing system. Intracellular overexpression of TRX was detected in transformed cells related to virus infection. Such transforming viruses include HTLV-1, EBV, hepatitis B virus, and papilloma virus. TRX is induced by oxidative stress, including hydrogen peroxide. Both glutathione and TRX also have been reported to have a protective effect against apoptosis.

Dysregulation of TRX is observed in tissues of humans infected with HIV, and TRX-expressing cells are reported to be lost in the lymph nodes of AIDS patients. Exogenous TRX also can protect cells against oxidative stress induced by hydrogen peroxide and activated neutrophils. The mechanisms of the cytoprotection are not clear, but one unique effect of exogenous TRX is to promote the uptake of cysteine into cells that are necessary for glutathione synthesis and the upregulation of the intracellular levels of glutathione.

**EVIDENCE OF OXIDATIVE STRESS DURING VIRAL INFECTIONS**

Oxidative stress is implicated in the pathogenesis of several viral infections, like hepatitis, influenza, and HIV, by increasing production of ROS and weakening the antioxidant defense system. ROS can play a role in the eradication or containment of the viral infection, but they may also favor the selection of viral mutants and enhance viral replication by activating transcription factors such as NF-κB. Therefore, the interplay between ROS production, the antioxidant defense system, and viral infections is extremely complex. Recent observations indicate that clear deficits in antioxidants can
have quite a dramatic effect on the outcome of viral infections. Therefore, use of antioxidants in the therapy of viral diseases may be of benefit.

**Influenza**

Influenza virus is a segmented RNA virus that is responsible for a significant morbidity and mortality worldwide. It can damage both the lungs and airways due to inflammatory responses. Influenza virus directly activates monocytes and polymorphonuclear neutrophils (PMN) to generate ROS, and ROS can act as stimulators for T-cell proliferation, playing a role in the symptoms and pathology of the infection. Influenza also can induce the expression of a variety of cytokines and proapoptotic genes in infected cells. This process is likely mediated by ROS because it is inhibited by an antioxidant, dithiocarbamate. Knobil et al. reported that influenza induces nuclear translocation and DNA binding of NF-κB factors as determined by electrophoretic mobility shift assay, and increases the production of intracellular ROS. Studies have shown that neutrophils and macrophages are important cellular sources of superoxide anion in the lungs of mice infected with the influenza virus. This is probably due to the upregulation of xanthine oxidase, an enzyme-generating superoxide that is released into alveolar spaces during the infection.

The damage that occurs during an influenza virus infection is caused in part by the host cell inflammatory reaction due to recruitment of inflammatory cells to the lung. Activated neutrophils and macrophages, by producing ROS, induce an oxidative stress that may play a role by increasing the local expression of chemokines, low-molecular-weight proteins that act as chemoattractants for immune cells.

The role of nitric oxide in the pathogenesis of influenza virus-induced pneumonia in mice also was investigated. Results showed that there was a significant amount of nitric oxide generated in the lungs, induced via production of interferon-γ, and formation of peroxynitrite through the reaction of nitric oxide with superoxide anion generated by alveolar phagocytic cells and xanthine oxidase. Administration of an inhibitor of nitric oxide resulted in a significant improvement in the survival rate of virus-infected mice without appreciable suppression of their antiviral defenses. This suggests that nitric oxide, together with superoxide anion and the formation of reactive peroxynitrite, is an important pathogenic factor in influenza virus-induced pneumonia in mice.

Influenza viral infection also has an effect on the antioxidant defense system. Infection of human airway epithelial cells with the virus results in an increase in expression of mRNA for Mn-superoxide dismutase (Mn-SOD).
In infected mice, there was also an increase in mRNA for Mn-SOD. However, no effect was seen for Zn, Cu-SOD and catalase mRNA. Treatment of influenza-infected mice with superoxide dismutase protected the mice from a lethal infection. In another study in mice, cells lavaged from the lungs were shown to be primed for enhanced ROS production. The number of phagocytes increased in the fluid and produced ROS. The activity of xanthine oxidase, an enzyme-generating superoxide, also was shown to be increased. Furthermore, the concentrations of antioxidants decreased in the lungs during infection, indicating that the antioxidant-buffering capacity diminished during the influenza infection. There was a decrease in the total concentration of lung glutathione and the antioxidant vitamins C and E.

There is one report on the efficacy of antioxidant therapy in influenza virus pneumonia. Oda et al. demonstrated that administration of pyran polymer-conjugated superoxide dismutase protected mice against potentially lethal doses of influenza A virus.

In humans, there is also evidence of oxidative stress during influenza infection. Children with Reye’s syndrome exhibit increased serum lipid peroxides and lipofuscin-like substances in their livers. It is hypothesized that this oxidative stress may induce lipid peroxidation in mitochondrial membranes, leading to mitochondrial dysfunction. This was supported by the fact that hepatocytes infected with influenza B depressed mitochondrial respiration when incubated with activated macrophages. This effect was inhibited by anti-TNF and vitamin E. However, another study with a mouse model infected with influenza B and Reye’s syndrome did not show increased lipid peroxidation in hepatic mitochondria or microsomes.

Coxsackievirus

Beck et al. have reported on the effect of a selenium-deficient diet on another RNA virus, coxsackievirus B3. These experiments provided the first persuasive evidence that the redox status of the host cell may have an effect on the viral genetic composition. In these studies, mice deficient in selenium and vitamin E had extensive myocarditis due to this coxsackievirus. However, the same strain was harmless in animals with sufficient selenium and vitamin E. Interestingly, inoculation of the virus from the deficient animals into the nondeficient mice also produced myocarditis, suggesting that the antioxidant status of the host may have influenced viral evolution. Therefore, dietary oxidative stress due to either selenium or vitamin E allowed a normally benign (i.e., amyocarditic) coxsackievirus B3 to convert to virulence and cause heart damage. This conversion to virulence was due to a nucleotide sequence change in the genome of the benign virus, which then resembled more closely the nucleotide sequence...
of virulent strains. This was the first report of host nutrition affecting the genetic sequence of a pathogen.

Based on the murine myocarditis model with the coxsackievirus, nutritionally induced oxidative stress may play a role in the emergence of more virulent infection. One possible example suggested by Beck et al. is the emergence of a virulent coxsackievirus responsible for the etiology of Keshan disease in the low selenium regions of China. There, using the technique of polymerase chain reaction, enteroviral RNA has been detected in paraffin-embedded myocardial specimens from Keshan disease victims. Another example of emerging virus possibly due to poor antioxidant status is the epidemic of optic and peripheral neuropathy that affected more than 50,000 people in Cuba, where 84% of the cerebrospinal fluid yielded viruses resembling enterovirus. Extensive epidemiologic studies have established that the disease was associated with an unbalanced diet low in animal protein, fat, and B-group and other vitamins. In particular, impairment of protective antioxidant pathways was suggested because patients had lower levels of riboflavin, vitamin E, selenium, α- and β-carotenes, and especially the carotenoid lycopene. Smoking, which also increases oxidative stress, was also a risk factor. Most patients responded to parenteral vitamin therapy followed by oral supplementation.

**Cytomegalovirus (CMV)**

Oxidative stress is also associated with CMV activation. TNF-α-associated ROS production was shown to directly induce CMV replication. In cell cultures, it was shown that TNF-α stimulated the transfected CMV immediate-early genes enhancer-promoter via induction of the transcription factor NF-κB. The antioxidant defense system is also affected. Intracellular levels of reduced glutathione are inversely correlated with CMV permissiveness in different cell types, and when the cellular glutathione level is reduced experimentally, it leads to activation of CMV. N-acetylcysteine was found to inhibit CMV infection in vitro.

**Viral Hepatitis**

In children with chronic viral active hepatitis B, there is an increased production of superoxide anion by resting neutrophils. Stimulated neutrophils, however, produced decreased amounts of superoxide anion compared to controls. Experiments using transgenic animals to study hepatitis B also have suggested that ROS from activated phagocytes or Kupffer cells may produce DNA oxidation, and may play a role in hepatocarcinogenesis. In another model using woodchuck hepatitis virus that resembles hepatitis B virus, Liu et al. reported increased urinary excretion of nitrate.
and the hepatocarcinogen N-nitrosodimethylamine when administering arginine and lipopolysaccharide. This suggests increased production of nitric oxide which, combined with superoxide anion, produced peroxynitrate. Furthermore, hepatocytes from infected animals produced increased amounts of the hepatocarcinogen N-nitrosomorpholine, also attributable to increased production of nitric oxide.

Iron overload, known to increase lipid peroxidation in the liver, may exacerbate hepatotoxicity due to viral hepatitis, or may perhaps synergize the hepatocarcinogenicity caused by chronic viral hepatitis. Senba et al. noted a strong correlation between the presence of hepatitis B surface antigen and iron deposition in the Kupffer cells and spleens of infected individuals. In addition, there seems to be a weakened antioxidant defense system along with increased iron. Bannister et al. demonstrated that the human hepatoma cell line Hep 3B, which has the hepatitis B virus genome, has markedly decreased copper/zinc as well as decreased manganese superoxide dismutase activity, decreased catalase, and absent glutathione peroxidase and glutathione-S-transferase. On the other hand, there was a more than 270-fold increase in ferritin, and a 25-fold increase in intracellular iron compared to normal autopsy liver. Interestingly, in patients with chronic viral hepatitis C who have previously not responded to interferon, iron reduction by phlebotomy as adjuvant was associated with less liver injury. This was manifested by a decrease in serum transaminase activity and a slight improvement in liver histopathology.

For hepatitis B virus and other similar viruses (woodchuck hepatitis virus, Peking duck virus), there are surprisingly few studies on the effect of antioxidants. Hagen et al. attempted to prevent oxidative hepatic DNA damage in the transgenic mouse model of the large envelope-protein of hepatitis B virus using vitamin E, but prevention was not effective. Some antivirals like α-interferon for hepatitis C may have antioxidant effects. Serum lipid peroxides were increased in patients with chronic hepatitis. In those individuals responding to treatment with α-interferon, serum lipid peroxides declined to values comparable to those of controls.

**HIV Infection**

ROS also influence the pathogenesis of HIV. *In vitro*, manipulations that result in enhanced oxidative stress increase the replication of HIV. HIV replication is enhanced due to the activation of NF-κB by ROS. NF-κB, in turn, binds to and activates a κB-enhancer element in the HIV proviral long terminal repeat, leading to increased viral gene expression. Antioxidants also have been shown to inhibit stimulation of HIV transcription.
HIV also may stimulate ROS production by various mechanisms, including interaction of gp125 with the cell membrane, and via Tat, the transactivating protein. Furthermore, opportunistic infections such as mycoplasmas, which are known to stimulate HIV replication, also may act by increasing oxidative stress, probably from the release of hydrogen peroxide from activated phagocytes. The effect of oxidants on viral replication has been confirmed by experiments in which both water- and lipid-soluble antioxidants were shown to inhibit HIV replication in vitro.

Antioxidants that demonstrated antiviral activity include N-acetylcysteine, cysteine, glutathione and glutathione esters, ascorbate, vitamin E, lipoic acid, and several other chemical compounds. However, the activity of antioxidants differs across various cell culture systems. In vitro studies with lymphocytes also indicate that selenium supplementation decreased NF-κB activation by TNF, and suppressed TNF-induced HIV replication.

Oxidative stress also may induce apoptosis. In HIV, excess hydrogen peroxide combined with deficiencies in catalase and glutathione peroxidase may lead to overproduction of hydroxyl radicals and lipid peroxides, and subsequently may induce apoptosis. The reasons for the progressive loss of CD4 lymphocytes in HIV-infected patients are unclear, but apoptosis may contribute to this decline. Adding TNF and hydrogen peroxide to HIV-infected cells in vitro increased cell death, while adding antioxidants such as vitamin E and catalase decreased apoptosis in these cells.

Evidence of Oxidative Stress in Humans Infected with HIV

Several studies have shown that asymptomatic HIV-infected or AIDS patients have increased oxidative stress, demonstrated mainly by elevated plasma metabolites of lipid peroxidation and reduced antioxidant vitamin levels. Several groups also have demonstrated depleted cysteine or glutathione in the tissues of HIV-infected patients, or disturbance in glutathione homoeostasis. Glutathione deficiency in CD4 T-cells and low total serum thiol levels were shown to be associated with decreased survival. Serum selenium, plasma glutathione, and red-cell glutathione peroxidase activity are further decreased at each successive stage of HIV. Baum et al. showed that selenium deficiency (<85 µg/ml) was a significant predictor of HIV-related mortality.

Plasma levels of TRX were also shown to be significantly elevated in HIV-infected individuals who are oxidatively stressed. HIV-infected individuals with higher levels of plasma TRX tend to have lower CD4 counts and lower intracellular glutathione levels. Moreover, among HIV-infected individuals with CD4 counts less than 200/µL, those with higher levels of plasma TRX survived a shorter time than those with lower levels of TRX.
Therefore, chronic oxidative stress appears to accompany the infection with HIV and, in general, there is a decline in the status of several antioxidants that seems to correlate with the severity of the disease.

Several epidemiologic studies have examined the relation between dietary and supplemental micronutrient intake and subsequent mortality among HIV-infected subjects. In terms of antioxidants, low levels of vitamins A, E, C, carotenoids, and selenium are associated with adverse clinical outcomes during HIV infection. However, randomized controlled trials on antioxidant supplementation are scarce. One group investigated the effect of one year of supplementation of selenium or β-carotene on the blood enzymatic antioxidant systems of HIV subjects. This was a non-randomized study. Superoxide dismutase, glutathione peroxidase, catalase, glutathione status, and plasma selenium were measured. No significant difference was observed for superoxide dismutase activity compared with baseline. However, glutathione peroxidase activity increased significantly after selenium treatment, whereas only a slight increase was found after β-carotene. A significant increase in glutathione was observed when either selenium or β-carotene was given. Finally, we conducted a randomized, double-blind, placebo-controlled trial using vitamin E (dl-α-tocopherol acetate) 800 IU/d and vitamin C 1000 mg/d for 3 months. We demonstrated that vitamin supplementation resulted in a significant decrease in oxidative stress parameters and a trend toward a decrease in viral load.

CONCLUSION

Oxidative stress can be generated by various types of infections. Studies have demonstrated that oxidative stress can directly activate transcription factors such as NF-κB and increase the replication of certain viruses. Antioxidant added to cell systems can inhibit this effect and significantly reduce viral transcription. During viral infections in animals and humans, there is evidence of increased oxidative stress based on elevation of lipid peroxidation metabolites. In addition, plasma and tissue levels of glutathione, antioxidant vitamins, and/or enzymes are generally low, suggesting weak antioxidant defense systems.

The approach of utilizing antioxidants to both decrease viral replication and decrease virus-induced oxidant injury may be useful in improving clinical outcomes. This will require further clinical studies.

REFERENCES


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VITAMIN A, CAROTENOIDs, AND HIV INFECTION

Richard D. Semba

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INTRODUCTION

The nutritionally acquired immune deficiency syndromes (NAIDS) are the leading cause of immune deficiency worldwide. Vitamin A deficiency is perhaps the most common and best understood of these. Vitamin A, or all-trans-retinol, plays an essential role in immunity, reproduction, maintenance of epithelial surfaces, growth, and vision. Dietary sources of vitamin A are found either as preformed vitamin A in foods such as dairy products, liver, and fish liver oils, or as provitamin A carotenoids in foods such as spinach, papayas, mangos, and carrots. Over 600 carotenoids from natural sources have been characterized and, of these, about 50 may be metabolized to vitamin A. The main dietary carotenoids found in human tissue and blood are β-carotene, α-carotene, and β-cryptoxanthin — which have some provitamin A activity — and lycopene, lutein, and zeaxanthin. Vitamin A and the carotenoids have aroused much interest in the area of HIV/AIDS because of the important roles these substances play in immune function and as antioxidants. Vitamin A deficiency may worsen the immune deficiency associated with HIV infection, theoretically through potentiating the impairment of the immune function. Although there are many populations at risk for both HIV infection and vitamin A deficiency, the clinical consequences of the two associated conditions have not been completely characterized.

Vitamin A deficiency, as assessed by low plasma vitamin A concentrations, has been associated with anemia, increased adult mortality, and increased infant mortality during HIV infection (Table 4.1). HIV-infected pregnant women with vitamin A deficiency had higher risks of mother-to-child transmission of HIV, HIV in breast milk, and HIV shedding in the vagina. Recent clinical trials in Tanzania, South Africa, and Malawi (Kumwenda, unpublished data) have not shown an impact of vitamin A supplementation on mother-to-child transmission of HIV, but there may be benefits for pregnancy outcomes, including low birth weight (Kumwenda, unpublished data) and pre-term birth.

HISTORICAL BACKGROUND

Vitamin A, in the form of fish liver oils, has long been recognized to influence the morbidity and mortality of infectious diseases. As early as the eighteenth century, cod-liver oil, a potent source of vitamins A and D, was known to have therapeutic value in the treatment of anemia, tuberculosis, measles, malaria, and other disorders. Over the last 200 years, naval and army surgeons observed the association between night blindness and increased morbidity and mortality. The existence of vitamin A was demonstrated through a long incremental process that included contributions by
over two dozen investigators, including the nutritional deprivation experiments in dogs conducted in 1816 by François Magendie (1783–1855); feeding experiments in rats by Nicolas Lunin (1853–1937); the demonstration of the fat-soluble nature of vitamin A by Wilhelm Stepp (1882–1964), Elmer McCollum (1879–1967), Thomas Osborne (1859–1929), and Lafayette Mendel (1872–1935); the description of its chemical structure; and the eventual isolation and crystallization of vitamin A in 1937 by Holmes and Corbet.

Nutritional deprivation experiments suggested that vitamin A deficiency increased the risk of infections, and vitamin A was termed the “anti-infective” vitamin in 1928. Between 1928 and 1940, at least 30 trials were conducted to determine whether vitamin A could reduce the morbidity and mortality from respiratory infections, measles, puerperal sepsis, tuberculosis, and other infections. The positive results of these trials were emphasized by the vitamin manufacturers, and the promotion of vitamin A and cod-liver oil was widespread in Europe and the United States through the 1950s. Interest from the scientific community in the use of vitamin A as

Table 4.1 Adverse Outcomes Associated with Vitamin A Deficiency and Poor Carotenoid Status during HIV Infection

<table>
<thead>
<tr>
<th>Outcome</th>
<th>Location</th>
<th>Association</th>
<th>Ref.</th>
</tr>
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<tr>
<td>Low birth weight</td>
<td>Malawi</td>
<td>With low maternal plasma vitamin A during pregnancy</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>U.S.</td>
<td>With low maternal plasma vitamin A during pregnancy</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>Malawi</td>
<td>Reduced by vitamin A supplementation during pregnancy</td>
<td>(Kumwenda, unpublished)</td>
</tr>
<tr>
<td>Child growth failure</td>
<td>Malawi</td>
<td>With low maternal plasma vitamin A during pregnancy</td>
<td>99</td>
</tr>
<tr>
<td>Pre-term birth</td>
<td>South Africa</td>
<td>Reduced by vitamin A supplementation during pregnancy</td>
<td>16</td>
</tr>
<tr>
<td>Viral shedding</td>
<td>Kenya</td>
<td>With low maternal plasma vitamin A concentrations</td>
<td>14</td>
</tr>
<tr>
<td>HIV in breast milk</td>
<td>Kenya</td>
<td>With low maternal plasma vitamin A concentrations</td>
<td>13</td>
</tr>
<tr>
<td>Infant mortality</td>
<td>Malawi</td>
<td>With low maternal plasma vitamin A during pregnancy</td>
<td>11</td>
</tr>
<tr>
<td>Low CD4 lymphocytes</td>
<td>U.S.</td>
<td>With low plasma vitamin A concentrations</td>
<td>8</td>
</tr>
<tr>
<td>Anemia</td>
<td>U.S.</td>
<td>With low plasma vitamin A concentrations in adults</td>
<td>9</td>
</tr>
<tr>
<td></td>
<td>Tanzania</td>
<td>With low plasma vitamin A during pregnancy</td>
<td>9</td>
</tr>
<tr>
<td>Adult mortality</td>
<td>U.S.</td>
<td>With low plasma vitamin A concentrations</td>
<td>10</td>
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anti-infective therapy appeared to wane with the advent of the sulfa antibiotics and improvements in diet in industrialized countries in the late 1930s.

In 1919, Harry Steenbock (1886–1967) and colleagues proposed that there was a connection between yellow plant pigments (carotene) and vitamin A, an observation suggested by the appearance of vitamin A deficiency in a rat colony when white corn was substituted for yellow corn in the regular animal feed. Crystalline carotin was found to have growth-promoting properties similar to vitamin A, and carotin was found to have anti-infective properties like vitamin A. Thomas Moore found that purified carotin restored growth and cured ophthalmia, leading to the conclusion that carotin was a precursor to vitamin A. Carotin was described in human adipose tissue, milk, and serum. The chemical structure of β-carotene was described by Paul Karrer and colleagues in 1930. Two pathways for the cleavage of β-carotene to vitamin A — central cleavage to yield two molecules of retinal, and asymmetrical cleavage to yield a short and long β-apocarotenol — were suggested in 1960.

Vitamin A underwent a renewed period of investigation in the 1980s after a longitudinal study of preschool children in Indonesia suggested that Bitot’s spots and night blindness were associated with increased mortality. This observation led to the first large community-based study, demonstrating that regular vitamin A supplementation reduced childhood mortality by about one third in Indonesia. The Indonesian study was followed by a series of clinical trials in Indonesia, India, Nepal, Sudan, and Ghana. These studies demonstrated that periodic vitamin A supplementation or fortification reduced child mortality in malnourished children. A study conducted in the Sudan differed in its findings in that high-dose vitamin A capsules had no impact on either child mortality or on vitamin A deficiency itself. Further meta-analysis of these studies suggested that vitamin A supplementation primarily reduced the morbidity and mortality of diarrheal disease rather than lower respiratory tract infections. These trials were important in showing that vitamin A had therapeutic value for diarrheal disease.

The impact of vitamin A supplementation on morbidity and mortality during measles is striking. Worldwide, measles affects approximately 70 million children per year, and up to 2 million children may die annually from measles and associated complications. In developing countries, case-fatality rates of 10 to 20% are not uncommon in malnourished populations, and a measles episode may result in diarrhea, pneumonia, encephalitis, blindness, and delayed morbidity and mortality after an attack. High-dose vitamin A supplementation may reduce mortality by 50% or greater when given to children with acute, complicated measles. Similarly, the morbidity of diarrhea and pneumonia associated with measles is reduced by vitamin A supplementation. High-dose vitamin A supplementation reduces morbidity and mortality in children who have measles but no clinical signs of vitamin A deficiency.
EPIDEMIOLOGY

The HIV-infected populations at the highest risk for vitamin A deficiency and poor carotenoid status are pregnant women, infants, preschool children, and individuals with advanced HIV infection. Low plasma or serum vitamin A concentrations considered consistent with deficiency have been reported in 2 to 11% of homosexual men,52,53 15% of injection drug users,8 and 30 to 60% of pregnant women in developing countries.10,54,55 Frequency distributions of plasma or serum vitamin A concentrations from several different HIV-infected populations have been published and, as might be expected, the highest prevalence of vitamin A deficiency in HIV-infected populations is found in developing countries.6 Normal serum vitamin A concentrations were described in a cohort of HIV-infected children in North America56 and in another cohort in Baltimore.57

Abnormally low plasma or serum provitamin A carotenoids occur in about 30 to 80% of HIV-infected adults.54,58-61 Lower plasma β-carotene has been found in HIV-positive adults as compared with controls.62,63 HIV-infected children in Italy had lower β-carotene concentrations than healthy control children,64 and β-carotene concentrations appear to decrease with advanced HIV disease.65 Poor carotenoid status has been reported among HIV-infected pregnant women in Malawi66 and in adults in the United Kingdom.67

METABOLISM

Rich dietary sources of preformed vitamin A include egg yolk, liver, butter, cheese, and whole milk. In many developing countries, the consumption of foods containing preformed vitamin A is limited, and provitamin A carotenoids may be the major source. Dietary sources that are rich in α-carotene and β-carotene include dark green leafy vegetables, carrots, sweet potatoes, mangoes, and papayas; β-cryptoxanthin is found in oranges and tangerines; lycopene is the red pigment in tomatoes, and zeaxanthin is found in yellow maize. The profile of the major dietary carotenoids may differ greatly between populations, as these will reflect local differences in diet. For example, chromatograms of plasma carotenoids in HIV-positive injection drug users in Baltimore show relatively low concentrations of α-carotene and β-carotene and high concentrations of lycopene, which is found in tomato sauce and ketchup (Figure 4.1). In contrast, among HIV-positive pregnant women in Malawi, the major dietary carotenoids are lutein and zeaxanthin, found in nsima, a corn porridge staple (Figure 4.2). In Uganda, local vegetables appear to be richer in α-carotene than β-carotene, and the dominant carotenoid in the chromatograms is α-carotene (Figure 4.3). Plasma carotenoids show
Figure 4.1 Plasma carotenoids in an HIV-positive injection drug user, Baltimore, Maryland. Lycopene is a dominant carotenoid. AU is Absorbance Units.

Figure 4.2 Plasma carotenoids in an HIV-positive pregnant woman from Blantyre, Malawi. Lutein/zeaxanthin are dominant dietary carotenoids, probably reflecting common use of corn porridge as a staple food. AU is Absorbance Units.
seasonal variation that is related to the local availability of certain fruits and vegetables.\textsuperscript{66}

Digested foods that contain preformed vitamin A are emulsified with bile salts and lipids. Retinol is esterified in the intestinal mucosa, packaged into chylomicra, and carried to the liver via the lymphatic circulation.\textsuperscript{68} Provitamin A carotenoids such as $\beta$-carotene may be converted to retinaldehyde through cleavage by carotenoid-15,15$^\prime$-dioxygenase, or by an asymmetrical cleavage pathway. The bioavailability of provitamin A carotenoids is less than preformed vitamin A due to a variety of factors, including differences in efficacy of absorption and biochemical conversion.\textsuperscript{69-71} Vitamin A is also less potent an antioxidant than $\beta$-carotene. Although carotenoids, such as $\beta$-carotene, and vitamin A are often popularly regarded to be equivalent, there may be large differences in the biological functions of these two nutrients.

Approximately 90\% of the vitamin A in the body is stored in the liver as retinyl esters.\textsuperscript{72} The liver has the capacity to store enough vitamin A to last for several months, with a longer storage capacity among adults than among children. Periodic high doses of vitamin A are effective in preventing vitamin A deficiency because of the liver’s ability to uptake and store

\textbf{Figure 4.3} Plasma carotenoids in an HIV-positive adult male in Kampala, Uganda. $\alpha$-carotene, rather than $\beta$-carotene, is a dominant dietary carotenoid in this population. AU is Absorbance Units.
vitamin A for prolonged periods. Retinol is released from the liver in combination with plasma retinol-binding protein (RBP) and transthyretin (TTR). Retinol is poorly soluble in water, and is carried in the blood sequestered inside the carrier proteins RBP and TTR. Retinol seems to enter cells via specific receptors, although it is unclear whether all cells contain these receptors.

Vitamin A exerts its effects through retinoid receptors in the nucleus of the cell. In the cytoplasm, retinol is converted to all-trans-retinoic acid and 9-cis-retinoic acid. Retinoic acid influences gene activation through specific receptors that belong to the superfamily of thyroid and steroid receptors. Retinoic acid receptors act as transcriptional activators for many specific target genes. The retinoic acid receptor (RAR) is expressed as several isoforms, referred to as RARα, β, and γ. Retinoid-X receptor (RXR) is also expressed as several isoforms, referred to as RXRα, β, and γ. All-trans-retinoic acid is a ligand for RARs, whereas 9-cis-retinoic acid is a ligand for both RARs and RXRs. 9-Cis-retinoic acid seems to be functionally distinct from all-trans-retinoic acid, and interconversion may exist between the two isomers. Each RAR and RXR has a specific DNA-binding domain by which these nuclear receptors may affect transcriptional activity.

The DNA sequences that interact with RAR and RXR are known as retinoic acid response elements (RAREs). RAR and RXR receptors form heterodimers that bind to DNA and control gene expression. In addition, RXR receptors can form heterodimers with the thyroid hormone receptor, vitamin D₃ receptor, peroxisome proliferator activator receptor receptors, and a number of newly described “orphan receptors.” Most RAREs seem to occur in the regulatory region of genes. In the presence of 9-cis-retinoic acid, RXR/RXR homodimers may form and recognize a subset of RAREs or inhibit the formation of certain heterodimers. Orphan receptors such as chicken ovalbumin upstream promoter transcription factor (COUP-TF), ARP-1, TAK1, RVR, RZR, and thymus orphan receptor (TOR) may repress or modulate the induction of genes by retinoic acid. Thus, RARs and RXRs may interact with multiple transcriptional mediators and/or co-repressors, adding an enormous level of complexity to the regulation of retinoic acid responses. Other vitamin A metabolites in the retroretinoid family may support biological functions via a pathway that is distinct from the retinoic acid pathway. 14-Hydroxy-4,14-retro-retinol supports, whereas anhydroretinol inhibits cell growth. In addition, the oxoretinoids may play a role as retinoic acid receptor ligands.
BIOLOGICAL FUNCTIONS OF VITAMIN A AND CAROTENOIDS

Immunity

Vitamin A deficiency may compromise mucosal immunity through pathological changes in the epithelia of the respiratory, gastrointestinal, and urinary tracts. Keratinizing metaplasia and loss of goblet cells and mucus may occur. Loss of intestinal brush border and goblet cells and squamous metaplasia, and destruction of ciliated epithelia in the respiratory system have been reported in vitamin A-deficient animals. Decreased levels of secretory IgA in saliva have been reported in vitamin A-deficient children. Atrophy of the thymus, spleen, and lymphoid tissues has been observed in children who died with vitamin A deficiency, but these changes are also associated with severe malnutrition. Mild vitamin A deficiency is associated with underlying alterations in circulating T-cell subpopulations such as decreased CD4+CD45RA+ T-cells, or “naive” CD4 T-cells, and decreased CD4/CD8 ratios.

Vitamin A and its metabolites are essential for immune effector cell function, including T-cells of the thymus, lymphocytes in lymphoid tissue, and peripheral blood lymphocytes. Retinoic acid is involved in the expression of IL-2 receptor on T-cells. Retinol is essential for growth and differentiation of B-cells and the production of antibodies. Retinoic acid may enhance immunoglobulin synthesis by cord blood mononuclear cells through increased T-cell help, i.e., modulation of cytokines, which induce B-cells to differentiate into greater numbers of immunoglobulin-secreting cells. In vitro studies suggest that vitamin A modulates the growth and function of T- and B-cells, either directly or through its active metabolite, all-trans-retinoic acid.

Vitamin A and β-carotene supplementation are associated with changes in lymphocyte numbers and T-cell subpopulations. The depression in circulating lymphocytes following surgery can be reversed by administration of high-dose vitamin A to adults. Children with clinical and subclinical vitamin A deficiency who received vitamin A had increases in circulating CD4 T-cells, especially CD4CD45RA or naive T-cells, and an increase in the CD4/CD8 ratio 5 weeks after dosing. Measles infection is characterized by immune suppression, leukopenia, and decreased circulating CD4 T-cells. Administration of high-dose vitamin A to children with measles has been shown to increase total circulating lymphocytes, as well as to enhance IgG responses to measles. Vitamin A and related metabolites are potent enhancers of cellular differentiation, and a possible mech-
anism for the increase in CD4 T-cells may be vitamin A-related differentiation of lymphocytes.

Vitamin A deficiency is characterized by the impaired ability to mount an antibody response against T-cell-dependent protein antigens. Other types of immune responses may be unaffected by vitamin A deficiency. The antibody response to immunization with tetanus toxoid or other antigens has been used to examine immune competence. Vitamin A-deficient children in Indonesia had reduced antibody responses to tetanus toxoid. Supplementation with high-dose vitamin A, 200,000 IU (60 mg retinol equivalent) was associated with a more than twofold enhancement of both primary and secondary IgG responses to tetanus toxoid as compared to children who received a placebo. Vitamin A potentiated IgG1 subclass responses to tetanus toxoid, which is the subclass that is usually involved in the protective antibody response to tetanus. These findings suggest that the ability to mount an IgG response to T-cell-dependent antigens is improved by the administration of vitamin A or related retinoids.

The impact of vitamin A supplementation on immunity during HIV infection has not been well characterized. A recent clinical trial of vitamin A supplementation to HIV-infected injection drug users in Baltimore showed that vitamin A supplementation, 200,000 IU, given in two consecutive doses did not enhance the antibody responses to pneumococcal and Haemophilus influenzae antigens following pneumococcal and Haemophilus influenzae B (HiB) immunization (Deloria, unpublished). Vitamin A supplementation seemed to protect against a decline in circulating CD4+ lymphocyte counts over time, and was associated with a decline in plasma HIV load compared with placebo. This clinical trial suggests that vitamin A may have promise in enhancing immunity during HIV infection, but the population of injection drug users in Baltimore was relatively healthy and well nourished compared with other populations at risk for HIV infection. Further studies are needed to examine the effects of vitamin A supplementation on immunity in populations with a high prevalence of vitamin A deficiency. Clinical trials among relatively well-nourished HIV-infected adults in Portland, Oregon, showed no apparent impact of daily β-carotene supplementation on circulating CD4+ lymphocytes.

Growth

Improvement of vitamin A status through supplementation or fortification has been shown to have a nonspecific effect on child growth; factors influencing the effect of vitamin A on growth include the baseline prevalence of stunting and underweight, the burden of infectious diseases, and the prevalence and severity of vitamin A deficiency in the population. Clinical vitamin A deficiency is associated with impairment of both linear and
ponderal growth. A controlled trial in Indonesia showed that vitamin A fortification was associated with about a 1-cm increase in linear growth at each year of age in preschool children compared with controls. In Indonesia, vitamin A supplements increased linear growth 0.4 cm/4 mo among children with low baseline plasma vitamin A concentrations. In other trials, vitamin A supplementation has been shown to improve weight but not height in preschool children. Vitamin A, through its active metabolite, all-trans-retinoic acid, is involved in the regulation of growth hormone.

Among HIV-infected pregnant women in Malawi, vitamin A deficiency has been associated with low birth weight and child growth failure. A multicenter study in the United States also showed an association between low plasma vitamin A concentrations during pregnancy, and low birth weight during HIV infection. A recent clinical trial involving daily vitamin A supplementation, 10,000 IU, to HIV-infected pregnant women showed an impact of maternal vitamin A supplementation on infant growth failure (Kumwenda, unpublished). It is unclear whether vitamin A supplementation will improve growth in HIV-infected infants.

**Reproduction and Pregnancy**

Vitamin A is essential for normal reproduction. Animals deficient in vitamin A are unable to produce sperm, and its deficiency may affect fertility in the female. Vitamin A deficiency was associated with increased placental infections in both humans and animal models. Pregnancy increases the risk of vitamin A deficiency for both mothers and newborns. Epidemics of night blindness among pregnant women were well-known in Europe in the early 1900s, and it is so common in some developing countries that it has been considered a normal associate of pregnancy, as with morning sickness. Low serum vitamin A concentrations in HIV-negative pregnant women have also been associated with increased infant mortality, but a recent trial did not show any impact of antenatal vitamin A supplementation on infant mortality.

The intake of carotenoids may be important for pregnant women; a recent clinical trial in Nepal suggests that antenatal supplementation with β-carotene can reduce maternal mortality by about 50%. Decreased circulating carotenoid levels have been described in pregnant women with preeclampsia in the United States and Nigeria, and low serum β-carotene concentrations have been found in pregnant women with preterm rupture of fetal membranes.
Hematopoiesis

Vitamin A plays a role in hematopoiesis and influences the metabolism of iron. Controlled trials show that vitamin A supplementation or fortification increases hemoglobin concentrations in children. Daily supplementation of pregnant women in Indonesia with iron (60 mg elemental iron) and vitamin A (2.4 mg retinol equivalent) lessened anemia more effectively than iron supplementation alone. Pregnant women in India who received daily iron (60 mg elemental iron), folate (500 µg), and a single dose of vitamin A (60 mg retinol equivalent) showed a higher increase in mean hemoglobin than women who received iron and folate. In Bangladesh, pregnant women supplemented with daily iron (60 mg elemental iron) and zinc (15 mg) plus a single dose of vitamin A (60 mg retinol equivalent) significantly improved the anemia in 2 months compared to women receiving iron plus zinc, or daily iron alone. Retinoic acid has been shown to increase the production of erythropoietin in a human hepatoma cell line, as well as to increase serum erythropoietin in vitamin A-depleted rats. Vitamin A also was associated with a dose-dependent increase in erythropoietin in a human hepatoma cell line. It is unclear whether improving vitamin A or carotenoid status will have any influence on erythropoietin production. A recent study shows that vitamin A supplementation does not influence erythropoietin production in pregnant women (Semba, unpublished).

Vision

Vitamin A deficiency is the leading cause of blindness among children worldwide. Xerophthalmia describes the wide spectrum of eye disease associated with vitamin A deficiency, and keratomalacia refers to the most severe stage in which the cornea undergoes ulceration, often resulting in blindness. Vitamin A is essential for the generation of rhodopsin, a necessary visual pigment. The earliest clinical manifestation of vitamin A deficiency is night blindness. Vitamin A deficiency in general has an effect on mucosal epithelia, including the conjunctiva and cornea. Mild vitamin A deficiency causes squamous metaplasia of the conjunctiva, which may be detectable microscopically, or which may form a Bitot’s spot, a well-demarcated area of keratinizing squamous metaplasia on the temporal or nasal bulbar conjunctiva. Bitot’s spots are considered pathognomonic for mild vitamin A deficiency. Severe xerophthalmia is characterized by classic, punched-out, full-thickness corneal ulceration. These ulcers may be sterile, or may rapidly become secondarily infected with melting of the corneal stroma. The ocular manifestations of vitamin A deficiency are described in detail elsewhere.
PATHOPHYSIOLOGY OF VITAMIN A DEFICIENCY

Insufficient Dietary Intake
Dietary intake of vitamin A and carotenoids may be affected by anorexia, CNS disease, dysphagia, and odynophagia (painful swallowing) during HIV infection. In HIV-infected adults in the Ivory Coast, loss of appetite, aversion to food, and dysphagia were commonly reported. Esophageal candidiasis is not infrequent during HIV infection, and will usually cause dysphagia and odynophagia. Decreased food intake may occur even in asymptomatic HIV-infected adults and has been associated with significant weight loss. Among patients with advanced HIV infection and AIDS, anorexia, nausea, and vomiting may be common and severe. Chronic fatigue may interfere with shopping, cooking, and consumption of regular meals.

Malabsorption and Diarrhea
Diarrhea and malabsorption of fats, carbohydrates, and vitamin B₁₂ appear to be common in all stages of HIV infection. Cryptosporidia, Microsporidia, cytomegalovirus, and Mycobacterium avium-intracellulare are major causes of diarrhea in patients with AIDS, and many pathogens are resistant to treatment and lead to severe weight loss and death. In London, 60% of HIV-infected homosexual men at all stages of infection had fat malabsorption. A study of 61 HIV-infected adults with and without diarrhea showed that 50% had steatorrhea. Malabsorption of fat reduces the absorption of vitamin A. Jejunal and duodenal villous atrophy, with or without crypt hyperplasia, occurs in all stages of HIV disease, and alterations or reduction of the brush border of enterocytes may interfere with the metabolism of vitamin A. Physiologic studies of human duodenal biopsies show that HIV-infected patients with diarrhea have epithelial barrier defects, suggesting that a passive leak of ions, substrates, and water could contribute to diarrhea during HIV infection.

Impaired Storage and Altered Metabolism
The liver is a site for storage of vitamin A. Hepatitis B and C are extremely common in HIV-infected adults, and are associated with more rapid progression to cirrhosis and decreased survival. Although hepatitis and cirrhosis are known to interfere with metabolism of vitamin A and RBP, the relationship between liver disease and the storage of vitamin A during HIV infection has not been examined. There have been no necropsy studies...
that examined micronutrient concentrations in the livers of individuals who died with HIV infection. HIV-infected patients are at higher risk of developing renal disease, including acute renal failure, fluid-electrolyte and acid-base disturbances, HIV-associated nephropathy, and other glomerulopathies. A low-molecular-weight proteinuria appears to be common during HIV infection — even during asymptomatic infection — leading to losses of retinol-binding protein and albumin. In patients with AIDS and acute infection, there may be significant losses of retinol and RBP in the urine, which may hasten the depletion of body stores of vitamin A. During the acute phase response, TTR can dissociate from the TTR-RBP-retinol complex, thus allowing the 21kD RBP-retinol complex to be lost into the urine.

**Vitamin A and Plasma HIV Load**

Maternal vitamin A deficiency has been associated with a higher risk of mother-to-child transmission of HIV. The association between low plasma vitamin A concentrations during pregnancy and mother-to-child transmission of HIV has not been consistently observed in the United States. Recent clinical trials have shown that that association is not a causal one. In Kenya, vitamin A deficiency has been associated with HIV in breast milk and HIV shedding in the vagina. Although it was recently speculated that plasma vitamin A concentrations may be only a marker for HIV load, previously published investigations show no association between plasma vitamin A concentrations and plasma HIV load, and vitamin A supplementation seem to influence plasma HIV load.

**ASSESSMENT OF VITAMIN A AND CAROTENOYD STATUS**

The most common method for assessing vitamin A and carotenoid status during HIV infection has been the measurement of plasma or serum vitamin A and carotenoid concentrations. Another method involves the assessment of dietary intake of vitamin A and carotenoids. Vitamin A deficiency, infections, and the acute phase response are closely associated. The measurement of acute phase proteins has been advocated to improve the interpretation of low plasma vitamin A concentrations in populations with a high risk of infection. Acute phase proteins, however, do not provide information that would distinguish low vitamin A concentrations due to an acute phase response and infection in a healthy person without vitamin A deficiency from a person with vitamin A deficiency. Low plasma vitamin A concentrations, elevated acute phase proteins, and infections were closely associated in women with night blindness in Nepal. Similarly, among preschool children with Bitot’s spots
and night blindness, low plasma vitamin A concentrations, elevated acute phase proteins, and infectious disease morbidity were closely associated. In a recent controlled trial, HIV-positive pregnant women with low plasma vitamin A concentrations and elevated acute phase proteins had significantly higher plasma and breast milk vitamin A concentrations following vitamin A supplementation. This clinical trial provides additional evidence that vitamin A deficiency and elevated acute phase proteins are closely associated. It was proposed that the ratio of retinol-binding protein to transthyretin (RBP:TTR ratio) could be used in the assessment of vitamin A deficiency in the presence of infections, but a recent study shows that the RBP:TTR ratio has both poor sensitivity and specificity in the diagnosis of vitamin A deficiency.

**PREVENTION OF VITAMIN A DEFICIENCY**

Vitamin A deficiency can be prevented either directly or indirectly. Direct prevention includes measures to ensure the adequate consumption of preformed vitamin A or provitamin A carotenoids. Improving the intake of preformed vitamin A, as found in milk, eggs, dairy products, and liver, may be difficult in impoverished communities and in places where these foods are not customarily eaten. A more practical approach has been to encourage the consumption of dark green leafy vegetables, carrots, and orange or yellow fruits such as papaya and mango. The bioavailability of provitamin A carotenoids may be somewhat less than previously believed, and this remains an active area of investigation. Indirect approaches to reduce vitamin A deficiency include better immunization coverage, especially for measles, and improved management of infectious diseases such as diarrhea. That is because infectious diseases can negatively affect the intake, absorption, utilization, and metabolic losses of vitamin A. The role of periodic high-dose vitamin A supplementation as targeted therapy for HIV infection has not been established, although improving the vitamin A status of women and children in developing countries is encouraged. β-Carotene supplementation has had equivocal results in improving morbidity during HIV infection.

**CONCLUSIONS**

Vitamin A has been known for many decades to have anti-infective properties, and is known to mitigate the morbidity and mortality from measles and diarrhea in preschool children. It is still unclear whether such benefits will extend to individuals with HIV infection in high-risk populations for vitamin A deficiency in developing countries. There appears to be little use for vitamin A supplementation for HIV-infected individuals in industrialized
countries. Further studies are currently in progress to determine whether periodic or daily vitamin A supplementation will lessen morbidity and mortality in HIV-infected children and pregnant women. The current strategy to improve micronutrient malnutrition in developing countries is shifting from a paradigm of single micronutrients—vitamin A, zinc, iodine, or iron—to multiple micronutrients. It is likely that this shift in thinking will influence the approaches taken for micronutrient interventions in HIV infection.

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REFERENCES


B VITAMINS AND HIV INFECTION

Miriam Garland and Wafaie W. Fawzi

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INTRODUCTION

The B vitamins are critical to cellular metabolism and may play an important role in immune function. Therefore, it is plausible that they may be beneficial in terms of slowing the progression of HIV disease or preventing the transmission of HIV infection from mother to child. In addition, there are data to suggest that one specific B vitamin, vitamin B<sub>12</sub>, may be helpful in improving the neurologic symptoms associated with HIV disease and in preventing AZT-related bone marrow toxicity.

HISTORY, BIOLOGICAL FUNCTIONS, AND DIETARY SOURCES

The B vitamins are present in all cells and tissues and (except for vitamin B<sub>12</sub>) are not stored. Therefore, they generally have low toxicity, although long-term exposure to high doses of certain B vitamins (such as vitamin B<sub>6</sub>) may have harmful effects.

The B vitamins are important in the metabolic processes of all cells, as they are involved in reactions in glycolysis and the Krebs cycle as well as in other metabolic pathways. The history, biological functions, and dietary sources of the B vitamins are shown in Table 5.1. The following brief description is based on previous reviews; the reader is referred to them for a more detailed discussion.

**Thiamin** acts as a coenzyme in decarboxylation reactions in glucose metabolism. Severe deficiency results in the disease beriberi characterized by disorders of the nervous and cardiovascular systems. Deficiency is common in some parts of Southeast Asia.

**Riboflavin** functions as part of two coenzymes in oxidation/reduction reactions. Deficiency, which is uncommon in Western countries, results in vascularization of the cornea, oral-buccal cavity lesions, a magenta tongue (glossitis), and seborrheic dermatitis.

**Niacin** is a component of nicotinamide adenine dinucleotide (NAD) and nicotinamide adenine dinucleotide phosphate (NADP), which are present in all cells and are involved in many metabolic pathways such as glycolysis, Krebs cycle-oxidative phosphorylation, and fatty acid synthesis and oxidation. Deficiency, a problem in parts of Africa and Asia, leads to the disease pellagra, which is characterized by dermatitis, diarrhea, and even dementia.

**Pantothenic acid** is a component of two coenzymes (coenzyme A and phosphopantetheine) involved in energy metabolism and lipid synthesis. Naturally occurring deficiency has not been reliably documented.

**Vitamin B<sub>6</sub>** is a coenzyme in numerous enzyme reactions, particularly amino acid metabolism and transport. Symptoms of vitamin B<sub>6</sub> deficiency
### Table 5.1 History, Functions, and Dietary Sources of the B Vitamins

<table>
<thead>
<tr>
<th>Vitamin</th>
<th>Discovery</th>
<th>Biological Functions</th>
<th>Dietary Sources</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thiamin (B₁)</td>
<td>1926</td>
<td>Coenzyme in decarboxylation reactions in glucose metabolism</td>
<td>Meat, legumes, whole grains, seeds, nuts</td>
</tr>
<tr>
<td>Riboflavin (B₂)</td>
<td>1933</td>
<td>Coenzyme in oxidation/reduction reactions</td>
<td>Meat, poultry, fish, dairy products, some green vegetables</td>
</tr>
<tr>
<td>Niacin (B₃)</td>
<td>1937</td>
<td>Component of NAD and NADP, which are present in all cells and are involved in many metabolic pathways such as glycolysis, Krebs cycle-oxidative phosphorylation, and fatty acid synthesis</td>
<td>High-protein foods (due to conversion of tryptophan to niacin), whole grains, seeds, nuts</td>
</tr>
<tr>
<td>Pantothenic acid (B₅)</td>
<td>1939</td>
<td>Constituent of two coenzymes, coenzyme A and phosphopantetheine involved in energy metabolism and lipid synthesis</td>
<td>Liver, kidney, egg yolk, legumes, whole grains</td>
</tr>
<tr>
<td>Vitamin B₆</td>
<td>1938</td>
<td>Coenzyme in numerous enzyme reactions, particularly amino acid metabolism and transport</td>
<td>Yeast, liver, kidney, chicken, fish, beans, wheat germ, unmilled rice, oats, whole-wheat products, peanuts, walnuts nuts, avocados, bananas</td>
</tr>
<tr>
<td>Folate</td>
<td>1943</td>
<td>Coenzymes involved in transport of single carbon fragments in amino acid metabolism and nucleic acid synthesis</td>
<td>Yeast, liver, legumes, leafy vegetables, some fruits</td>
</tr>
<tr>
<td>Vitamin B₁₂</td>
<td>1948</td>
<td>Coenzyme involved in transmethylation from methylfolate to homocysteine, thus releasing unmethylated folate for reactions critical to nucleic acid synthesis (see folate, above); also a coenzyme involved in amino acid and odd-chain fatty acid catabolism</td>
<td>Meats (especially liver), poultry, fish, seafood, dairy products, eggs</td>
</tr>
<tr>
<td>Biotin</td>
<td>1948</td>
<td>Coenzyme involved in transport of carboxyl units and “carbon dioxide fixing” reactions of energy metabolism, including gluconeogenesis</td>
<td>Yeast, liver, egg yolk, soybeans, rice, bran, tomatoes</td>
</tr>
</tbody>
</table>

include skin lesions, hypochromic anemia, irritability, nervousness, insomnia, and convulsions (in infants). Isolated vitamin B₆ deficiency is rare.

**Folate** acts as a coenzyme involved in the transport of single carbon fragments in amino acid metabolism and nucleic acid synthesis, deficiency therefore leads to impaired cell proliferation. Folate deficiency is common in developing countries.⁵

The function of **B₁₂** is closely linked to that of folate. It functions as a coenzyme that catalyzes the methylation of homocysteine to form methionine, thereby regenerating unmethylated folate, which is necessary for reactions critical for nucleic acid synthesis. Vitamin B₁₂ also functions as a coenzyme involved in amino acid and odd-chain fatty acid catabolism. Deficiency is most frequently associated with inadequate absorption; dietary deficiency is rare. Vitamin B₁₂ deficiency can lead to neurological symptoms, neuropsychiatric disorders, and macrocytic, megaloblastic anemia.

Finally, **biotin** functions as a coenzyme involved in the transport of carboxyl units and “carbon dioxide fixing” reactions of energy metabolism, including gluconeogenesis. Deficiency results in anorexia, nausea, vomiting, glossitis, pallor, depression, alopecia, and dermatitis.

In epidemiologic studies, dietary intake of B vitamins can be assessed using a food frequency questionnaire. An alternative approach is to utilize biochemical markers. For example, blood levels of vitamin B₆ have been shown to reflect dietary intake of this vitamin.⁶ Likewise, blood levels of vitamin B₁₂ have been shown to be associated with dietary vitamin B₁₂ intake.⁷ However, low blood levels of vitamin B₁₂ can also be due to malabsorption, or to other factors such as folate deficiency.⁵

Since the B vitamins play a central role in cellular metabolism, it is expected that they are important to cell populations undergoing rapid proliferation, such as cells involved in the immune response.

**IMMUNE FUNCTION**

In animal studies, thiamine deficiency has not been definitively linked to impaired immune function, although in some such studies, thiamine deficiency has been associated with increased susceptibility to *Salmonella typhimurium* infection, impaired hemagglutinating antibody response to human red blood cells, and inhibition of splenic plaque formation after immunization with sheep red blood cells.⁶ Similarly, early data from animal studies suggest that deficiencies of riboflavin and pantothenic acid may lead to depressed immune function. Limited *in vitro* data support a role for niacin in immune function.⁶

There are considerable data to support a role for vitamin B₆ in immune function, most of which are derived from animal studies. In animals, vitamin B₆ deficiency has been shown to be associated with atrophy of
lymphoid tissue, depletion of lymphocytes, decreased antibody production in response to a large number of antigens, prolonged allograft survival, depressed hypersensitivity reactions, and decreased cytotoxic T-cell responses. In a limited number of studies conducted among humans, vitamin B₆ deficiency has been associated with impaired proliferative responses, decreased antibody responses to vaccines, and decreased cell-mediated responses. In a study conducted among an elderly population, vitamin B₆ supplementation increased lymphocyte proliferative responses to T- and B-cell mitogens, as well as percentages of CD3 and CD4 (but not CD8) cells. In another study conducted among elderly persons, vitamin B₆ depletion resulted in a reduction in the percentage and total number of lymphocytes, proliferative responses of peripheral blood lymphocytes to T- and B-cell mitogens, and IL-2 production. Most parameters returned to normal upon normalization of intake.

In some (although not all) human studies, folate deficiency has been associated with impaired neutrophil function, while folate supplementation has been associated with improvement of neutrophil activity.

In in vitro studies, vitamin B₁₂ enhanced T-cell proliferation and pokeweed mitogen-induced antibody production by B-cells. Low serum levels of vitamin B₁₂ were associated with impaired neutrophil function in some (although not all) studies conducted in humans. Several investigators have examined the effects of vitamin B₁₂ deficiency anemia on lymphocyte sub-populations. For example, in a recent cross-sectional study, 14 subjects with vitamin B₁₂ deficiency anemia had significantly lower numbers of lymphocytes and CD8 cells, and significantly lower natural killer cell activity, than healthy controls. Following B₁₂ injection, an increase in the number of lymphocytes and CD8 cells was observed in both groups, and NK cell activity was increased among the subjects with vitamin B₁₂ deficiency anemia. Serum levels of immunoglobulins were not associated with vitamin B₁₂ deficiency or supplementation. In a small study of elderly subjects, those with low serum vitamin B₁₂ levels had impaired antibody responses to a pneumococcal vaccine as compared to those with normal levels.

Finally, results of studies in both animals and humans indicate that biotin deficiency leads to increased susceptibility to infections (particularly fungal infections), decreased antibody responses, and a decrease in circulating lymphocytes.

HIV INFECTION

Serum Levels of B Vitamins in HIV Infection

Among HIV-infected individuals, investigators have observed a high prevalence of deficiencies in several of the B vitamins, including riboflavin, vita-
min B₆, folate, and vitamin B₁₂.⁹ For example, among HIV-infected persons, the prevalence of low vitamin B₁₂ levels has generally been observed to be between 10 and 20%. Low vitamin B₁₂ levels are found most frequently in persons with AIDS, but are also found in asymptomatic individuals.⁴

Available data suggest that among HIV-infected individuals, intakes in multiples of the RDA may be required to raise plasma micronutrient values to normal levels. For example, in a study with 108 HIV-infected men, the HIV-infected individuals consuming adequate, recommended intakes of certain micronutrients had a relatively high prevalence of deficiencies of these micronutrients as compared to uninfected men with comparable intakes.¹⁷ However, in this study, there were moderate correlations between intakes and plasma levels of several micronutrients (vitamin E, vitamin B₆, vitamin B₁₂, and zinc) among both HIV-infected and uninfected men, and intakes at multiples of the RDA (Recommended Dietary Allowances) generally resulted in normal plasma micronutrient levels in the HIV-infected men. For example, whereas 56% of HIV-infected individuals with vitamin B₆ intake at the RDA level had inadequate vitamin B₆ levels, none of those consuming 11 times the RDA or greater had such inadequate levels.

The B Vitamins in Relation to HIV Disease Progression

Cross-Sectional Studies

The relationship between serum levels of B vitamins and immune function among HIV-infected individuals has been assessed in several cross-sectional studies. In one study conducted among asymptomatic HIV-infected men, those with vitamin B₆ deficiency had decreased lymphocyte responsiveness to mitogens and reduced natural killer cell cytotoxicity as compared to those who were not deficient in vitamin B₆.¹⁸ In another cross-sectional study conducted among 60 HIV-infected individuals, those with low serum vitamin B₁₂ levels had significantly lower total lymphocyte counts, CD₄ cell counts, and CD₄/CD₈ ratios compared to those with higher levels.¹⁹ The direction of causality is difficult to ascertain from cross-sectional studies. Although results of these studies are consistent with the hypothesis that deficiencies in B vitamins accelerate HIV disease progression, these results also may be due to lower intake or absorption of these nutrients in conjunction with advanced stages of HIV disease.

Prospective Studies

The relationships between blood levels of B vitamins and HIV disease progression have been examined in prospective studies. In a prospective study
conducted among 108 homosexual men over an 18-month period, development of vitamin B12 deficiency (as assessed by plasma levels) was associated with a significant decline in CD4 cell counts, while normalization of vitamin B12 levels was associated with significantly higher CD4 cell counts. In the Multicenter AIDS Cohort Study (MACS), a prospective study conducted among HIV-infected homo-/bisexual men, in which 163 cases of AIDS were diagnosed during the study period, low serum vitamin B12 levels (but not vitamin B6 or folate levels) were significantly associated with an increased risk of developing AIDS. The relative hazard associated with low vitamin B12 levels, as compared to adequate levels, was 1.89 (95% confidence interval [CI], 1.15–3.10).

Several investigators have studied the relationship between dietary intake of B vitamins and HIV disease progression. In a cohort study conducted among 296 homo-/bisexual men in San Francisco in which 107 cases of AIDS were diagnosed during the study period, increased dietary intakes of thiamin, riboflavin, niacin, and folic acid were each associated with a decreased risk of progression to AIDS; this relationship was statistically significant for riboflavin. In this study, the median intake from food and supplements of each of these B vitamins was greater than 100% of the recommended dietary allowance. In the MACS study mentioned above, a prospective study conducted among 281 HIV-infected homo-/bisexual men, in which 108 cases of AIDS were diagnosed during the study period, intakes of several micronutrients were assessed by a food frequency questionnaire. Consumption of micronutrients was quite high in this cohort, with median levels generally far exceeding recommended dietary intakes. High levels of consumption of several of the B vitamins were found to be associated with a decreased risk of progression to AIDS. For example, for niacin, the relative hazard for the highest quartile of dietary intake, compared to the lowest three quartiles, was 0.52 (95% CI, 0.31–0.86). However, the authors noted that for the B vitamins, intercorrelations were high (r > 0.90). Thus, it is difficult to attribute a protective effect to a specific B vitamin or set of B vitamins. Similar results were obtained when intakes of B vitamins were examined in relation to survival in this same cohort, and much of the protective effect appeared to be attributable to B vitamins derived from supplements and not from food. In a cohort study conducted among 2179 HIV-infected individuals from South Africa, those who had taken B vitamins had a significantly increased median survival compared to those who had not taken any vitamin supplements. Analyses of dose or duration were not presented, however.

Results from prospective studies support a protective role of B vitamins in HIV disease progression. However, several inconsistencies and questions remain. First of all, because intakes of micronutrients are highly intercorrelated, it is difficult to attribute a protective role to a specific micronutrient. As observed in the MACS study, dietary intakes of B vitamins in particular
are highly intercorrelated, so it may be prohibitively difficult to disentangle the individual effects of specific B vitamins from observational data. Another important limitation of studies conducted to date is that they were generally conducted among populations where intakes of B vitamins and other micronutrients were high. Thus, data are lacking from populations with lower micronutrient intakes. Another issue is one of confounding, particularly by duration of infection, which was unknown in these cohort studies. Although various surrogates for infection duration (such as baseline signs and symptoms and CD4 counts) were generally accounted for, residual confounding is still a possible explanation for certain of the above findings.

**Randomized Trials**

In a randomized, placebo-controlled trial conducted among pregnant, HIV-infected women in Tanzania, 1075 women were assigned in a two-by-two factorial design to a daily oral dose of placebo, vitamin A (30 mg of β-carotene and 5000 IU of preformed vitamin A), multivitamins excluding vitamin A (20 mg B1, 20 mg B2, 25 mg B6, 100 mg niacin, 5 μg B12, 500 mg C, 30 mg E, and 0.8 mg folic acid), or multivitamins including vitamin A. Multivitamin supplementation, but not vitamin A supplementation, was found to be associated with a significant increase in CD4, CD8, and CD3 cell counts. The results from this large, well-designed trial suggest that multivitamin supplementation (including relatively high doses of B vitamins) may have some beneficial effects among HIV-infected women. Future analyses from this study hopefully will shed light on the effect of the multivitamin supplements on the clinical progression of HIV disease. Because the B vitamins were administered in conjunction with other nutrients (vitamin C and vitamin E), it will not be possible to determine the specific effect of the B vitamins from this study.

**The B Vitamins in Relation to Vertical Transmission of HIV and Other Adverse Pregnancy Outcomes**

In the randomized trial discussed above, multivitamin supplementation, but not vitamin A supplementation, was found to be associated with a significantly decreased risk of fetal death and other adverse pregnancy outcomes. A subsequent analysis from this same study suggested that neither multivitamin supplementation nor vitamin A supplementation had an effect on *in utero*, intrapartum, or early postpartum vertical HIV transmission. These results therefore suggest that, among HIV-infected women, multivitamin supplementation reduces the risk of low birth weight and other adverse pregnancy outcomes, but does not affect the risk of *in utero*, intrapartum, or early postpartum vertical HIV transmission. Future analyses
from this study will help to elucidate the effect of the multivitamins on transmission through breast-feeding. As discussed above, it will not be possible in this study to examine the effects of the B vitamins separately from the effects of the other micronutrients contained in the multivitamins.

**Vitamin B₁₂ in Relation to Outcomes in HIV Disease: Neurologic Symptoms and AZT-Related Bone Marrow Toxicity**

Several studies have been conducted on the relationship between vitamin B₁₂ and neurologic symptoms associated with HIV disease; these data have recently been reviewed.⁵ Inconsistent results have been reported from several cross-sectional studies that examined the relationship between serum vitamin B₁₂ levels and neurologic abnormalities.⁵ In one prospective study, normalization of plasma vitamin B₁₂ levels over an 18-month study period was associated with a significant improvement in cognitive function. ²⁹ Although B₁₂ injection therapy has appeared promising in terms of improving neurologic symptoms in several small uncontrolled studies, no definitive conclusions can be reached in the absence of well-designed trials.⁵

The data on vitamin B₁₂ and AZT-related bone marrow toxicity also have recently been reviewed.⁵ In one placebo-controlled trial of AZT in HIV disease,³⁰ subjects with low baseline serum vitamin B₁₂ levels were most susceptible to adverse hematologic effects of AZT. Although no benefit of vitamin B₁₂ injection therapy was seen in several trials conducted among HIV-infected persons being treated with AZT, these trials have included individuals with normal vitamin B₁₂ levels;⁵ thus, any beneficial effect among vitamin B₁₂-deficient individuals may have been obscured.

**CONCLUSIONS**

The B vitamins are likely to be important for immune function, supporting the plausibility of a role for these vitamins in HIV disease. Available data from prospective studies are consistent with a protective role of B vitamins in HIV disease progression, although confounding by infection duration or other factors is still a possible explanation for some of these findings. Because confounding can be circumvented in randomized trials, it is critical to conduct such trials to establish more definitively the effect of B vitamins on the progression of HIV disease. In a recent, well-designed trial of multivitamin supplements among HIV-infected women, the group receiving the supplements (which included high doses of several of the B vitamins) had a significant increase in CD4, CD8, and CD3 cell counts. Future analyses from this study will provide information on the effect of the multivitamin supplements on the clinical progression of HIV disease. At present, it is not possible to attribute a protective effect to a specific B vitamin or set
of B vitamins. In light of the current state of knowledge and the urgent need for affordable interventions in the developing world, where expensive antiretroviral drugs are virtually unavailable, the first priority should be to examine the effect of the B vitamins in combination. Administration of B vitamins may be particularly beneficial in the developing world because of the high prevalence of vitamin B deficiencies in that setting.

Although the data are more limited, the B vitamins have been examined in relation to several other outcomes among HIV-infected individuals. Results from one trial suggest that multivitamin supplementation (including high levels of B vitamins) may be beneficial in preventing infant low birth weight and other adverse pregnancy outcomes. Although the multivitamins did not appear to prevent in utero, intrapartum, or early postpartum vertical HIV transmission, further follow-up of this cohort will yield important information on the effect of the multivitamins on later postpartum transmission through breast-feeding. Available data, although limited, suggest that vitamin B₁₂ may play a role in improving HIV-related neurologic symptoms. Finally, it is possible that vitamin B₁₂ therapy may help to prevent AZT-related bone marrow toxicity. As with studies of the role of B vitamins in HIV disease progression, the role of B vitamins in preventing these other outcomes should be examined in well-designed randomized trials.

One important issue in trials of B vitamins among HIV-infected persons is that of appropriate dosages. As discussed above, dosages in multiples of the RDA may be required in order to raise plasma micronutrient values to normal levels. Thus, trials of B vitamins among HIV-infected persons should utilize relatively high dosages, although obviously within safe limits.

In summary, B vitamins appear to have promise in helping to prevent certain adverse outcomes in HIV-infected persons. Well-designed randomized trials of the administration of B vitamins to HIV-infected individuals will provide critically important information. These trials are urgently needed since affordable interventions in the developing world are currently lacking.

ACKNOWLEDGMENTS

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REFERENCES


VITAMINS C AND E, AND HIV INFECTION

Alice M. Tang and Ellen Smit

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INTRODUCTION

Early in the HIV epidemic, several studies reported significantly lower serum levels of vitamins C and E in HIV-positive subjects compared to HIV-negative subjects. Deficiencies of these vitamins were purported to play an important role in hastening the progression of HIV infection to AIDS. These hypotheses were supported by the established role of both vitamins as antioxidants. As such, they have been shown to boost host immunity by diminishing the adverse effects of oxidative stress on immune function.

Vitamins C and E function both independently and synergistically as antioxidants. Vitamin C, the main water-soluble antioxidant in humans, scavenges aqueous free radicals. Vitamin E, the main lipid-soluble antioxidant in humans, quenches free radicals in cell membranes. In addition, vitamin C is important for regenerating vitamin E through the reduction of vitamin E radicals formed during antioxidant reactions.1 The radical-scavenging ability of vitamin E becomes less efficient in the absence of vitamin C.

This chapter briefly reviews the basic properties of both vitamins, their functions in the context of infectious disease, and the current state of knowledge of their roles in HIV disease progression.

VITAMIN C

History

In 1747, James Lind, a Scottish surgeon, performed the first nutrition clinical trial. He observed that sailors frequently developed a disease now known as scurvy, characterized by bleeding gums, swollen joints, muscle weakness, and blotchy skin. He subsequently selected 12 sailors with scurvy and divided them into six pairs. Each pair received a diet supplement of one of the following: vinegar, cider, sulfuric acid, seawater, oranges and lemons, or a mix of spices. Those receiving the lemons and oranges quickly recovered. It was not until the late 1920s and early 1930s, however, that Albert Szent-Gyorgy isolated a substance from cabbage, oranges, and adrenal glands that could prevent scurvy. This substance was later identified as vitamin C, or ascorbic acid.2,3

Metabolism and Function

Vitamin C is the generic term for all compounds with the biologic activity of ascorbic acid. It is a water-soluble, six-carbon compound with the chemical name 2,3-didehydro-L-threohexano-1,4-lactone. Normally, vitamin C is in the form of ascorbic acid, which can be easily and reversibly oxidized
to dehydroascorbic acid.² Five percent of the plasma pool of vitamin C is usually in the form of dehydroascorbate. Both ascorbate and dehydroascorbate are absorbed in the human intestine through energy-dependent active transport. Approximately 80 to 95% of dietary ascorbic acid is absorbed at intakes up to 100 mg/d, after which absorption rapidly decreases to about 50% of a 1.5-g dose and 25% of a 6-g dose.⁴

The highest levels of vitamin C in body tissues are found in the pituitary glands, the adrenals, and leukocytes. The lowest levels are found in plasma and saliva.² Approximately 70% of vitamin C in the blood is found in plasma and erythrocytes; the remainder is found in high concentrations in various white cells.⁴

Vitamin C is an antioxidant and, as such, prevents oxidative damage to tissues. It also acts as the first line of defense against free radicals in extracellular fluids.⁵ Additionally, it plays a role in collagen formation, synthesis of carnitine, and iron absorption; promotes resistance to infection; converts dopamine to norepinephrine; and has multiple other functions as a coenzyme or cofactor.²

**Assessment of Vitamin C Status**

The most commonly used index of vitamin C status in humans is the concentration in the serum.⁶ Serum levels are influenced by recent intakes of vitamin C, so fasting blood samples are recommended. Serum ascorbic acid concentrations rarely increase beyond a threshold of 1.4 mg/dL, as renal clearance increases rapidly with daily intakes over 100 mg. Serum levels are, therefore, not appropriate for identifying persons who regularly consume excessive amounts of vitamin C. However, in those who consume chronically low amounts of vitamin C, serum levels are an accurate reflection of body ascorbic acid content. Several nonnutritional factors may lower serum ascorbic acid levels, including acute stress, oral contraceptives, acute and chronic infections, and cigarette smoking.⁶ Cutoff values for low serum vitamin C levels vary widely. In the United States, low vitamin C levels are generally defined as below 0.29 mg/dL, with levels below 0.20 mg/dL considered deficient.

There are several other indices of vitamin C status that can be measured; however, each has its limitations. Leukocyte ascorbic acid concentrations are a more reliable measure of tissue stores and are less responsive to recent vitamin C intakes, but the assay is difficult and requires relatively large samples of blood. Urinary excretion of ascorbic acid is influenced by recent dietary intake, requires 24-hour urine samples, and has relatively low sensitivity and specificity. The most reliable method of assessing the total body pool size of vitamin C requires radioactive isotope dilution methods.
Recommended Intake and Food Sources

The Dietary Reference Intake (DRI) for vitamin C is 75 mg/d for females and 90 mg/d for males. Smoking has an oxidant effect, and smokers are, therefore, recommended to increase their intake by 35 mg/d. The tolerable upper intake level for adults is 2000 mg/d. Food sources high in vitamin C include fruits and vegetables, specifically citrus fruits, broccoli, dark green vegetables, cantaloupe, strawberries, peppers, tomatoes, and potatoes.

Deficiency and Host Defense

Vitamin C deficiency produces a number of small changes in immune function, including impairment of the inflammatory response and cytotoxic T-cell activity. However, the literature shows that vitamin C deficiency exerts its main effect on the phagocytic function of neutrophils and macrophages. Vitamin C is normally present in large concentrations in phagocytic cells. It plays a major role in the synthesis and assembly of tubulin, an intracellular protein that facilitates cells to change shape and move to sites where they are needed. Without tubulin, phagocytic cells are unable to move to sites of localized infections. Vitamin C deficiency, therefore, results in a decrease in the mobility of phagocytic cells, although the microbicidal activity of these cells generally remains effective.

VITAMIN C AND THE COMMON COLD

Vitamin C supplementation and its role in the prevention of common colds has been studied extensively. In well-nourished individuals, vitamin C supplementation does not appear to prevent colds. Hemilä et al. pooled the results of six randomized, placebo-controlled clinical trials that were selected based on their study design (randomized controlled trial), levels of vitamin supplementation (≥ 1 g/d), and sample sizes (each had ≥ 200 common cold episodes over the 2- to 9-month study periods). They found that the pooled relative risk for colds comparing those on vitamin C to those on a placebo was 0.99 (95% confidence interval (CI): 0.93–1.04). In other words, these six major studies showed no reduction in common cold incidence with vitamin C supplementation in the general population. However, it was then hypothesized that vitamin C supplementation would have a greater effect on common cold incidence in specific groups of people. This was later demonstrated in a pooled analysis of three randomized, placebo-controlled clinical trials involving individuals undergoing acute physical stress. Combining the results of these three studies, all of subjects engaging in heavy exercise, Hemilä found a significant 50% reduction in
the incidence of common colds. Subjects in these trials were taking 0.6 to 1 g/d of vitamin C or a placebo for 1 to 2 weeks.

Studies of the effects of vitamin C supplementation on the duration and severity of colds have shown mixed results. In general, there appears to be a slight to moderate effect of adding vitamin C at the onset of symptoms to reduce their duration and severity. Hemilä pooled 23 studies with regular vitamin C supplementation and found that the decrease in duration of a cold was greater in children than in adults. He also found that there was a greater benefit with a higher vitamin C dose (2 g or more per day compared to 1 g a day). In another review of 30 randomized and nonrandomized trials of vitamin C supplementation, it was found that vitamin C in doses as high as 1 g/d for several winter months had modest therapeutic effects on the duration of cold symptoms, again with larger doses producing greater benefits than lower doses.

**VITAMIN C DEFICIENCY AND HIV INFECTION**

A few studies have examined serum vitamin C levels in HIV-infected populations (Table 6.1). There does not seem to be any consensus among studies, however, as to the definition of subnormal vitamin C levels, with cut-offs ranging from 1.5 to 22.7 µmol/L. In the early 1990s, three studies found no significant differences in vitamin C levels among HIV-infected subjects at various stages of infection. At around the same time, Beach et al. published a study which showed that although dietary intake of vitamin C was higher among HIV-positive participants, plasma levels were not significantly different among 100 HIV-positive and 42 HIV-negative subjects in Miami, Florida.

In the latter half of the 1990s, three other studies demonstrated significantly lower vitamin C levels in HIV-infected subjects compared to HIV-negative controls. Lacey et al. assessed plasma vitamin C levels in 35 HIV-positive subjects and 38 HIV-negative controls in the United Kingdom. They found lower vitamin C levels in HIV-positive subjects than in the controls. However, vitamin C levels were not associated with CD4 cell counts among the HIV-positive subjects. Allard et al. also found lower plasma vitamin C levels in 49 HIV-positive subjects than 15 healthy controls in Canada. Finally, Treitinger et al. studied the antioxidant defense system in 75 subjects with HIV infection and 26 HIV-negative controls in Brazil, and found lower levels of plasma ascorbate in HIV-positive patients than in HIV-negative controls. After classifying the HIV-positive patients according to the Walter Reed Army Institute system for disease stage, they found a nonsignificant trend for lower plasma ascorbate levels with increasing progression of HIV infection.
### Table 6.1 Mean Serum Vitamin C Levels and Prevalence of Low Serum Vitamin C Levels in HIV-Positive and HIV-Negative Study Subjects

<table>
<thead>
<tr>
<th>Author</th>
<th>Year</th>
<th>Place of Study</th>
<th>Study Subjects</th>
<th>Mean ± SD (µmol/L)</th>
<th>% Subnormal</th>
<th>Definition of Subnormal (µmol/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bogden16</td>
<td>1990</td>
<td>United States</td>
<td>6 HIV+, asymp.</td>
<td>29.5 ± 9.1</td>
<td>27</td>
<td>≤22.7</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>17 HIV+, ARC</td>
<td>50.0 ± 10.2</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>7 HIV+, AIDS</td>
<td>51.7 ± 9.7</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baum17</td>
<td>1991</td>
<td>United States</td>
<td>15 HIV+, HS, asymp. (pre-ZDV treatment)</td>
<td>48.3 ± 22.7</td>
<td>7</td>
<td>≤17.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>22 HIV+, HS, asymp. (no ZDV treatment)</td>
<td>48.3 ± 17.0</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>Beach19</td>
<td>1992</td>
<td>United States</td>
<td>100 HIV+, HS, asymp.</td>
<td>57.9 ± 36.3</td>
<td>7</td>
<td>≤22.7</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>42 HIV–, HS, controls</td>
<td>62.5 ± 19.9</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Coodley18</td>
<td>1993</td>
<td>United States</td>
<td>15 HIV+, wasted</td>
<td>49.4 ± 35.8</td>
<td>23</td>
<td>≤11.4</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>11 HIV+, CD4&lt;200</td>
<td>63.0 ± 35.4</td>
<td>12</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>21 HIV+, CD4&gt;200</td>
<td>61.9 ± 28.4</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td>Lacey20</td>
<td>1996</td>
<td>United Kingdom</td>
<td>35 HIV+</td>
<td>38.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>38 HIV–, controls</td>
<td>58.0</td>
<td></td>
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<tr>
<td>Allard21</td>
<td>1998</td>
<td>Canada</td>
<td>49 HIV+</td>
<td>40.7 ± 3.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>15 HIV–, controls</td>
<td>75.7 ± 4.3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Van Staden55</td>
<td>1998</td>
<td>South Africa</td>
<td>22 HIV+, CD4&lt;200</td>
<td>0</td>
<td></td>
<td>≤1.5</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>37 HIV+, CD4: 200–499</td>
<td>0</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>17 HIV+, CD4≥500</td>
<td>0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Treitinger22</td>
<td>2000</td>
<td>Brazil</td>
<td>75 HIV+</td>
<td>19.6 ± 14.3</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>26 HIV–, controls</td>
<td>33.4 ± 20.0</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Median levels reported.*

ARC, AIDS-related complex; HS, homosexuals; RH, relative hazard
Only a few studies have examined dietary intake of vitamin C among HIV-infected individuals. Dannhauser et al. examined dietary intake among 81 HIV-positive subjects in South Africa and found that 75% of the subjects consumed less than 67% of the RDA. However, there was no HIV-negative comparison group in this study.

In a U.S. study of inner-city injection drug users, we found that intake of dietary vitamin C was lower in 45 HIV-positive subjects than in 59 HIV-negative controls, although the difference was not statistically significant. Mean vitamin C intake in both groups was above the RDA. In another study we compared total vitamin C intake (diet and supplement) among HIV-positive homosexual men in the U.S. and found that intake above the median was somewhat associated with a lower risk of progression to AIDS than was intake below the median (relative hazard 0.59, 95% CI: 0.34–1.03).

Studies on plasma vitamin C levels and HIV infection seem to show that, although vitamin C levels are generally lower among HIV-positive individuals than HIV-negative individuals, levels among HIV-positive individuals do not differ with stage of infection. Results on dietary vitamin C intake in HIV infection have been varied, possibly due to differences in study participants, geographic location, study design, and methods.

**Vitamin C Supplementation**

Early in the AIDS epidemic, Cathcart reported preliminary data describing clinical improvements consisting of suppression of symptoms in AIDS patients after supplementing with vitamin C at levels of 50 to 200 g per day. This study was based on an anecdotal group of 90 AIDS patients who reported taking high doses of vitamin C on their own, and 12 patients who were given either high doses of vitamin C or intravenous ascorbate. Although suggestive, there was no control group or scientific data presented in the paper.

Several studies have reported the *in vitro* effects of vitamin C supplementation on HIV replication using infected cell lines. Harakeh et al. examined the effects of noncytotoxic concentrations of ascorbic acid on HIV replication in chronically and acutely infected T-lymphocytes. The investigators found that addition of ascorbic acid to cell cultures suppressed HIV replication through inhibition of extracellular reverse transcriptase production. Suppression of HIV replication continued as long as ascorbate supplementation was maintained. However, viral replication resumed as soon as ascorbate was removed from the system.

The activation of a transcription-enhancing factor, nuclear factor κB (NF-κB), by reactive oxygen species is believed to play a key role in HIV activation. Certain stimuli such as tumor promoters (e.g., PMA) and cytokines (e.g., tumor necrosis factor-α (TNF-α)) are associated with
increased oxidative stress levels and HIV viral production. In further experiments, Harakeh et al. showed that ascorbic acid inhibited virus reactivation in HIV-infected T-lymphocytic cells following stimulation with PMA and TNF-α. In contrast, however, Munoz et al. found that ascorbate increased the activation of NF-κB in TNF-α-stimulated T-cells.

The effect of ascorbate on HIV may be dependent on dosage. Rivas et al. showed that vitamin C at pharmacological levels decreased HIV production, yet at levels within normal range there was no effect on cell proliferation or viral production in vitro.

In 1998, Allard et al. published the results from a randomized, placebo-controlled trial in which 49 subjects were supplemented with α-tocopherol (800 IU per day) and vitamin C (1000 mg per day) or placebo for 3 months. After the supplementation phase, plasma vitamins C and E levels were higher, while oxidative stress levels (breath pentane, plasma lipid peroxides, and malondialdehyde levels) were lower in the supplemented group than in the placebo group. There was also a trend toward a reduction in viral load in the vitamin-supplemented group. This is one of the first trials in humans to show that vitamin C supplementation, along with vitamin E supplementation, reduces the level of reactive oxygen species, and perhaps viral load, in HIV-infected patients.

VITAMIN E

History

Vitamin E was discovered in 1922 as a lipid-soluble substance essential for reproduction and resorption in rats fed a rancid lard diet. Later, it was rediscovered by Schwarz as playing an important role in cellular antioxidant systems. It has since been shown to be effective in preventing lipid peroxidation and other free radical-induced oxidative stress.

Metabolism and Function

Vitamin E is the collective term for all compounds known as tocopherols and tocotrienols, of which α-tocopherol has the greatest biologic activity. Four tocopherols and four tocotrienols exist naturally, differing only in the number and position of methyl groups on the chromanol ring. In addition, there are several types of synthetic α-tocopherols available commercially in fortified foods and vitamin supplements.

Tocopherols and tocotrienols are absorbed from the small intestine along with dietary lipids. In humans, between 50 and 70% of α-tocopherol is absorbed at dietary levels (0.4 to 1 mg), while only about 10% of
pharmacologic doses (≥ 200 mg) are absorbed.\(^3^6\) Once absorbed, vitamin E is distributed in plasma in association with lipoproteins. Plasma concentrations of vitamin E, therefore, tend to vary according to the amount of lipid present. \(\alpha\)-Tocopherol is taken up by most tissues but is continuously sequestered in adipose tissue. Vitamin E compounds are found mostly in cell structures containing phospholipid membranes, such as mitochondria, microsomes, and plasma membranes. Tocopherol metabolites are mainly excreted in the feces, possibly in association with bile secretion.

Vitamin E is the major lipid-soluble, chain-breaking antioxidant found in cell membranes. Its main function is to act as a free radical scavenger to protect membrane lipids from oxidative damage.\(^3^6,^3^7\) Several nonantioxidant functions of vitamin E also have been suggested, although their mechanisms have not been elucidated as clearly. Vitamin E has been reported to modulate the activities of microsomal enzymes, to inhibit protein kinase C activity and cell proliferation, and to regulate immune response.\(^7,^3^7\)

**Assessment of Vitamin E Status**

Although most frequently used by researchers, serum tocopherol concentrations are questionable as a measure of vitamin E status because they are highly correlated with serum lipid levels. Serum tocopherol levels also vary according to age, physiological state, and method of analysis. In general, serum total tocopherol levels of less than 0.5 mg/dL (<11.6 \(\mu\)mol/L) have been associated with low vitamin E status in adults. Children under 12 years of age have lower serum tocopherol levels than adults, and with no evidence of vitamin E deficiency. The use of tocopherol/lipid ratios has been suggested as a better indicator of vitamin E nutritional status.\(^6\) This ratio, though still controversial, is recommended to prevent those with low serum lipid levels from being misclassified as vitamin E deficient, and those with elevated lipid levels from being misclassified as normal despite vitamin E deficiency. A ratio of 0.6 mg total tocopherols per gram of total serum lipids is used as the cutoff for adequate vitamin E status. Vitamin E levels in liver or adipose tissue biopsy samples provide good measures of tissue stores of vitamin E; however, these methods are too invasive for use in population-based studies.

High performance liquid chromatography (HPLC) is the method most commonly used to measure the tocopherols in the blood and tissues. Normally, about 90% of circulating vitamin E is in the form of \(\alpha\)-tocopherol, but large amounts of \(\beta\)- and \(\gamma\)-tocopherols may be found in some circumstances.\(^3^6\) HPLC techniques are able to quantify the amount of \(\alpha\)-tocopherol separately from the \(\beta\) and \(\gamma\) isomers.
Recommended Intake and Food Sources

The RDA for α-tocopherol is 15 mg (35 µmol) per day for both men and women. The other naturally occurring forms of vitamin E (β-, γ-, and δ-tocopherols and the tocotrienols) do not contribute to the vitamin E requirement because of their relatively low biologic activity. The tolerable upper intake level for adults is set at 1000 mg (2325 µmol) per day of any form of supplemental α-tocopherol. Major food sources of vitamin E include vegetable and seed oils (such as soybean, corn, cottonseed, and safflower) and the products made from them (such as margarine and shortening), nuts, seeds, wheat germ, and green leafy vegetables (such as spinach and collard greens).

Deficiency and Host Defense

There exists a large body of literature on the immunoenhancing actions of vitamin E that is only briefly summarized here. Several recent articles have been published that review the animal and human studies on vitamin E and immune function.38-41

In general, deficiencies of vitamin E are associated with a reduction in T-cells, natural killer cells, and phagocytic responses.38 Severe vitamin E deficiency results in the impairment of both cell-mediated and humoral immunity. In both human and animal studies, vitamin E supplementation in two- to tenfold excess of what is generally recommended significantly increased humoral and cell-mediated immune responses to antigens and enhanced phagocytic functions.42,43

The main mechanism by which vitamin E enhances immune response and phagocytosis is through its antioxidant properties.43 Rapidly proliferating cells of the stimulated immune system are highly prone to peroxidative damage by free radicals, peroxides, and superoxides.42 Furthermore, oxidative metabolites of activated macrophages have been shown to suppress lymphocyte proliferation.44 Vitamin E can, therefore, improve immune function by virtue of its role as a free-radical scavenger. Vitamin E also modulates the biosynthesis and activity of prostaglandins (in particular, PGE₂). Prostaglandins help to regulate lymphocyte proliferation and lymphokine synthesis, and are immunosuppressive at high levels.44 Vitamin E is an effective inhibitor of prostaglandin synthetase, and so serves indirectly as an enhancer of cell-mediated immunity.

Supplementation in the Elderly

Many elderly persons experience waning immunity as part of the aging process. Documented immune dysfunction associated with aging includes
decreased delayed hypersensitivity, reduced IL-2 production, decreased lymphocyte response to mitogens, and decreased antibody production after vaccination. Furthermore, it has been reported that over 40% of the elderly have inadequate dietary intake of vitamin E. In a randomized, placebo-controlled trial, De Waart et al. examined the effects over 3 months of a daily dose of 100 mg dl-α-tocopherol acetate on the immune response of elderly subjects aged 65 years and over. They found that vitamin E supplementation had no effect on the in vitro proliferative response of peripheral blood mononuclear cells to mitogens, nor on antibody levels against several common antigens. In another randomized controlled trial, Pallast et al. studied the effects of 50- and 100-mg vitamin E supplements on immune response in subjects aged 65 to 80 years old. They found that subjects who received 100 mg of vitamin E, and who had low baseline DTH reactivity or who were physically less active, had a larger increase in DTH reactivity than the placebo group. This study showed that 100 mg of vitamin E supplementation may be more beneficial for specific subgroups of the elderly. In another clinical trial using a much higher dose of vitamin E, daily supplementation of healthy elderly individuals with 800 mg dl-α-tocopherol acetate for 1 month significantly improved delayed-type hypersensitivity reactions and enhanced in vitro response to the mitogen, concavalin A. The results of these studies suggest that there may be some beneficial effects of high doses of vitamin E supplements on immune response in the elderly. It is not yet clear, however, whether these improvements are associated with increased resistance to infections or improvements in morbidity and mortality in this population.

VITAMIN E DEFICIENCY AND HIV INFECTION

Since vitamin E appears to be an important immunomodulator, it is not surprising that vitamin E deficiency has been associated with HIV infection and more rapid disease progression. Serum vitamin E levels in individuals infected with HIV have been measured in several published studies. These studies are listed in Table 6.2. The first studies in the early 1990s examined the prevalence of low serum/plasma vitamin E levels as part of a panel of micronutrients or antioxidants that were being assessed. The main aim of these studies was to determine the prevalence of specific nutrient abnormalities in those infected with HIV. Although a few studies used cutoff levels for vitamin E that were somewhat higher, the majority of studies used 11.6 μmol/L as the cutoff to indicate low serum vitamin E levels.

In one of the earliest studies, Bogden et al. measured plasma concentrations of 21 micronutrients to determine the prevalence of abnormalities in HIV-positive patients, and to compare levels between individuals at various stages of infection. The investigators found that 12% of 30
Table 6.2 Mean Serum Vitamin E Levels and Prevalence of Low Serum Vitamin E Levels in HIV-Positive and HIV-Negative Study Subjects

<table>
<thead>
<tr>
<th>Author</th>
<th>Year</th>
<th>Place of Study</th>
<th>Study Subjects</th>
<th>Mean ± SD (µmol/L)</th>
<th>% Subnormal</th>
<th>Definition of Subnormal (µmol/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bogden16</td>
<td>1990</td>
<td>United States</td>
<td>HIV+, asymp. 6</td>
<td>21.6 ± 2.3</td>
<td>12</td>
<td>≤13.9</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>HIV+, ARC 17</td>
<td>25.3 ± 3.5</td>
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<td>HIV–, controls</td>
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<td>1997</td>
<td>HIV+, no AIDS, baseline</td>
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<td>22.3</td>
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<td></td>
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<td>Subset of above patients, 12 mos. later</td>
<td>13.7 ± 1.2</td>
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<tr>
<td>Tang 66</td>
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<td>1997</td>
<td>HIV+, HS</td>
<td>19.4 ± 11.7</td>
<td>22</td>
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<tr>
<td>Jordao, Jr. 71</td>
<td>Brazil</td>
<td>1998</td>
<td>HIV+, AIDS</td>
<td>15.3 ± 12.2</td>
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<td>26.4 ± 17.0</td>
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<td>HIV–, controls</td>
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<td>Allard 81</td>
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<td>HIV–, controls</td>
<td>26.6 ± 2.6</td>
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### Table 1: Micronutrients and HIV Infection

<table>
<thead>
<tr>
<th>Author</th>
<th>Year</th>
<th>Place of Study</th>
<th>Study Subjects</th>
<th>Mean ± SD (µmol/L)</th>
<th>% Subnormal</th>
<th>Definition of Subnormal (µmol/L)</th>
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<tr>
<td>Van Staden</td>
<td>1998</td>
<td>South Africa</td>
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<tr>
<td>Kelly</td>
<td>1999</td>
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<td>122 HIV+, persistent diarrhea</td>
<td>11.6</td>
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<tr>
<td>Wiratchai</td>
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<td>Thailand</td>
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<td>≤11.6</td>
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<td>58 HIV+, mothers w/ HIV-infants</td>
<td>27.8 ± 11.4</td>
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<td>Monteiro</td>
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<td>20.0 ± 7.2</td>
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<td>30 HIV+, IDU, comb. w/ PI</td>
<td>25.0 ± 10.9</td>
<td>6.7</td>
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<td>210 HIV–, IDU</td>
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<td>26 HIV–, controls</td>
<td>8.0 ± 4.6</td>
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* Unpublished data.

ARC, AIDS-related complex; HS, homosexuals; IDU, intravenous drug user; ART, antiretroviral treatment; PI, protease inhibitor; comb., combination therapy.
HIV-infected patients had low plasma vitamin E levels, although no differences in vitamin E levels were found between HIV-positive subjects who were asymptomatic or symptomatic, and those with AIDS.

Beach et al.\(^48\) reported serum vitamin E levels in 100 asymptomatic HIV-infected men and 42 HIV-negative controls. They found that 26% of the HIV-negative controls were consuming less than the RDA for vitamin E compared to only 10% of the HIV-positive men. However, 19% of the HIV-positive subjects had serum vitamin E levels below 11.6 μmol/l as compared to none of the HIV-negative controls, a statistically significant difference. In another study by the same research group, Baum et al.\(^17\) found no effect of zidovudine treatment on vitamin E levels in HIV-positive subjects, but found a 14 to 16% prevalence of low serum vitamin E levels in treated and untreated HIV-positive subjects. In a later study, Baum et al.\(^49\) evaluated plasma micronutrient levels in relation to patterns of dietary intake in the same study population. Although mean vitamin E levels did not differ between HIV-positive and HIV-negative subjects, a significantly larger proportion of HIV-positive subjects who consumed more than the RDA for vitamin E had inadequate plasma levels than did HIV-negative subjects. In fact, 7% of HIV-positive subjects who consumed vitamin E at more than 25 times the RDA still had inadequate plasma vitamin E levels compared to none of HIV-negative subjects who were consuming more than 25 times the RDA.

Favier et al.\(^50\) studied the antioxidant status of HIV-positive and HIV-negative subjects over a 6-month period. There were no differences in mean serum tocopherol levels between HIV-negative subjects and HIV-positive subjects who were asymptomatic. However, mean serum tocopherol levels were significantly lower in patients with AIDS. In all three groups, mean serum vitamin E levels were well above the cutoff for deficiency (11.6 μmol/L); no information was given on the prevalence of low vitamin E levels in each of the study groups.

Periquet et al.\(^51\) evaluated the prevalence of specific nutrient abnormalities in HIV-infected children at various stages of infection. The authors reported significantly lower vitamin E levels in HIV-positive children with AIDS than in HIV-negative controls. In another study in children, Mastroiacovo et al.\(^52\) evaluated vitamin E levels in 20 hospitalized HIV-positive children, ages 1 to 6 years, who were administered a multivitamin supplement including 1.5 mg vitamin E. Twenty clinically healthy HIV-negative children of the same age, who were taking the same multivitamin preparation, were included as controls. Mean vitamin E levels were significantly lower in the HIV-positive children than in the HIV-negative children, despite the intake of multivitamin supplements in both groups; however, dietary vitamin E intake in both groups was not reported.
In a recent longitudinal study, Pacht et al.\textsuperscript{53} followed a group of HIV-infected subjects in the United States for 12 months to determine changes in vitamin E levels. The authors found that mean vitamin E levels significantly decreased after 12 months of follow-up, indicating depletion of vitamin E levels with HIV disease progression. Furthermore, 22.3\% of subjects at baseline had vitamin E levels below the cutoff for deficiency.

In two studies in African populations,\textsuperscript{54,55} low vitamin E levels were found in extraordinarily high proportions (from 50 to 78\%) of HIV-infected subjects. Although neither of these studies reported vitamin E levels in HIV-negative control groups, it can be expected, based on results from the United States and Europe, that HIV infection in already malnourished populations would increase the risk of vitamin E deficiency.

In contrast to the above results, a few studies found no significant differences in the prevalence of vitamin E deficiencies in HIV-positive populations. Coodley et al.\textsuperscript{18} found no evidence of low serum vitamin E levels in a study of HIV-positive patients at various stages of infection. In this study, concentrations of several micronutrients were measured to determine if deficiencies were associated with the HIV wasting syndrome. The authors found that none of the 39 HIV-infected subjects had serum vitamin E levels below 11.6 \(\mu\text{mol/L}\). However, mean vitamin E levels were substantially higher in this study population than in those reported in other studies, possibly due to the different assay that was used to measure vitamin E levels (atomic absorption spectrophotometry vs. high performance liquid chromatography). Similarly, Omene et al.\textsuperscript{56} found no difference in vitamin E levels in HIV-positive children compared to HIV-negative children. Again, mean vitamin E levels reported in this study were substantially higher than those reported in other studies. Constans et al.\textsuperscript{57} measured vitamin E levels in 95 HIV-positive patients and 20 healthy controls. Mean plasma vitamin E levels did not differ between HIV-positive patients at various levels of CD4\(^+\) cell counts and HIV-negative controls. Levels for all CD4 groups were within the normal range.

In the mid-1990s, researchers began to study the antioxidant effects of vitamin E in relation to HIV infection. Malvy et al.\textsuperscript{58} conducted a study to investigate the association between HIV infection and increased lipid peroxidation. Plasma levels of malondialdehyde (an indicator of lipid peroxidation), vitamin E, and a few other antioxidants were compared between 10 HIV-positive hemophiliac boys (aged 6 to 11 years); 10 HIV-negative hemophiliac boys matched on age, type, and severity of hemophilia; and 20 healthy age-matched controls. The authors found that HIV-positive boys had significantly higher plasma concentrations of malondialdehyde and reduced plasma \(\alpha\)-tocopherol levels compared to both HIV-negative control groups. This suggested that HIV-infected subjects have higher oxidative stress and lower antioxidant levels.
In 1998, Allard et al.\textsuperscript{21} showed that plasma levels of $\alpha$- and $\gamma$-tocopherol, along with several other antioxidants, were significantly lower in HIV-positive patients than in HIV-negative controls. In the same subjects, mean oxidative stress levels were significantly higher in the HIV-positive group than in the HIV-negative group.

Recently, we examined antioxidant levels in HIV-positive and HIV-negative injection drug users (IDUs) in the era of highly active antiretroviral therapies (HAART).\textsuperscript{59} We were surprised to find that serum $\alpha$-tocopherol levels were significantly higher in HIV-positive IDUs than in HIV-negative IDUs. However, among the HIV-positive subjects, there were significant differences in antioxidant levels by the various antiretroviral therapy regimens. Serum vitamin E levels were significantly higher in those taking combination therapies including protease inhibitors than in those who were on therapies without a protease inhibitor, or those who were not taking any antiretroviral therapies at all. This difference remained significant even after adjusting for differences in dietary and supplement intake and lifestyle changes. These results suggest that there is a reduction of oxidative stress levels in patients on protease inhibitor therapies, thereby allowing antioxidant levels to return to normal levels.

**Vitamin E Supplementation**

While many of the above studies demonstrated that low vitamin E levels are associated with HIV infection, few studies have examined the possible beneficial effects of vitamin E supplementation on HIV disease progression. As with vitamin C, the benefits of vitamin E supplementation on retrovirus-induced immune dysfunctions have largely been studied using \textit{in vitro} systems and animal models. In 1991, Odeleye and Watson\textsuperscript{60} showed that components of the immune system that become dysfunctional in HIV infection are similar to those that can be stimulated and/or restored by pharmacologic doses of vitamin E.

It has also been hypothesized that vitamin E, through its antioxidant properties, may prove effective in inhibiting NF-$\kappa$B, thereby inhibiting the subsequent transcription of HIV. Suzuki and Packer\textsuperscript{61} examined the effects of vitamin E and its derivatives on TNF-$\alpha$-induced NF-$\kappa$B activation using an \textit{in vitro} system. Although three of the vitamin E derivatives they tested ($\alpha$-tocopheryl succinate, vitamin E acetate, and 2,2,5,7,8-pentamethyl-6-hydroxycchromane (PMC)) were able to inhibit NF-$\kappa$B activation through what appeared to be different processes, $\alpha$-tocopherol was not completely successful. These results imply that some of the naturally occurring vitamin E derivatives could be useful in combination with other AIDS therapies; however, more research is needed to determine the mechanisms through which they work.
In a series of studies in a murine AIDS model, researchers at the University of Arizona in Tucson investigated whether vitamin E supplementation, alone or in combination with other therapies, would be able to modulate cytokine production and restore many of the immune dysfunctions caused by retrovirus infection. Although the murine retrovirus used in these studies (LP-BM5) is different from HIV, it shares many of the same characteristics and has a similar pathogenesis to HIV. Mice infected with the LP-BM5 retrovirus are found to have inhibited release of IL-2 and IFN-γ; increased secretion of IL-4, IL-5, IL-6, and TNF-α; and decreased hepatic and serum levels of vitamin E. This abnormal cytokine profile mimics that which is found in HIV-infected patients. In these studies, the authors found that the cytokine and immune dysregulation induced by the murine retrovirus infection could be reversed with a 15- to 450-fold increase in dietary intake of vitamin E, or an increased intake of vitamin E plus IFN-γ. These findings still need to be demonstrated in humans infected with HIV.

In a 9-year longitudinal study of 310 HIV-positive homosexual men in Baltimore, we found that subjects in the highest quartile of serum vitamin E level (≥ 23.5 μmol/L) had a significant decrease in the risk of progression to AIDS compared to those in the lower 3 quartiles combined. The association remained significant even after adjusting for several potential confounders, including dietary intake. In addition, there was a strong correlation between intake of vitamin E supplements and serum vitamin E levels in our subjects, suggesting that oral vitamin E supplements may be an effective means of raising vitamin E levels. Finally, as reported earlier, a randomized controlled trial of vitamins C and E showed that 3 months of daily supplementation reduced oxidative stress levels.

CONCLUSION

Early prevalence studies demonstrated that factors in addition to dietary intake, such as malabsorption and altered metabolism, may play a role in the abnormal vitamin C and E levels observed in HIV-infected populations. More recent studies in humans have provided evidence that supplementation with antioxidants such as vitamins C and E can be successful in reducing oxidative stress levels. The most compelling study to date has been the randomized controlled trial conducted by Allard et al., where they demonstrated that vitamins E and C may play a direct role in decreasing oxidative stress levels. While most other studies have tried to separate the antioxidative effects of vitamins C and E, both vitamins were included in this trial, presumably to enhance the antioxidative properties of both through their synergistic interaction. The results of this trial give promise that therapeutic levels of these vitamins could be helpful in inhibiting HIV activation and replication. However, larger trials over
longer time periods should be undertaken for more definitive results on the ability of vitamins C and E to reduce HIV viral load levels. Furthermore, this trial was conducted before the widespread use of HAART. Our more recent results from an observational study (reported earlier)\textsuperscript{59} suggest that the role of antioxidants in HIV disease progression may differ depending on the type of antiretroviral treatment regimen.

REFERENCES


IRON AND HIV INFECTION

Geoffrey A. Weinberg, Johan R. Boelaert, and Eugene D. Weinberg

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INTRODUCTION

“Gold is for the mistress – silver for the maid –
copper for the craftsman cunning at his trade.”
“Good!” said the Baron, sitting in his hall,
but Iron – Cold Iron – is the master of them all.”

Rudyard Kipling

Iron is the second most abundant metallic element, and the fourth most abundant of any chemical element, in the earth’s crust (after silicon, oxygen, and aluminum). The concentration of environmental iron spans a tremendous range, from millimolar to molar quantities in some rocky soil, to nanomolar quantities in seawater. However, both prokaryotic and eukaryotic cells have essentially the same narrow growth requirement, approximately 0.3 to 10 μM, despite whatever environmental niche they may occupy. Thus, it is readily appreciated that iron availability may affect the balance between growth of a microbial pathogen and effective immune effector cell clearance of such a pathogen by the host.

The pivotal role of iron in host-pathogen relations is no less important in HIV infection, although it has been less appreciated as the overwhelming HIV/AIDS knowledge base has been rapidly expanding. In this chapter, we review the available data concerning the reciprocal interactions between host iron status and HIV infection, as well as the role iron status plays in the development of opportunistic infections in the HIV-infected individual. We begin with a brief history of the recognition of iron as a critical micronutrient, followed by a survey of iron balance in human health.

HISTORICAL BACKGROUND

Benefits and Hazards of Iron

Of all the micronutrients now known to be essential for most forms of life, iron was the first to be discovered. As early as 1750, iron was detected in the ash of blood.2 During the next 150 years, patients suspected of having “chlorosis” (now considered to be probable moderate-to-severe hypochromic microcytic anemia) were treated with oral iron salts. Not surprisingly, overdoses were frequent; moreover, many persons presumed to be chlorotic were not iron deficient.

In an 1870 lecture on “true and false chlorosis,” Trousseau, a noted Parisian professor of clinical medicine, warned his students of the danger of feeding iron preparations to patients with quiescent tuberculosis. He
appeared certain that such a procedure could activate clinical episodes of the disease. In 1988, Trousseau’s astute observations were confirmed in animal models.

The biochemical utility of iron began to be identified early in the twentieth century. Awareness of heme iron as an oxygen carrier in hemoglobin and myoglobin, and as an electron carrier in the cytochromes, had developed by 1930. During the middle of the twentieth century, such non-heme iron molecules as the iron-sulfur proteins and ribonucleotide reductase were characterized. Also recognized at that time was the catalytic role of iron in DNA synthesis, ATP generation, nitrogen fixation, denitrification, and chlorophyll synthesis.

Iron is required for nearly all forms of microbial, plant, and animal life. Remarkably, only a single group of nonpathogenic microorganisms (Lactobacillus sp. and a few closely related forms) have been found to practice total iron abstinence. These bacteria employ manganese and cobalt as mineral catalysts, and they thrive in ecological niches in which little or no iron is available. Until very recently, no pathogenic microorganism was known to be able to eliminate the need for iron, but it appears that Borrelia burgdorferi also utilizes manganese in the few metalloproteins coded for in its limited genome.

The manifold chemical attributes of iron that provide diversified metabolic utility likewise render the metal dangerous for iron-dependant cells to manipulate. By 1980, these biochemical hazards of iron had become apparent. The metal catalyzes the generation of hydroxyl radicals and can thus seriously exacerbate oxidative stress. Cellular consequences include enhancement of radiosensitivity, mutation, chromosomal aberrations, neoplasia, degenerative aging, and death.

Still another hazard of iron began to be described in the 1960s. This is the ability of the metal to serve as a growth-essential nutrient for microbial and neoplastic cell invaders. Efficient acquisition of host iron is a requisite for high invasiveness of microbial pathogens (Table 7.1), as well as for rapid growth of neoplastic cells.

The Iron-Withholding Defense System

For protection from the various biochemical and microbial growth hazards of iron, vertebrate hosts have evolved an iron-withholding defense system (Table 7.2). An early recognition of a major component of this system is recorded in Shakespeare’s tragedy, King Lear. In Act III, Scene 7, raw egg white is employed as an anti-infective agent. The effective principle in egg white was shown in the 1940s to be an extremely powerful iron-binding protein that is remarkably active in blood plasma as well. The protein was
Table 7.1  Microbial Genera Whose Growth in Body Fluids, Cells, or Tissues of Intact Vertebrate Hosts is Stimulated by Excess Iron

<table>
<thead>
<tr>
<th>Gram-Positive and Acid-Fast Bacteria</th>
<th>Gram-Negative Bacteria</th>
<th>Fungi</th>
<th>Protozoa</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bacillus</td>
<td>Acinetobacter</td>
<td>Klebsiella</td>
<td>Candida</td>
</tr>
<tr>
<td>Clostridium</td>
<td>Aeromonas</td>
<td>Legionella</td>
<td>Cryptococcus</td>
</tr>
<tr>
<td>Corynebacterium</td>
<td>Alcaligenes</td>
<td>Moraxella</td>
<td>Histoplasma</td>
</tr>
<tr>
<td>Erysipelothrix</td>
<td>Campylobacter</td>
<td>Neisseria</td>
<td>Paracoccidioides</td>
</tr>
<tr>
<td>Listeria</td>
<td>Capnocytophaga</td>
<td>Pasteurella</td>
<td>Penicillium</td>
</tr>
<tr>
<td>Mycobacterium</td>
<td>Chlamydia</td>
<td>Proteus</td>
<td>Pneumocystis</td>
</tr>
<tr>
<td>Staphylococcus</td>
<td>Coxiella</td>
<td>Pseudomonas</td>
<td>Pythium</td>
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<tr>
<td>Streptococcus</td>
<td>Ehrlichia</td>
<td>Salmonella</td>
<td>Rhizopus</td>
</tr>
<tr>
<td>Tropheryma</td>
<td>Enterobacter</td>
<td>Shigella</td>
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<tr>
<td></td>
<td>Escherichia</td>
<td>Vibrio</td>
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<td></td>
<td>Helicobacter</td>
<td>Yersinia</td>
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</tbody>
</table>

Modified from Ref. 10.

Table 7.2  The Iron-Withholding Defense System

**Constitutive Components:**
- Siderophilins in fluids and secretions
  - Transferrin in plasma, lymphatic fluids, cerebrospinal fluid
  - Lactoferrin in secretions of lacrimal and mammary glands, and of respiratory, gastrointestinal, and genital tracts
- Ferritin and hemosiderin within cells

**Process Induced at Time of Invasive Infection (Activating Cytokines, if Known):**
- Suppression of assimilation of up to 80% of dietary iron (interleukin-1 [IL-1], IL-6, or tumor necrosis α [TNF-α])
- Suppression of iron efflux from macrophages that have digested senescent erythrocytes, resulting in up to 70% reduction in plasma iron (IL-1, IL-6, TNF-α)
- Increased synthesis of ferritin to sequester withheld iron (IL-1, IL-6, TNF-α)
- Release of neutrophils from bone marrow into circulation and ultimately into the site of infection, with release of neutrophil granule apolactoferrin which binds iron locally (IL-1, IL-6, TNF-α)
- Macrophage scavenging of ferrated lactoferrin in areas of infection and neoplasia
- Hepatic release of haptoglobin and hemopexin (which bind extracellular heme, respectively)
- Macrophage synthesis of nitric oxide from L-arginine, which disrupts microbial iron metabolism (IFN-γ)
- Macrophage downregulation of transferrin receptor expression and increased synthesis of Nramp1, leading to suppression of intracellular microbial growth (IFN-γ)
- B lymphocyte synthesis of antibodies to microbial iron-repressible cell surface proteins (e.g., outer membrane proteins which bind heme, ferrated siderophilins, or ferrated siderophores)

Modified from Ref. 10.
named siderophilin; later the name was changed to transferrin; and today the term siderophilin is used to refer to the class of iron-carrying proteins.

Between 1940 and 1960, a second indispensable iron-chelating protein, lactoferrin, was discovered in a variety of body fluids and in neutrophils. Additionally, ferritin and hemosiderin, intracellular packagers of excessive iron deposits, were delineated. The pronounced shifts in host iron metabolism during threatened microbial and neoplastic cell invasion, as well as in response to other physiological insults, began to be identified in the early 1930s. Hormonal control of these shifts is now fairly well characterized.9,14

In the 1990s it was recognized that, in response to invasion by the intracellular pathogens, macrophages modify their iron metabolism.14 Th-1 lymphocytes produce interferon-gamma (IFN-γ), which activates macrophages to (1) suppress their expression of transferrin receptors, (2) synthesize nitric oxide, and (3) increase formation of natural resistance-associated macrophage protein 1 (Nramp1). These macrophage actions, respectively, dampen iron influx, disrupt iron metabolism of microbial invaders, and modify intracellular distribution of the metal.

Certain microbial invaders can subvert host iron defense. For instance, such obligate intracellular pathogens as Coxiella burnetii and Ehrlichia chafeensis induce upregulation of host-cell transferrin receptor expression.14 Nitric oxide synthesis by macrophages can be inhibited by lipophosphoglycan, a surface molecule of Leishmania. Moreover, a multitude of studies from 1960 to 2000 described the ability of extracellular pathogens to obtain iron from such host proteins as transferrin, lactoferrin, hemoglobin, and haptoglobin. Indeed, a consistent hallmark of host-pathogen encounters is that pathogen ascendency is favored by iron-loading of hosts.10

**IRON BALANCE**

**Dietary Requirements and Food Sources**

The iron content of normal adult males is approximately 35 to 45 mg/kg body weight, with that of premenopausal women being somewhat less because of menstruation.15 About 50 to 60% of the metal is present in hemoglobin, 7.5% in myoglobin, and 35% in intracellular deposits of ferritin and hemosiderin.15 About 5% is utilized in cellular enzymes and less than 0.1% is in transit in combination with transferrin. Iron distribution in premenopausal females is similar to that in males except for a considerably smaller deposit in intracellular ferritin and hemosiderin.1

In normal persons, the total iron content of the body tends to remain within relatively narrow limits. In postmenopausal women and in men,
approximately 1 mg is lost daily in perspiration and shed skin, urine, and feces. This quantity is replaced by the absorption of 1 mg from the diet. Menstruating women absorb about 1.5 mg daily.

The recommended total daily allowance for iron is 6 to 10 mg for children under 1 years, 10 mg for children 1 to 11 years, 12 mg for male teens, 15 mg for female teens and adult premenopausal females, and 10 mg for postmenopausal females and all adult males. Regulation of iron balance occurs mainly in the gastrointestinal tract through absorption. The percentage of the metal absorbed from food varies with the amount of body iron deposits and with dietary composition. When deposits are low, absorption of non-heme iron increases; when deposits are sufficient, absorption decreases.

Iron bioavailability also depends on dietary components. Heme iron contained in mammalian meat, poultry, and fish is 3 to 10 times more absorbable than non-heme iron. Moreover, the bioavailability/absorption of heme iron is much less responsive to body iron deposits. A typical serving of liver contributes 6 to 8 mg of iron to the diet; of beef, 2 to 3.5 mg; of pork, 1 to 2 mg; of poultry, 0.5 to 1.5 mg; and of fish, 0.5 to 1 mg. A typical serving of eggs, vegetable, or wine each contributes 1 mg of iron. Cereals can contribute 1 to 6 mg depending on the amount and form of inorganic iron added by the processor. Non-heme iron is contained in plant-based foods, iron-fortified processed foods, and in iron supplements. A doubling of the absorption of non-heme iron can occur if heme iron is present concurrently.

Other enhancers of non-heme iron absorption include such reducing agents as ascorbic acid and cysteine-containing peptides, and promoters of gastric acid secretion such as ethanol. Suppressors of non-heme iron absorption include phytic acid in grains, legumes, and nuts; polyphenols in tea and coffee; and calcium in dairy products.

Pregnancy

In women who begin pregnancy with absent or minimal ferritin deposits of iron, about 6 mg/d of dietary iron is required to be absorbed in the second and third trimesters to supply the needs of the mother, the placenta, and the fetus. To accommodate these needs, a normal, marked expansion of iron absorption occurs in the second and third trimesters. For instance, a study of 12 healthy pregnant women on a regular, non-iron-supplemented diet found the amount of the metal absorbed from food was increased 5-fold at 24 weeks of gestation, and 9-fold at 36 weeks as compared with the quantity absorbed at 7 weeks.

For many decades, large numbers of pregnant women have been professionally advised to routinely take a supplement of up to 65 mg of
inorganic iron daily, even without laboratory evidence of iron deficiency. This routine procedure, even if unnecessary, is often considered to be harmless and perhaps to have some benefit. However, in 1993, a U.S. Preventive Services Task Force of the Office of Disease Prevention and Health Promotion, U.S. Public Health Service, carefully reviewed the published studies available and concluded that adequately controlled trials failed to demonstrate that routine iron supplementation of this magnitude during pregnancy yielded significant improvements in maternal or fetal outcome.

There is agreement, however, that those pregnant women who have subnormal hematologic values and who are iron deficient should take supplemental iron. Moreover, such underlying conditions that might be responsible for low iron values as malaria, hookworm infestation, or other causes of hemolysis or bleeding should be treated promptly.

Of increasing concern are observations that excessively high, as well as excessively low, hematologic values can be detrimental to the mother or fetus. For example, a study in Wales of 54,000 pregnancies found that perinatal mortality, low birth weight, and preterm births were more common in women with hemoglobin values either less than 10.4 g/dL or greater than 13.2 g/dL than in normal women with hemoglobin values of 10.4 to 13.2 g/dL. Similarly, in a U.S. study of 22,000 pregnancies with unfavorable outcomes, perinatal mortality was as much as twice as high in women with a lowest hemoglobin concentration of <9 g/dL during pregnancy, and up to five times higher in women with lowest hemoglobin concentrations of >13 g/dL than in women with a lowest hemoglobin concentration of 11 g/dL. In that study, low birth weight and neonatal prematurity also were elevated, although to a lesser extent, in women with lowest pregnancy hemoglobin concentrations of <9 or >11 g/dL.

Few data are currently available on whether HIV infection or AIDS affects the pregnant woman’s iron balance differently than that of the non-pregnant woman, adult male, or child. This would seem to be an important area of research, as often greater than 10 to 25% of pregnant women are infected with HIV in a number of sub-Saharan African nations, according to the latest estimates of the World Health Organization. Indeed, Friis et al. reported recently that HIV infection alters micronutrients status in pregnant women in Zimbabwe. Nearly 1700 pregnant women were studied in a nonendemic region for malaria (nonendemic because of high altitude) but where HIV seroprevalence in the women studied was 31.5%. HIV infection in pregnant women was associated with lower concentrations of serum folate, ferritin, and hemoglobin when compared to non-HIV-infected pregnant women; the effects on hemoglobin were particularly striking in HIV-infected women with adequate iron stores but low serum retinol.
Iron and HIV Infection

HIV Infection Leads to Iron Loading

During HIV infection, particularly in its more advanced stages, iron has been repeatedly observed to accumulate in several tissues, such as the bone marrow, the brain, muscle, liver, and spleen. The types of cells most involved with iron uptake are macrophages, microglia, endothelial cells, and myocytes. The main cause of this iron loading is the chronic inflammatory response that involves iron withholding and is accompanied by retention of iron in the reticulo-endothelial system. Indeed, iron traffic is modified during inflammation, so that the metal is diverted from both the circulation and the sites of erythropoiesis to the storage compartment in the reticulo-endothelial system. Although the precise mechanism is not fully elucidated, both Th-1- and Th-2-derived cytokines seem to cooperate, the primary effect of the Th-2-derived cytokines being an increased iron uptake in the activated macrophage, and the primary effect of the Th-1-derived cytokines an increased iron storage in ferritin.

A second important cause of iron accumulation in HIV infection may be transfusion of packed red cells. Another potential cause of iron loading during HIV infection is tobacco smoking, as previously reported by our group; smoking may increase the local iron burden in bronchoalveolar macrophages. Iron in excess in these cells is particularly evident when tobacco smoking is associated with chronic bronchitis.

Consequences of Iron Loading

Laboratory Studies

In view of the paramount importance of continued viral replication (generally measured as its surrogate, the plasma HIV-1 RNA copy level or “viral load”) as the primary determinant of the outcome of HIV-1 infection, the first step is to consider whether an excess of iron, as observed in advanced HIV disease, increases the replication of HIV-1. Indeed, iron does appear to enhance transcription of HIV-1 by increasing oxidative stress, leading to activation of nuclear transcription factor kappa B (NF-κB). For example, we reported that, in a model of provirally infected cells of the monocyte/macrophage lineage (U1 cells), induction of oxidative stress by
hydrogen peroxide resulted in a 20-fold activation of NF-κB, increased HIV-1 reverse transcriptase activity, and decreased cell survival. Pre-incubation of such U1 cells with iron nitriloacetate (50 $M$) prior to hydrogen peroxide exposure resulted in a 1.7-fold further increase in reverse transcriptase activity as compared to hydrogen peroxide exposure alone.$^{25,27}$ Pretreatment with iron chelators such as deferoxamine (at 2.5 to 5 $M$) decreased the activation of NF-κB and the resultant enhanced transcription of HIV-1.$^{27}$ More recently, another group confirmed the inhibitory effect of iron chelators upon HIV-1 replication as assayed by a p24 assay. However, the authors found that HIV-1 inhibition was mirrored by a decrease in cell proliferation, and therefore questioned whether the observed effect is due to iron chelation-mediated inhibition of oxygen radical generation or by the antiproliferative effect of iron chelation.$^{28}$

A second and well-known consequence of iron loading is the stimulation of the growth of many microorganisms, including opportunistic pathogens (see References 23, 29, 30). Of special concern is the fact that such iron-promoted increase in microbial growth may in itself upregulate the transcription of HIV, as has been elegantly demonstrated in the case of human tuberculosis/HIV coinfection.$^{31}$

A third consequence of iron loading is direct alteration of immune function. Decreased function of polymorphonuclear granulocytes,$^{32}$ macrophages,$^{33}$ and cell-mediated immune mechanisms$^{34,35}$ have all been reported. In addition, shifts in the balance of Th-1/Th-2 immune responses have been reported in an experimental model of murine candidiasis in iron-overloaded animals.$^{36}$ In the latter study, mice were overloaded by iron dextran injections, and were inoculated with a *Candida albicans* strain of low virulence. Iron treatment greatly increased the susceptibility of mice to *C. albicans* infection, and led to a decrease in Th-1 protective antifungal responses.$^{36}$

Finally, an excess of iron may increase the risk of HIV-related neoplasia.$^{12}$ For example, spindle cells isolated from AIDS-associated Kaposi’s sarcoma lesions grow in cell culture better with increasing concentrations of iron, and their growth is inhibited by iron chelators.$^{37}$ This has led to the suggestion that iron may be a cofactor in the pathogenesis of Kaposi’s sarcoma.$^{37}$ That the prevalence of endemic Kaposi’s sarcoma in Africa conforms geographically to the presence of volcanic soils containing iron oxides also may suggest a link between iron excess and this AIDS-related tumor.$^{38}$ However, the exact role that iron excess plays as a co-factor in this complex condition is not yet known, because recently it has become clear that human herpes virus 8 infection is directly related to the occurrence of Kaposi’s sarcoma in the majority of both HIV-infected and noninfected patients.$^{39,40}$
Clinical Studies

To date, four retrospective clinical studies have directly or indirectly examined the effects of iron status on the outcome of HIV-1 infection. First, a study of 64 HIV-1-infected thalassemia major patients concluded that the rate of progression of HIV disease among them was inversely proportional to the deferoxamine dose.\textsuperscript{41} Thalassemic patients treated with a deferoxamine dosage recognized to be effective at reducing body iron had slower HIV progression (albeit graded fairly crudely, by clinical progression to CDC Stage IV) than those treated with lower doses.\textsuperscript{41} In a follow-up report by the same group, the worst outcome in the lower deferoxamine dosage group was related to higher serum ferritin concentrations, with each 1000 µg/L increase in serum ferritin being associated with a 1.4-fold increase in the adjusted estimated risk of progression of HIV disease, and a 1.6-fold increase in the estimated risk of death. Because serum ferritin tends to reflect body iron status, the authors concluded that iron excess due to thalassemia may favor the progression of HIV infection.\textsuperscript{42}

A French comparative study of dapsone vs. aerosolized pentamidine for the prophylaxis of \textit{Pneumocystis carinii} pneumonia yielded interesting indirect data in support of the adverse role of iron loading in HIV infection.\textsuperscript{43} The oral dapsone preparation used in France also contained 30 mg of elemental iron per dose. After a mean follow-up of 13 months, mortality was greater in the group given dapsone (and thus iron) than in that of the pentamidine group.\textsuperscript{43} This result is in direct contrast with two other large prospective comparative trials using iron-free dapsone, in which no excess mortality was found. Thus, we and others have suggested that the inadvertent administration of low daily doses of oral iron in the French dapsone trial explains the excess mortality observed.\textsuperscript{44,45}

A polymorphism in the haptoglobin gene has been reported to influence iron metabolism, so that both uninfected and HIV-infected individuals exhibiting the haptoglobin 2-2 phenotype accumulate more iron and have greater serum ferritin concentrations than those exhibiting the haptoglobin 1-1 or 2-1 phenotypes.\textsuperscript{46} In addition, a subset of HIV-1-infected patients from Belgium and Luxembourg with the haptoglobin 2-2 phenotype presented signs of more oxidative stress and enhanced viral transcription, as shown by higher HIV-1 RNA plasma levels (patients were antiretroviral therapy-naïve). More importantly, a survival analysis of 653 HIV-1-infected patients showed that the haptoglobin 2-2 phenotype patients had a significantly shorter mean survival (7.3 years) compared to that of haptoglobin 1-1 and 2-1 phenotype patients (11.0 years).\textsuperscript{46}

A fourth study examined the relationship between iron stores and survival of HIV-1-infected patients.\textsuperscript{47} Bone marrow macrophage iron stores were graded on a scale from 0 to 5 in 348 HIV-positive patients who had
undergone a diagnostic bone marrow aspirate. The adjusted estimated rate of death was greater in the patients with grades 4 to 5 bone marrow aspirates (iron excess) than in those with grades 1 to 2 aspirates (normal or decreased iron), even when survival was studied from the time of seropositivity.\textsuperscript{47b} Infections caused by \textit{Candida} spp., \textit{P. carinii}, and \textit{Mycobacterium} spp. were significantly more common in the patients with the higher macrophage iron stores.\textsuperscript{47b}

Although these four retrospective clinical studies do not conclusively prove that greater iron stores result in a faster progression of HIV-1 infection, the data nevertheless converge and suggest the possibility that this indeed may be the case.

Three more cross-sectional clinical studies provide conflicting data on the role of iron stores and progression of HIV infection. The first study is a laboratory analysis of iron status markers and indicators of HIV disease in samples collected from 483 HIV-infected women in Malawi who were participating in a clinical trial of vitamin A supplementation during pregnancy.\textsuperscript{47a} In this analysis, Semba et al. failed to find any correlation between iron status and markers of HIV disease severity.\textsuperscript{47a} The authors suggested that iron supplementation to achieve normal iron status is probably safe in HIV-positive pregnant women in developing countries.\textsuperscript{47a} However, the study did not adjust for changes of ferritin as an acute phase reactant, and essentially all of the subjects appeared to have iron deficiency anemia. It is possible that a more significant correlation would be unmasked if ferritin levels were adjusted for acute phase response, or if comparisons were made between subjects with normal iron status and iron overload (because subjects with true iron deficiency might be expected to have already decreased immune function, confounding studies of iron-HIV interactions).\textsuperscript{106-108}

In contrast, in two cross-sectional analyses of iron status and HIV infection performed on samples from pregnant HIV-infected women and uninfected pregnant controls in Zimbabwe, significant associations were found between iron status and HIV infection.\textsuperscript{22a} In these studies, 1669 pregnant women, of whom 526 were HIV-infected, were assessed for markers of iron and other micronutrient status, as well as haptoglobin phenotype and viral load. Importantly, the effects of changes in ferritin concentration because of the acute phase response were controlled for by simultaneously analyzing another acute phase reactant, α₁-antichymotrypsin; in addition, comparisons were made across a broader range of iron deficiency/sufficiency in both HIV-infected and uninfected women.\textsuperscript{22a} Pregnant women with HIV infection had lower serum folate, ferritin, and hemoglobin concentrations than did non-HIV-infected pregnant women.\textsuperscript{22a} The effects of HIV infection upon low hemoglobin were strongest for those women with nondepleted iron stores (serum ferritin ≥ 12 µg/L) and low serum retinol (< 0.70 µmol/L).\textsuperscript{22a} Further analysis showed that women with haptoglobin phenotype 2-2 had
twice-elevated HIV RNA viral loads compared to those with haptoglobin phenotype 1-1; these data are in accord with those of the study of Delanghe et al. in a predominantly male population. Finally, HIV RNA viral load levels were twice as great in infected pregnant women with nondepleted or high iron stores (serum ferritin ≥ 24 µg/L) than those with iron depletion (serum ferritin < 6 µg/L).

Thus, the majority (but not all) of the available retrospective and cross-sectional data suggests that iron overload may prove harmful in individuals with HIV infection. In view of the worldwide standard practice of iron supplementation of pregnant women, many of whom may be HIV-infected, controlled clinical trials are needed urgently to resolve this potential public health claim.

**Therapeutic Strategies**

The results of the laboratory and clinical studies discussed above imply that prevention of iron loading in HIV-1-infected patients may be beneficial. Specific patient subpopulations, such as those carrying the haptoglobin 2-2 phenotype, should perhaps be targeted for iron restriction. Several strategies are available to accomplish this goal.

First, general behavioral measures, previously reported by our group, include restricting iron intake through the parenteral, alimentary, and respiratory routes. These simple, rather nonspecific measures could be applied on a broad scale without extra requirements as long as iron deficiency is excluded. For example, excessive iron intake may be avoided by reducing unnecessary blood transfusions, decreasing consumption of red meats and alcohol (which facilitates iron absorption), and reducing or eliminating iron supplements (including attention to processed foods, which may have been iron-supplemented, or plants genetically modified to incorporate extra iron). Increasing intake of dietary iron-chelating plant foods (e.g., soy products, cereals, and teas containing phytic acid, tannins, and polyphenols) also may decrease gastrointestinal iron absorption. Finally, restriction of respiratory iron may be achieved by reducing exposure to tobacco smoke, asbestos fibers, and urban air particulates.

Whether there is a role for the more specific strategy of iron chelation as an adjunctive therapy in the HIV-infected patient with established excessive iron burden is not yet certain. The only available study concerned thalassemic HIV-infected patients in whom effective therapy with deferoxamine slowed progression of HIV-1 infection. No other study has been reported on the long-term use of iron chelation in (nonthalassemic) HIV-positive patients. Although iron chelators from several chemical classes (i.e., deferoxamine and deferiprone) inhibited HIV-1 transcription in vitro, the difficulty related to the subcutaneous administration of deferoxamine
and the risk of bone marrow toxicity and neutropenia related to deferiprone have hindered the design of clinical chelation studies. It is possible that newer derivatives such as hydroxyethyl starch-bound deferoxamine, newer hydroxypyridinone compounds, or other chelating agents will be found to have greater affinity to cellular iron compartments but less myelotoxicity.\textsuperscript{49-51} Such agents may facilitate iron chelation studies in iron-loaded HIV-1 infected patients in the future.

The old antimalarial drug chloroquine has been found to limit the availability of iron to intracellular compartments, and this may be of help in HIV-positive patients by limiting the deposition of iron in the reticuloendothelial system.\textsuperscript{52} We recently reported that chronic chloroquine administration to rats decreases both hepatic iron content and macrophage iron loading caused by experimental iron dextran administration.\textsuperscript{53} Apart from its effect upon iron metabolism, the use of chloroquine in HIV-infected individuals may offer other advantages. First, at least experimentally, chloroquine exhibits an inhibitory effect toward several important pathogens that cause opportunistic infection in AIDS.\textsuperscript{54-57} Second, chloroquine and its derivative hydroxychloroquine are known to inhibit HIV-1, both \textit{in vitro}\textsuperscript{58-60} and in HIV-infected patients.\textsuperscript{61} Moreover, the addition of chloroquine to the combination of didanosine plus hydroxyurea had an additive anti-HIV effect \textit{in vitro}.\textsuperscript{62} This relatively inexpensive antiretroviral triple combination may, therefore, be of particular interest for HIV-therapy in resource-poor countries.\textsuperscript{62} Finally, chloroquine has anti-inflammatory properties and may decrease the secretion of pro-inflammatory cytokines, which appear to play a role in the progression of HIV-1 infection.

\textbf{IRON AND OPPORTUNISTIC INFECTIONS}

Whether HIV infection is present or absent, iron plays a clear role in determining the virulence of bacterial, fungal, parasitic, and possibly viral infections.\textsuperscript{1,11,23,24,29,30,63-68} HIV infection is characterized by the loss of cell-mediated immunity as well as dysregulation of humoral immunity. Emerging data suggest that iron status in HIV infection has an important role in the development of opportunistic infection in addition to HIV-modulated destruction of cell-mediated and humoral immunity (Table 7.3).

\textbf{Bacterial Infections}

Although not generally thought of as opportunistic infections, some bacterial infections such as those caused by \textit{Salmonella} spp., \textit{Haemophilus influenzae}, and \textit{Streptococcus pneumoniae} have increased incidences of disease among HIV-infected patients. It is possible that the iron loading of HIV infection may play a role in such infections, although direct data
proving this hypothesis are not available. More data are available concerning the increased susceptibility of HIV-infected patients to mycobacterial infections, including both tuberculous and nontuberculous infection.

Serum transferrin has long been known to be inhibitory against the growth of *Mycobacterium tuberculosis*, and administration of iron to mice increases splenic growth of *M. tuberculosis*. A recent reanalysis of data collected from African patients studied before the HIV pandemic began revealed an association between iron overload and tuberculosis. Increased bone marrow iron stores in HIV-infected patients in the United States also have been associated with increased risk of mycobacterial disease, as well as with decreased survival.

Infections with *M. avium-intracellularare* complex (MAC) are well-known to be increased among individuals coinfected with HIV. The growth rate of MAC in macrophage cultures *in vitro*, as well as in experimental animals, is dependent upon iron availability. Furthermore, mice naturally resistant to MAC infection by virtue of a specific genotype of the *Nramp1* gene lose control of MAC infection when iron overloaded, linking iron metabolism with genetic resistance to opportunistic infection. Again, human data show that both increased bone marrow and hepatic iron stores are associated with an increase in MAC infection.

### Fungal Infections

Several fungal infections have a markedly increased incidence in HIV infection and AIDS, especially those caused by *Candida* and *Cryptococcus*, but...
also endemic mycoses such as histoplasmosis, coccidioidomycosis, and *Penicillium marneffei* infection. The relationship between iron status and the growth of five such opportunistic fungal pathogens has been reported recently.

First, iron overload greatly increased susceptibility to disseminated *Candida albicans* infection in mice, while iron chelation with deferoxamine cured the infection and modulated murine Th-1/Th-2 lymphocyte balance. Similarly, iron-loaded mice infected intranasally with *Cryptococcus neoformans* had 10 times greater microbial loads in the lungs than did control mice. Third, intracellular and extracellular growth of *P. marneffei* were both enhanced by iron overload and reduced by iron chelation in cell culture studies. Intracellular iron depletion appears to be an effective growth inhibitor of *Histoplasma capsulatum*. In the latter study, iron depletion was achieved by using the antimalarial agent chloroquine, which raises intralysosomal pH, retarding intracellular iron release. Chloroquine has also exhibited activity against *C. neoformans*, although some investigators feel that iron depletion does not sufficiently explain the measured antifungal activity.

Finally, *Pneumocystis carinii* is one of the most common opportunistic organisms associated with AIDS. Recent data suggest that *P. carinii* is most likely a primitive atypical fungus, yet it shares some traits with parasites and is sometimes still grouped with them for historic reasons. Both Weinberg and Clarkson have studied the role of iron availability in *P. carinii* infections. Iron chelation with deferoxamine inhibits *P. carinii* growth in short-term *in vitro* cell co-culture models as well as in experimental animal models of rat and mouse *P. carinii* pneumonia. The effect of deferoxamine appears to be mediated by iron chelation rather than by nonspecific toxicity, as it is reversed by excess saturation of the chelator with iron but not zinc, calcium, or magnesium. Deferoxamine is effective against experimental *P. carinii* pneumonia in the rat when administered by intermittent injection or continuous infusion. Clarkson’s group found a greater effect of deferoxamine upon *P. carinii* trophozoites than upon cysts, although we have shown substantial deferoxamine activity against both trophozoites and cysts. Multiple iron chelators of diverse chemical classes such as hydroxypyridinones (e.g., deferiprone), desmethyldesferrithiocin, reversed siderophores (e.g., SF-1-ileu), and the ferrous iron chelator VUF-8514 show anti-*P. carinii* activity *in vitro*, but are inactive (deferiprone) or not yet tested in *in vivo* models. Whether the activity of the ferrous iron chelator implies that *P. carinii* iron acquisition involves membrane ferric iron reduction similar to that in *Saccharomyces cerevisiae* remains to be determined.
Parasitic Infections

Leishmaniasis presents as either a cutaneous or visceral disease in immuno-
competent hosts, but appears to present only as severe visceral disease in
HIV-infected individuals. Soteriadou and colleagues have demonstrated that
Leishmania infantum promastigotes express a human transferrin receptor
and bind transferrin during growth. The same group of investigators has
also shown that iron chelation reduces promastigote growth in both
axenic systems and in macrophage culture. L. chagasii may also acquire
iron from transferrin and lactoferrin. Preliminary data suggest that the iron
status of L. major-infected mice drives the balance between Th-1 and Th-2
immune responses, and thus may affect clinical expression of disease.

Viral Infections

Although viral replication does not require micronutrients in the traditional
sense, the iron status of the host cells in which replication is taking place
appears to be important. At the cellular level, acute HIV-1 infection also
appears intertwined with iron metabolism, in that lymphoid cell lines
infected with HIV-1 downregulate expression of the transferrin receptor
(CD71) compared to uninfected control cells, and chelation with citrate
prevents such downregulation and modulates viral cytopathic effects.

Human cytomegalovirus replication in fibroblast cultures in vitro is
inhibited by deferoxamine as well as by other chelators such as diethyl-
etriaminepentaacetic acid (DTPA). Preliminary data in four patients
with refractory cytomegalovirus retinitis suggested that deferoxamine also
might be a useful adjunctive therapy.

Another important coinfecting virus in HIV-infected individuals is
hepatitis C virus (HCV). The inverse relationship of hepatic iron status and
response to interferon-α has been demonstrated in three recent papers
(summarized in Reference 104). Reduction of iron loading by phlebotomy
has resulted in reduction of serum transaminase activity in HCV-infected
patients, although changes in plasma HCV RNA levels are less consistent.
These studies have generally been performed in non-HIV-infected patients
with chronic HCV infection; similar data are not yet available for patients
coinfected with both viruses.

PERSPECTIVES AND INFECTIONS

Whether one studies bacterial, fungal, parasitic, or viral opportunisti
infections in HIV-infected individuals, or even the cell biologic and immuno-
logic consequences of HIV infection itself, iron appears to be one of the
most important micronutrients of concern (if not the most important one –
For selected patients participating in controlled investigational research studies, iron chelation might serve as a useful adjunct to antiretroviral and/or antimicrobial chemotherapy. Decreasing iron loading by chelation might be expected to reduce host cell damage, opportunistic pathogen virulence, and, perhaps, retroviral replication. However, this approach is not entirely risk-free, as altering iron balance also can lead to microbial overgrowth and heightened cell damage if iron is transferred to inappropriate tissue compartments rather than being excreted. In addition, there are an estimated 500 million people in the world with iron-deficiency anemia, and certainly at least as many or twice as many with lesser degrees of iron deficiency. Severe iron deficiency, besides causing anemia, can contribute to decreased immune function. As the majority of iron-deficient people reside in developing countries with significant and increasing prevalences of HIV infection, widespread iron chelation without regard to an individual’s iron balance and immune competence also might be harmful. Clearly, more work is required in this important area. Further clinical advances may be made in combating the AIDS pandemic.

ACKNOWLEDGMENTS

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ZINC AND HIV INFECTION

Henrik Friis and Brittmarie Sandström

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HISTORICAL BACKGROUND

Zinc is a trace element essential to microorganisms, plants, and animals. In 1934, it was shown to be essential to the growth of animals. In Holstein-Fresian cattle, a mutation (A-46) impairing the absorption of zinc and followed by severe thymic atrophy was described in 1971. In man, the name acrodermatitis enteropatica (AE) was suggested in 1942 for a syndrome characterized by dermatitis, diarrhea, and impaired growth, later found to be an inborn error of metabolism resulting in impaired zinc absorption. In addition to thymic atrophy, AE patients are susceptible to bacterial, viral, and fungal infections. The first thorough account of endemic zinc deficiency in man was given in 1963 by Prasad, with the description of a syndrome of dwarfism, hypogonadism, and anemia among Iranian males. Finally, in the 3rd Report of the World Nutrition Situation in 1997, zinc deficiency was recognized as an important threat to infant, child, and maternal health, responsible for stunting and increased infectious disease morbidity and mortality.

METABOLISM AND BIOLOGICAL FUNCTIONS

The fundamental reliance of biological systems on zinc is due to its ubiquity, its ability to bind and rapidly exchange ligands, its flexible coordination geometry, and its ability to accept electrons rather than participating in redox reactions that could cause oxidative damage. As such, zinc has a multitude of structural, catalytic, and regulatory roles in processes like the synthesis of protein and DNA, mitosis, apoptosis, gene expression, and neurological function. Zinc is important in stabilizing cell and organelle membranes, and in folding proteins by zinc-fingers to enable them to function as DNA transcription factors, hormone receptors, and enzymes. It is essential to the activity of more than 300 enzymes where, in addition to maintaining structure and stability, the zinc ion serves as a cofactor in reactions that require a redox-stable ion that is able to accept a pair of electrons.

Of particular interest in the context of infectious diseases is the role of zinc in apoptosis and oxidative stress. Apoptosis, also called programmed or gene-directed cell death, is a mechanism required for the removal of cells that are infected or otherwise damaged or superfluous, thus serving to maintain cellular homeostasis. In addition, apoptosis is involved in the pathogenesis of many diseases, such as autoimmune and malignant diseases, as well as HIV infection. Several studies have demonstrated that zinc deficiency leads to an increased rate of apoptosis in thymic lymphocytes and other cells. Similarly, zinc supplementation prevents apoptosis after exposure of laboratory animals or cells in vitro to radiation, ischemia, and chemicals, although a high dose of zinc may itself lead to apoptosis. Zinc plays a role in protection against oxidative stress through the
antioxidant enzyme Zn,Cu superoxide dismutase, and there is compelling evidence that zinc per se has antioxidant properties. Consequently, important body functions such as reproduction, growth, immunity, and resistance to infections are vulnerable to inadequate zinc intake.

Response to Inadequate Intake

The human body contains less than 3 g of zinc, of which more than 95% is intracellular. Bone and skeletal muscle account for 80 to 90% of total body zinc. The liver, the most important organ in terms of zinc metabolism, contains 6%, and plasma only 0.1%. Zinc homeostasis is maintained through regulation of absorption of exogenous zinc in the small intestine, and excretion of endogenous zinc. The intracellular metal-binding protein metallothionein (MT) and zinc transporter proteins participate in the protection of the body against inadequate and excessive intake, and keep the free and tissue zinc concentrations within narrow ranges. The MT protein contains two domains where zinc atoms can be bound tetrahedrally to cysteine through sulfur ligands. Although zinc is itself redox inert, oxidation or reduction of the cysteine sulfur ligands to zinc lead to conformational changes followed, respectively, by the release or binding of zinc. MT may thus enhance availability of zinc to biochemical functions. Hepatic, intestinal, and lymphocytic MT synthesis is increased during times of inadequate intake and reduced in times of excessive intake.

Because the body pool of readily mobilizable zinc is small, deficiency develops rapidly in laboratory animals on low-zinc diets. It impairs appetite, and causes growth retardation in growing animals and loss of lean body mass in adult animals. This is followed by immune deficiency and increased susceptibility to infections; impaired wound healing; hair, nail, and skin abnormalities; hypogonadism; neurological disturbances; and delayed sexual development (Table 8.1). Specific symptoms are only seen in severe zinc deficiency, which is a rare condition in humans. In contrast, mild zinc deficiency is probably widespread, although there are neither specific manifestations of mild zinc deficiency nor any satisfactory methods to diagnose it.

Golden has suggested that nutrients should be classified according to the response to inadequate intake. For vitamins, iron, and selenium, the response to inadequate intake is a decline in the body stores, followed by impairment of the specific metabolic pathways involved and development of specific clinical signs or symptoms. Growth, however, is not primarily affected. Such nutrients are called Type I nutrients. In contrast, the response to a deficiency of a so-called Type II or growth nutrient, such as zinc, is immediate cessation of growth or the breakdown of lean tissue as soon as homeostatic mechanisms are exhausted. This is due to the fact
that Type II nutrients have no functional body stores and are involved in DNA synthesis and other basic metabolic pathways. Consequently, the concentrations of zinc in tissues and body fluids can be maintained over long periods of low intake, during which there will be a negative balance of any other Type II nutrients, as they will be in relative surplus.\textsuperscript{20}

**Response to Infections**

Zinc metabolism is dramatically affected by infections, trauma, burns, and other conditions eliciting an acute phase response.\textsuperscript{21} The acute phase response is mediated by a cascade of cytokines, initiated by interleukin-1 (IL-1) released by activated macrophages.\textsuperscript{22} In rats injected with IL-1, serum zinc declined by 75% after 6 hours, but returned to normal levels after 12 hours. This decline in serum zinc was found to be due to a redistribution of zinc caused by increased uptake by metallothionein in the liver, bone marrow, and thymus.\textsuperscript{21} In contrast to iron, the absorption of zinc was increased during infections,\textsuperscript{23} although this remains to be demonstrated in man.

<table>
<thead>
<tr>
<th>Table 8.1 Signs and Symptoms of Zinc Deficiency</th>
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<tr>
<td><strong>Mild</strong></td>
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<tr>
<td>Cessation of growth (children)</td>
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<tr>
<td>Loss of lean body mass (adults)</td>
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<tr>
<td>Reduced appetite and food intake</td>
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<tr>
<td>Impaired immune functions</td>
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<tr>
<td>Impaired resistance to infections</td>
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<tr>
<td>Impaired taste and smell</td>
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<tr>
<td>Night blindness</td>
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<tr>
<td>Delayed wound healing</td>
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<tr>
<td>Skin, nail, hair changes</td>
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<tr>
<td>Reduced physical activity</td>
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<tr>
<td>Behavioral disturbances</td>
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<tr>
<td>Delayed sexual maturation</td>
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<tr>
<td>Hypogonadism</td>
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<td>Hypospermia</td>
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<td>Diarrhea</td>
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<tr>
<td>Alopecia</td>
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<tr>
<td>Acro-orificial lesion</td>
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<tr>
<td>Psychoneurological disturbances</td>
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<tr>
<td>Infections</td>
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<tr>
<td><strong>Severe</strong></td>
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<tr>
<td>Death</td>
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</tbody>
</table>
ASSESSMENT OF STATUS

By definition, the assessment of status of a Type II nutrient like zinc is difficult. In mild, and even in severe, overt zinc deficiency, tissue concentrations may be normal and total body zinc close to normal.\textsuperscript{24} Quoting from Golden: “Indeed, the very use of the word ‘status’ with respect to zinc nutrition requires careful definition, as it probably subsumes a presumption of the nutrient in question giving rise to a Type I deficiency.”\textsuperscript{24} Yet, the concentration of zinc in serum, white blood cells, hair, etc. is still widely used as a measure of zinc “status,” and values below a certain cut-off are used to define zinc deficiency. This is unfortunate, because the concentration of zinc in these fluids and tissues is little and unpredictably affected by zinc intake, and affected further by several other factors.

Because the growth of hair and the composition of white blood cells depend on zinc status, zinc concentrations in these tissues are inherently invalid as measures of status, although they may respond to supplementation. Mean serum zinc in a population may give an indication of the risk of zinc deficiency in the population,\textsuperscript{25} although when zinc repletion leads to an anabolic phase, serum zinc may decline. Serum zinc also may increase during acute starvation,\textsuperscript{26} while it declines in response to meals,\textsuperscript{27} stress, pregnancy, and oral contraceptives.\textsuperscript{28} Of particular concern is the fact that the acute phase response to infections and other conditions leads to a redistribution of zinc from serum (and bone, skin, and intestine) to liver, marrow, and thymus.\textsuperscript{21} Thus, the only method to diagnose zinc deficiency is to assess the effect of zinc supplements on suspected zinc deficiency symptoms, or on growth or any other zinc-dependent body functions, preferably compared to placebo in a randomized, controlled trial.

DIETARY REQUIREMENTS AND FOOD SOURCES

Establishment of zinc requirements and recommended dietary allowances (RDA) has been difficult due to the lack of sensitive indicators of zinc status, the strict homoeostatic regulation, and the highly variable bioavailability of zinc in different diets. Recent estimates of the amount of zinc that needs to be absorbed, the physiological requirements,\textsuperscript{29} are based on the so-called factorial approach, i.e., estimates of the obligatory losses of zinc via skin, urine, and feces, with an addition of zinc required for the growth of new tissue when appropriate. The data used for these estimates are based on metabolic balance studies with low zinc intakes in small groups of presumably initially well-nourished subjects. As it is well documented that zinc losses are reduced at longer periods of low intakes, values obtained in the initial deprivation phase are selected. These losses are estimated at 1.4 and 1.0 mg/d for an adult man and woman, respectively.
additional zinc requirements for growth are, on average, rather small (≈ 30 μg/kg per day), but can be substantially increased in periods of growth spurt or catch-up growth after periods of malnutrition. The pubertal growth spurt of adolescent males is associated with a zinc increment of 0.5 mg/d. In late pregnancy, an additional 0.7 mg zinc/d is required, and zinc lost in lactation corresponds to 1.4 and 0.5 mg/d during the first and second 6 months, respectively. Thus, the physiological requirement of zinc is highest during pubertal growth, late pregnancy, and early lactation.

To translate the physiological requirements to dietary intake recommendations, the diet composition has to be accounted for. The major determinants for this are the content of zinc, animal protein, and phytic acid. Typical diets can be classified as high, moderate, and low with regard to zinc availability, i.e., potentially absorbable zinc. Refined diets low in cereal fiber and animal-based formula diets show a high availability; mixed animal and vegetable protein diets with a phytic-acid-to-zinc molar ratio below 15 show a moderate availability; and cereal- or legume-based diets low in animal protein and with a molar ratio of phytic-acid-to-zinc >15 show a low availability. The recommended dietary allowances for zinc will consequently vary greatly. With a high availability, a population mean intake of 4 mg/d will cover the needs of adult nonpregnant, nonlactating women, while 13 mg/d is needed for the same population group if the diet has a low zinc availability. Good food sources of zinc include meat, liver, and eggs. Seafood, especially oysters, are particularly good sources of zinc. Unrefined cereals and legumes have a high zinc content, but bioavailability is low because of the high content of phytate and dietary fibers.

In the United States, 70% of the zinc consumed is from animal foods. Even in affluent Western countries, growth-limiting zinc deficiency has been demonstrated among infants, toddlers, and preschool children. In developing countries, zinc status is likely to be low, since the staple diet is cereal-based with a high phytate content and low in animal proteins. Using available data for zinc requirement and food intake in different countries, 68 to 94% of children in a number of developing countries were considered at risk for zinc deficiency compared to only 1% of Canadian children.

Toxicity after excessive oral zinc intake has been reported. Daily zinc intakes above 50 mg have been associated with increased copper excretion, and higher intakes may lead to copper deficiency with anemia, neutropenia, impaired immune functions, and adverse changes in cholesterol metabolism.

**HOST DEFENSE**

While zinc is required for the host immune system and other functions, it is also essential for the growth of bacteria and yeast, and for the replication of viruses. The substantial decline in serum zinc during the acute phase
response to infections\textsuperscript{21} may, therefore, be part of the host defense against microorganisms.\textsuperscript{41} Zinc is also made unavailable to bacteria and yeasts locally in the gastrointestinal tract, and in tissues invaded by pathogens.\textsuperscript{42} This is mediated by the protein calprotectin, released from degrading neutrophils, which chelates zinc and, as such, prevents the growth of bacteria and fungi in abscesses.\textsuperscript{43,44} Conversely, zinc may be microbicidal at high concentrations.\textsuperscript{45}

Zinc is probably the micronutrient of greatest importance to the immune system. It is essential to a wide range of nonspecific and specific immune functions, in particular cell-mediated immunity, as reviewed by Beisel in the chapter on nutritionally acquired immune deficiency syndromes. Thymulin is a nonapeptide hormone secreted by the thymus, a key immune organ, and involved in the differentiation of T-lymphocytes.\textsuperscript{46} Interestingly, thymulin is only active if bound to a zinc ion.\textsuperscript{47} In fact, the thymus can virtually be removed after a couple of weeks on a low-zinc diet, a phenomenon called “nutritional thymectomy,” due to the loss of precursor T- and B-cells.\textsuperscript{48} Puzzling results were reported from a study on pregnant mice on low-zinc diets: reduced immune functions were seen not only in the offspring fed a zinc-sufficient diet, but even in the second and third filial generations.\textsuperscript{49} The role zinc seems to play in the regulation of apoptosis\textsuperscript{13} is not least important in relation to the immune systems and the pathogenesis of infectious diseases.\textsuperscript{10,12}

Similarly, in malnourished children, thymus size\textsuperscript{50} and delayed type hypersensitivity reaction\textsuperscript{51} were markedly reduced, but increased significantly after the administration of zinc. Interestingly, human zinc deficiency also has been shown to lead to a Th-1 to Th-2 shift in cytokine profile, and a decreased recruitment of T-naïve and T-cytolytic cells, which may partially explain the impaired cell-mediated immunity in zinc deficiency.\textsuperscript{52}

Excessive intake also may impair the immune function,\textsuperscript{53} and this may be because of the effect of zinc on copper metabolism.\textsuperscript{54} Recently, the role of zinc in immunity has been reviewed comprehensively.\textsuperscript{45,48}

**INFECTION**

Because of the fundamental importance of zinc to the immune system, and the inadequate zinc nutriture among people in developing countries, zinc deficiency is likely to contribute substantially to the global burden of infectious diseases. However, due to the lack of a valid measure of zinc status, observational studies are of little use in assessing the relationship between zinc deficiency and infectious diseases. The randomized, placebo-controlled, double-blind intervention trial is not only the strongest tool to establish a cause–effect relationship, it is absolutely necessary when drawing valid inferences about the effects of deficiencies of zinc and other nutrients for which status cannot be measured. The proper design and
interpretation of randomized, controlled trials using micronutrient interventions, however, are more complex than those of drug trials, and should be based on a thorough understanding of the characteristics of the micronutrient in question.

In general, if a micronutrient deficiency is hypothesized to reduce host defense against infection, then a supplementary trial is only meaningful if deficiency of the micronutrient is widespread and the intervention effectively repletes the study population. For Type I nutrients this usually can be documented through assessment of status. In zinc studies, it is necessary to include a known zinc-dependent effect parameter, such as growth. On the other hand, a lack of effect of zinc supplementation in a particular study could be due to the limiting effect of another Type II nutrient. In accordance with the characteristics of Type II nutrients, the optimal intervention would be to give adequate amounts of all other Type II nutrients to all study participants, with or without zinc. However, immunosuppressive zinc deficiency is conceivable, even though another Type II nutrient is growth limiting. Conversely, although growth probably has a high rank in the hierarchy of zinc-dependent body functions, an effect of zinc on infectious disease morbidity is likely to contribute to the effect on growth or body composition.

Within the last decade, randomized, controlled zinc supplementation trials have documented a role of zinc in diarrhea, respiratory tract infections, and other infections. But, as discussed above, given the nature of micronutrients in general, and zinc in particular, results from randomized, controlled supplementation trials are bound to be inconsistent.

**Diarrhea**

Diarrhea may contribute to zinc deficiency via increased intestinal zinc loss\(^5\) and, as such, partially explains the consistent finding of an association between diarrhea and severe zinc deficiency. Conversely, zinc deficiency also may cause or contribute to diarrhea, either by changing the morphology\(^6\) and integrity\(^7\) of the intestinal mucosa, making the host more susceptible to intestinal pathogens through suppression of the gut mucosal immune system,\(^8\) or by upregulating enzymes mediating intestinal damage or secretion.\(^5\)\(^9\)-\(^6\)\(^1\) Accordingly, it has now been clearly demonstrated that zinc plays a role in the prevention\(^6\)\(^2\) and case management of diarrhea.\(^6\)\(^5\)

**Prevention**

A Mexican study among preschoolers showed that the number of reported annual episodes of acute diarrhea was 38% lower in children receiving 20 mg of zinc per day, whereas there was no effect on the duration of
Similarly, rural Guatemalan children had a 22% reduction in incidence if given 10 mg of zinc daily for 7 months. An Indian study showed that daily multivitamin supplements containing 10 mg of zinc, compared to multivitamins alone, for 6 months after an episode of diarrhea reduced the incidence of acute diarrhea in children above 12 months by 27%, while there was no effect on infants. A study among young, undernourished Vietnamese children reported that 10 mg of zinc per day for 5 months reduced the risk of diarrhea by 71%. Young Bangladeshi children with acute diarrhea who were given 20 mg zinc for 2 weeks had significantly fewer episodes and shorter durations of diarrhea in the subsequent 8 weeks compared to children in the control group. In contrast, 70 mg zinc twice weekly had no effect on the number of clinic visits for diarrhea in Gambian children, possibly due to inadequacy of the dosing regimen.

The role of zinc on persistent diarrhea and dysentery is of particular interest, because they are responsible for the majority of diarrhea-associated deaths. In a study from India, the effects of zinc depended on serum zinc, age, and sex, in that zinc supplementation reduced the incidence of persistent diarrhea in children above 11 months by 49%, and in children with low serum zinc by 78%. Similarly, the incidence of dysentery was reduced by 38%, but only in boys.

As reviewed by Black, a number of other studies have shown similar effects on acute diarrhea, while the results of studies on persistent diarrhea are less consistent. A pooled analysis of data from seven randomized, controlled trials showed that zinc supplementation reduced the incidence of diarrhea by 18%, and the number of days with diarrhea by 25%.

**Case Management**

Zinc supplementation has beneficial effects on recovery in children suffering from diarrhea. A community-based study among more than 900 young Indian children with acute diarrhea demonstrated that a daily multivitamin supplement with 10 mg zinc, compared to multivitamins alone, reduced the risk of continued diarrhea by 23%. If initiated within 3 days of onset, there was a 39% reduction in the proportion of diarrhea lasting more than 7 days. Further, the number of watery stools and the number of days with watery stools were reduced by 39 and 21%, respectively. In a hospital-based study among young, undernourished children admitted with diarrhea, zinc supplements reduced the stool output by 36%, although there was no significant effect on duration. In a study among young, undernourished children admitted with diarrhea, 20 mg zinc per day reduced the stool output by 28% and the duration of diarrhea by 14%, especially in the more stunted children and the children with low serum zinc. In a community-based study in Peru, 20 mg zinc per day to children with persistent diarrhea
reduced the duration of diarrhea by 28%.\textsuperscript{75} Recently, pooled analyses were done on data from studies on the effect of zinc supplementation to children with acute and persistent diarrhea.\textsuperscript{76} Zinc supplementation reduced the risk of continuing diarrhea by 15% among those with acute diarrhea, and by 24% among those with persistent diarrhea. Furthermore, the risk of treatment failure or death was reduced by 42%.

Since diarrheal diseases lead to a 3-fold increase in intestinal zinc loss,\textsuperscript{55} a vicious circle between zinc deficiency and diarrhea may develop, which may explain some of the 3,000,000 diarrhea-related deaths among infants and children each year. Although food-based measures to ensure adequate zinc intake and bioavailability throughout life should be promoted, the addition of zinc to oral rehydration fluid has been suggested to break the vicious circle.\textsuperscript{77}

**Respiratory Tract Infections**

Because the common cold has been estimated to cost the United States more than $3.5 billion per year, there is considerable interest in measures to reduce its frequency or severity.\textsuperscript{15} \textit{In vitro} studies have suggested that zinc inhibits rhinovirus replication\textsuperscript{78} or binding of rhinovirus to the cells.\textsuperscript{80} Further, it is likely that immune suppression or cell membrane instability due to zinc deficiency could make the host susceptible to rhinovirus and its effects. Nevertheless, a recent review concluded that evidence for an effect of zinc lozenges on the common cold is still lacking,\textsuperscript{15} but this does not preclude an effect on the common cold in populations with a low intake of zinc.

Among populations of developing countries, the contribution of respiratory tract infections to the global burden of disease is substantial,\textsuperscript{81} and a considerable proportion of this seems to be attributable to zinc deficiency. In growth-retarded young Vietnamese children, a 10-mg daily zinc supplement reduced the relative risk of, predominantly lower, respiratory tract infections by 59%.\textsuperscript{67} In young Indian children, zinc supplementation reduced the incidence of lower respiratory infections by 45%.\textsuperscript{82} When data from these and other randomized, controlled trials were pooled and reanalyzed, zinc supplementation was found to reduce the incidence of pneumonia by as much as 41%.\textsuperscript{71} Nevertheless, an increase in the incidence of acute lower respiratory tract infections has recently been reported from a study among young Bangladeshi children\textsuperscript{83} where the daily dose was 20 mg.

**Other Infections**

In rural Zimbabwe, schoolchildren receiving zinc supplements on school-days for 12 months after treatment of schistosomiasis had significantly
lower intensities of *Schistosoma mansoni* reinfections. Zinc deficiency is also involved in the pathogenesis of malaria infection. In the study among young children in Gambia, those receiving 70 mg zinc twice weekly had approximately 30% fewer clinic visits for microscopically verified malaria compared to those allocated a placebo, but the difference was not significant. In Papua New Guinea, preschool children were randomized to receive either 10 mg zinc or a placebo for 6 days a week over a period of 10 months. Zinc supplementation reduced not only health facility attendance by 29%, but also reduced *Plasmodium falciparum*-associated fevers by 40%. A study was recently conducted among 6- to 12-month-old, breast-fed, Ethiopian infants, where stunted as well as nonstunted infants were randomized to receive a supplement of 10 mg zinc or a placebo for 6 days a week for 6 months. Zinc supplementation increased length and weight in both stunted and nonstunted children, and also reduced the frequency of anorexia, cough, diarrhea, vomiting, and fever among the stunted children.

A zinc supplementation study was conducted among young, severely malnourished Bangladeshi children admitted for nutritional rehabilitation. The children were randomized to either 1.5 or 6.0 mg zinc per kg daily. No difference between the groups in growth or body composition was seen, but mortality was significantly higher in those receiving the high dose of zinc. Similarly, parental administration of zinc during an acute phase response was found to increase the febrile response. These results suggest the presence of a zinc–infection paradox, and that the reactive hypo-zincemia may be beneficial to the host.

Despite the convincingly demonstrated effects of zinc on immune functions and diarrheal and respiratory tract infections, and the preliminary data on mortality, the impact of zinc supplementation on other important infections, such as geohelminth, other parasite infections, and tuberculosis, remains to be established. Also, the role of zinc in HIV infection is insufficiently elucidated.

**HIV INFECTION**

Early in the pandemic, prior to the identification of HIV, zinc deficiency due to excessive losses of zinc via semen was suggested as a cause of AIDS in sexually very active homosexual men. Similarly, zinc deficiency was suggested as an important facilitating factor among malnourished Haitians, based on the observation that lower respiratory tract infections with the opportunistic pathogen *Pneumocystis carinii* occur in malnutrition. After the discovery of HIV as the cause of AIDS, and subsequent efforts to develop vaccines and drugs directed against the virus, little attention was devoted to clarifying the role of zinc in HIV transmission and progression.
This is unfortunate, because zinc interventions could prove to be simple and cost-effective complementary strategies to control the epidemic and its consequences.

**Zinc Status**

Hypozincemia in patients with HIV infection compared to controls has been reported in several studies, but not in others. However, given the difficulties in assessing zinc status, low concentrations of zinc in serum, cells, or other tissues, as well as correlations between zinc levels and clinical stage of HIV disease or CD4 count, provide neither evidence of low zinc status nor an effect of HIV infection on zinc status.

There is no doubt that zinc deficiency is common in HIV infection. For example, a case of a young child with AIDS manifesting as *acrodermatitis enteropathica* has been reported. The child had an oro-facial rash, diarrhea, and extreme hypozincemia, and responded to oral zinc with improvement in diarrhea and resolution of the rash. While growth is a major determinant of zinc requirements in children, the major nondietary risk factors of zinc deficiency in adults are excessive losses, primarily through the gastrointestinal tract, sweat, and semen. The latter is more than a curiosum, because the zinc content of semen is extremely high, from an average of 0.6 mg up to several mgs per ejaculation. This is considerable, as 3 mg of absorbed zinc is required to replace the normal losses. Obviously, if the loss through semen alone is increased to 2 to 5 mg per day in the most sexually active men, then the requirement will not be met through a normal diet. Because the high losses of zinc through semen occur in men likely to be most intensively exposed to HIV, then any effect of zinc deficiency on susceptibility or viral shedding could have played a pivotal role in the pandemic. Diarrhea increases zinc losses considerably, and is considered an important risk factor of zinc deficiency in individuals with a marginal intake. Diarrhea is common in HIV infection. In children in Zaire, the incidence of diarrhea was 2 to 4 times higher, and the diarrhea-specific mortality 11 times higher, in HIV-infected children when compared with HIV-uninfected children of HIV-infected mothers. As many as 30 to 50% of adult HIV-infected patients in developed countries, and nearly 90% in developing countries, develop diarrhea. If the intake of zinc does not compensate for the increased losses, then the zinc status may be exacerbated by anorexia and reduced food intake.

**Zinc and Infections in HIV Patients**

Analogous to the findings from animal studies and human studies among individuals without HIV infection, low zinc status and intake are likely to
increase morbidity to opportunistic and common infections among HIV-infected individuals. For example, the higher incidence of diarrhea among HIV patients in developing countries compared to developed countries\textsuperscript{111} may not only be attributable to the higher exposure to pathogens due to the lack of sanitary facilities and safe water, but also to a cereal-based diet with a lower amount of absorbable zinc.

The current understanding of the mechanisms behind the effect of zinc deficiency on diarrhea is based on experimental animal studies, suggesting that zinc deficiency may potentiate or even cause diarrhea via effects on intestinal gene expression.\textsuperscript{59-61} Interleukin (IL)-1\textsubscript{α} is known to induce an acute phase response and, as such, to increase expression of inducible nitric oxide synthase (iNOS), producing nitric oxide, a free radical gas that damages the intestine and may play a role in diarrhea.\textsuperscript{60} To assess the role of zinc in the pathogenesis of diarrhea, rats were kept on zinc-deficient diets for 4 weeks. Zinc deficiency was then documented by the presence of reduced food intake, growth retardation, and development of dermatitis and alopecia in the rats on zinc-deficient diets, compared to the pair-fed and \textit{ad libitum}-fed controls. The zinc-deficient rats expressed iNOS in the intestines\textsuperscript{60} and had a higher permeability and an increased number of apoptosis-positive cells in the intestinal mucosa.\textsuperscript{56} These findings suggest that zinc deficiency may lead to noninfectious diarrhea. Six hours after a subcutaneous injection of IL-1\textsubscript{α}, the zinc-deficient rats had a considerable increase in intestinal iNOS expression, and 66% of the zinc-deficient rats developed diarrhea, in contrast to 23% of pair-fed and none of the \textit{ad libitum}-fed controls. Zinc deficiency also has been shown to increase the expression of uroguanylin, a peptide hormone that activates the \textit{Escherichia coli} heat-stable enterotoxin receptor in the intestine, leading to increased transport of chloride and water into the gastrointestinal tract.\textsuperscript{59,61}

Few studies have been conducted to assess the effect of zinc supplementation on opportunistic infections in HIV infection. Among Italian AZT-treated patients in stages III and IV, 45 mg of elementary zinc daily for 30 days was reported to reduce the incidence of infections with \textit{Pneumocystis carinii} and \textit{Candida eosophagea}, but not cytomegalovirus and toxoplasma, in the subsequent 24 months.\textsuperscript{112} However, the study was apparently neither randomized nor placebo-controlled, and in the absence of any zinc stores it seems unlikely that zinc supplements have long-term effects. In Zambia, the effect of zinc, selenium, and vitamins A, C, and E was assessed in a randomized, placebo-controlled trial among 106 HIV patients with persistent diarrhea.\textsuperscript{113} The micronutrient supplement containing 200 mg of zinc or placebo was given daily for 2 weeks, and the patients were observed for 12 weeks. There were no effects of the supplement on diarrhea morbidity and mortality. However, the patients had advanced HIV disease with a 6-month median duration of diarrhea. Although low serum vitamin A was
a predictor of death, no increase was seen after 2 weeks of supplementation, suggesting severe malabsorption.

Randomized, controlled trials, preferably using a factorial design, are needed to assess the effects of zinc in diarrheal, respiratory tract, and other infections among HIV-infected individuals. Studies on the effect of zinc on mortality among patients with pulmonary tuberculosis, of whom most are co-infected with HIV, are in progress.\(^{114}\)

### Zinc and HIV Progression

Given the essentiality of zinc for host defense, as well as its antioxidative properties and its role in preventing apoptosis of immune cells, it is reasonable to assume that an improved zinc intake or status would beneficially affect not only the susceptibility to opportunistic and common infections in HIV-infected individuals, but also the course of HIV infection per se.

Patients with AIDS not only had low serum zinc, but also low or undetectable plasma concentrations of zinc-bound active thymulin,\(^ {115}\) whereas the total zinc-saturable (active and inactive) thymulin levels were normal.\(^ {97}\) This finding indicates not only that zinc status is low in HIV infection, but also that improved zinc status may partially restore the immune derangement in AIDS.\(^ {97}\) The importance of zinc in avoiding apoptosis of CD4 and other T-lymphocytes, by inhibiting the endogenous endonuclease responsible for the TNF-induced apoptosis,\(^ {10}\) is also in accord with a presumed advantageous effect of zinc in HIV infection. Similarly, zinc has antioxidant properties,\(^ {11}\) and is necessary for the antioxidant enzyme Cu,Zn superoxide dismutase that has been demonstrated to reduce viral replication.\(^ {116}\)

Zinc also may play a more direct role in viral replication as an essential component of structural and catalytic proteins of HIV. For example, zinc has been found to concentrate with HIV particles.\(^ {99}\) Zinc is bound to the nucleocapsid protein NCp7 and forms zinc-fingers, which are essential to viral structure, proviral DNA synthesis, and production of infectious viruses,\(^ {117}\) and is required for the activity of reverse transcriptase. While these effects provide biological plausibility to the adverse effects of zinc in HIV replication, zinc also may bind to the catalytic site of HIV protease, thereby potentially inhibiting HIV replication.\(^ {118}\) Nonetheless, the relevance of these observations to human nutrition is unknown.

Zinc status also may modify the effect of antiretroviral treatment. For example, the enzyme thymidine kinase, required for conversion of AZT to its active form, is zinc dependent.\(^ {119}\) Accordingly, in a study of 15 HIV-infected individuals given AZT, those with normal serum zinc had significant increases in the response of peripheral blood lymphocytes to mitogens, whereas this was not seen in those with low serum zinc.\(^ {119}\)
A few prospective studies have attempted to assess the relationship between zinc status or intake and the progression of HIV infection, using clinical outcome as an effect parameter. A nested case control study was conducted in the United States among homosexual men included in the Multicenter AIDS Cohort Study in 1984. Zinc and copper levels in serum, toenail, and diet were assessed in 54 HIV-infected men who later developed AIDS, and in 54 HIV-infected men who remained AIDS-free. The participants had all been free of diarrhea the previous 6 months, and the mean follow-up time was 2.5 years. Neither dietary intake of zinc and copper nor zinc and copper levels in toenails were significantly different between progressors and nonprogressors. However, serum zinc levels were significantly lower, and serum copper significantly higher, in the progressors. When the confounding effect of CD4 count at baseline was controlled for in logistic regression analysis, low serum zinc and high serum copper remained significantly associated with progression to AIDS. Since neither zinc nor copper levels in toenails was associated with progression, the authors rightly concluded that low serum zinc and high copper are markers of progression rather than causes of progression.

Based on data from the same cohort, the relationship between dietary and supplementary intakes of various micronutrients, and progression to AIDS and mortality over a 7- to 8-year follow-up period, were studied among 281 HIV-infected participants. The study controlled for age, symptoms, CD4 counts, energy intake, and treatment. Surprisingly, in addition to beneficial effects of vitamins A and B, a total intake of zinc only 30% above the RDA was associated with twice as high a risk of progression to AIDS, and twice as high a risk of mortality. However, the data should be interpreted with caution because the presence of infection may have influenced the reporting of dietary intake at baseline, and the dietary intake may have changed as the disease progressed. Nevertheless, the finding of a negative effect of zinc on HIV progression has been worrisome, and may have led to hesitancy to clarify the role of zinc through randomized, controlled trials among populations with low intake of absorbable zinc. This is unfortunate, since the finding, if causal, may merely reflect the adverse effects of a very high intake of absorbable zinc. In fact, the HIV-infected study participants had a mean zinc intake of 22.5 mg per day, well above the RDA, probably reflecting that some had large supplemental intakes that may have been immunosuppressive. Importantly, no adverse effect of zinc was found in a similar cohort study among 296 HIV-infected homosexual men (San Francisco Men’s Health Study) followed over 6 years. In fact, daily multivitamin use was associated with significantly reduced progression to AIDS.
CONCLUSION

Considering the importance of zinc for immune functions, and for resistance to diarrhea and lower respiratory tract infections, it is conceivable that improved zinc intake could be beneficial to individuals with HIV infection. However, there are few data to confirm this. In vitro studies provide biological plausibility to both beneficial and harmful effects of zinc on HIV infection. Due to the lack of a valid measure of zinc status, observational studies are of little use in assessing the relationship between zinc status and an HIV outcome. However, increased progression of HIV among those with a high zinc intake has been reported in one but not in other studies, yet it has not been studied in populations with low zinc dietary intake or bioavailability. Randomized, controlled trials are needed to assess the effect of zinc to define its role in HIV replication, progression, and transmission, as well as in morbidity from common and opportunistic infections.

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SELENIUM AND HIV INFECTION

Henrik Friis, Pernille Kæstel, Astrid N. K. Iversen, and Susanne Bügel

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HISTORICAL BACKGROUND

The trace mineral selenium was discovered in 1817 by the Swedish chemist Berzelius. Since the 1930s, selenium has been known as a toxin causing blind staggers and alkaline disease (subacute and chronic selenosis, respectively) in livestock grazing in selenium-rich soils. Later, impairment of growth and reproduction, and white-muscle disease due to selenium deficiency, were recognized in animals. In the 1950s, a factor in certain yeasts was shown to prevent liver necrosis in vitamin E-deficient rats. It was called “Factor 3,” and was later shown to be a selenium compound. The role of selenium in the enzyme glutathione peroxidase was shown in 1973. Only in 1979, when selenium supplementation was shown to prevent Keshan disease in China, was the essentiality of selenium for human nutrition demonstrated. Recommended Dietary Allowances for selenium were defined by the Food and Nutrition Board of the U.S. National Research Council in the 1989 revision. In recent years it has become increasingly clear that selenium is an essential nutrient of fundamental importance to human biology and health.

METABOLISM AND BIOLOGICAL FUNCTIONS

Selenium is an element with similarities to sulfur, and it naturally forms both inorganic and organic compounds. The inorganic forms comprise selenide (Se\(^2^–\)), selenite (SeO\(_3^2^–\)), and selenate (SeO\(_4^{2–}\)), similar to the compounds of sulfur. Plants take up inorganic selenium and incorporate it into the amino acid selenomethionine, the selenium analog to the sulfur amino acid methionine. In animals (Figure 9.1), the selenomethionine derived from the diet can be directly incorporated in various proteins in place of methionine. The selenium in the selenomethionine-containing proteins does not seem to have any biological function except to provide a pool of selenium. Alternatively, selenomethionine and other selenium compounds can be metabolized and enter a metabolically active pool of selenium in the selenide form (Se\(^2^–\)). The amino acid selenocysteine is synthesized in situ, and is incorporated via UGA codon into the biologically active selenoproteins, i.e., proteins that contain selenocysteine at the active site.

The growing number of selenoproteins identified includes the glutathione peroxidases (GSHPx), the iodothyronine deiodinases, selenophosphate synthetase, thioredoxin reductase, and the selenoproteins P and W (Table 9.1). The redox potential of selenocysteine is responsible for the activity of selenoproteins, and although they possess a variety of functions, selenocysteine’s antioxidant properties have been the primary focus of research.
The enzyme family of glutathione peroxidases is probably the most important group of selenoproteins; they act as catalysts for the reduction of peroxides to water and alcohols. The biological importance of these antioxidant reactions is to prevent generation of free radicals and, hence, oxidative damage to proteins, lipids, lipoproteins, and nucleic acid. In these reactions, reduced glutathione (GSH) provides the reducing equivalents as Selenocysteine, the principle form of selenium in animal foods, after intestinal absorption (50–80%) enters a central metabolic pool of selenium where it is metabolized and eventually reduced to the intermediate selenide (Se\(^{2−}\)). Through selenocysteine it is specifically incorporated into the biologically active selenoproteins. Excess selenium is excreted via the kidneys through trimethyl-selenonium ((CH\(_3\))\(_3\)Se\(^+\)), or exhaled through the volatile dimethyl-selenide ((CH\(_3\))\(_2\)Se). Selenomethionine, primarily derived from plant foods, is metabolized analogously to selenocysteine, but also can be directly incorporated into selenium-containing proteins, which serve as unregulated storage forms of selenium.

Figure 9.1 Human metabolism of the main dietary forms of selenium. Selenocysteine, the principle form of selenium in animal foods, after intestinal absorption (50–80%) enters a central metabolic pool of selenium where it is metabolized and eventually reduced to the intermediate selenide (Se\(^{2−}\)). Through selenocysteine it is specifically incorporated into the biologically active selenoproteins. Excess selenium is excreted via the kidneys through trimethyl-selenonium ((CH\(_3\))\(_3\)Se\(^+\)), or exhaled through the volatile dimethyl-selenide ((CH\(_3\))\(_2\)Se). Selenomethionine, primarily derived from plant foods, is metabolized analogously to selenocysteine, but also can be directly incorporated into selenium-containing proteins, which serve as unregulated storage forms of selenium.
shown in Figure 9.2. Moreover, GSH provides the reducing power for the maintenance of other antioxidants, e.g., ascorbic acid, vitamin E, and β-carotene.13

In iodine deficiency, coexisting selenium deficiency may protect against the neurological form of cretinism (neurological impairment, mental retardation, but normal growth and thyroid function) and change the clinical manifestation toward the myxoedematous form (overt hypothyroidism and stunting).14,15

<table>
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<tr>
<th>Table 9.1 Functions of the Major Known Selenoproteins</th>
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<tr>
<td>Selenoproteins</td>
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<td>Glutathione peroxidases</td>
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<tr>
<td>- Cellular</td>
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<td>- Extracellular</td>
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<tr>
<td>- Phospholipid hydroperoxide</td>
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<td>Thyroid hormone deiodinases</td>
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<td>Selenophosphate synthetase</td>
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<td>Thioredoxin reductase</td>
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<td>Selenoprotein P</td>
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Figure 9.2 Glutathione peroxidase catalyzes the reduction of hydrogen peroxide (A) to water, and of peroxides in general (B) to their corresponding alcohol and water through oxidation of reduced glutathione, GSH. Subsequently, GSH is regenerated from the oxidized form, GSSG, with NADPH as the reducing agent (C), a reaction that is catalyzed by the enzyme glutathione reductase.
ASSESSMENT OF STATUS

Selenium status may be assessed using static or functional indices. The concentration of selenium in plasma, serum, or whole blood is the most widely used static measure of selenium status. Plasma selenium levels in the range of 0.76 to 1.27 μmol/L are considered normal. Lower levels are found in pregnant and lactating women, as well as in infants, but it is not clear if this reflects different intakes relative to the requirements or the physiological states. The erythrocyte selenium correlates with plasma selenium at a level almost twice as high. Plasma selenium reflects short-term selenium status as it responds to changes in intake within a few weeks, whereas erythrocyte selenium reflects long-term status due to the 120-day life span of erythrocytes. Plasma selenium may not be a valid measure of status during the acute phase response to infections, injuries, etc., because it may decline in response to acute infections. Hair and nail selenium have been used as measures of selenium status, as they reflect long-term selenium intake. However, some major drawbacks limit their application as indicators of selenium status. First, hair selenium is vulnerable to contamination from certain selenium containing shampoos. Second, deposit and extraction of selenium from hair and nails depend on the dietary form of selenium. Changes of the selenium content in urine, its major excretory route, are suggested as a useful index of short-term changes in status at moderate levels of intake.

The most common functional indices of selenium status include activity of GSHPx in plasma, erythrocytes, or whole blood. Based on data from a large number of studies, Diplock concluded that blood or plasma selenium correlated well with erythrocyte GSHPx, but also that the correlation became progressively weaker with higher selenium concentrations as the enzyme became saturated. It is generally accepted that plasma GSHPx only increases in selenium-deficient subjects with daily intakes up to 40 μg, where it reaches a plateau. Erythrocyte GSHPx increases with intakes up to 60 to 80 μg/d, while platelet GSHPx only plateaus with intakes around 100 to 110 μg/d.

FOOD SOURCES AND DIETARY REQUIREMENTS

The selenium intake and status of human populations are influenced by the content and chemical form of selenium in the soil, i.e., at the beginning of the food chain. The solubility of selenium compounds is a major determinant of plant uptake of selenium. In general, elemental selenium and selenides are rather stable and poorly soluble, selenites are moderately soluble, and selenates are soluble. Also, the pH and texture of the soil influence the availability of selenium to plants. Under acidic conditions, the
selenite form is poorly soluble and binds tightly to clay particles, whereas the selenate form is stable and soluble under acidic as well as alkaline conditions.\textsuperscript{8} Further, the oxidation of selenites to soluble selenates is favored by alkaline conditions. Overall, the highest uptake of soil selenium is achieved from selenites or selenates in neutral or alkaline sandy soils.\textsuperscript{19}

Apparently, plants do not require selenium for their own metabolism, but take it up in place of sulfur proportional to the amount available in the soil.\textsuperscript{8} The selenium content of a plant grown in selenium-rich soils may, therefore, be many times higher than that of the same plant grown in selenium-poor soils. In soils with very high contents of selenium, the seleniferous soils of China, Yang found more than 1000-fold higher selenium content in plants when compared to similar plants grown in selenium-poor soils.\textsuperscript{20} Animals, in contrast to plants, need selenium for specific purposes, wherefore they are able to reduce excretion if the intake is low, and increase excretion if the intake is high. Due to this homoeostatic control, the selenium content of animal foods is less dependent on soil selenium. Rich dietary sources of selenium include fish, seafoods, organ and muscle meat, and cereals. However, due to the high intake, cereals represent the most important source of dietary selenium in most populations.\textsuperscript{1}

The U.S. RDA for selenium, first published in 1989,\textsuperscript{6} was based on the finding that 41 \( \mu \)g/d was required to saturate plasma GSHPx in Chinese men from areas affected by Keshan disease.\textsuperscript{21} Upon correction for differences in body weight and interindividual variation, the U.S. RDA was set at 70 \( \mu \)g/d for men, and 55 \( \mu \)g/d for women.\textsuperscript{6} Based on recent data from New Zealand,\textsuperscript{22} the RDA for selenium has been changed to 55 \( \mu \)g/d for both men and women.\textsuperscript{23} However, daily intakes of selenium as low as 19 \( \mu \)g in men and 13 \( \mu \)g in women seem to prevent Keshan disease.\textsuperscript{24} Nevertheless, it is still unknown what intakes are required for optimal health, including prevention of chronic degenerative or other diseases\textsuperscript{25} such as infections.

In countries with soils poor in selenium, there is a high risk of having low dietary intakes. China, Zaire, New Zealand, parts of Canada and Australia, and the Scandinavian and other European countries cover areas where the amount of selenium available in the soil is low.\textsuperscript{1,7,17,26} Finland used to have poor soil selenium, but since 1984 selenium fertilizers have been used for grain as well as fodder production.\textsuperscript{27} With this altered agricultural practice, the selenium intake of the Finnish population was increased threefold, and serum selenium was increased by 70%.

Soils with very high selenium content are found in the Dakotas, Wyoming, and a few other states in the United States, as well as in areas of Ireland, China, Venezuela, Colombia, and Israel.\textsuperscript{1} Clearly, excessive intake of selenium supplements should be avoided as toxicity may occur.\textsuperscript{12} Among humans, accidental intake of 27 mg/d for up to 2½ months in 13 individuals caused nausea in all, and abdominal pain and diarrhea in 7 of the 13.\textsuperscript{28}
Other symptoms were hair and nail changes, peripheral neuropathy, fatigue, and irritability. Among the population of Hubei Province in China, an endemic selenium intoxication occurred in the 1960s where daily intakes around 5 mg caused the loss of hair and nails. Nail abnormalities were reported among Chinese adults with intakes only above 850 μg/d. However, no evidence of toxicity was found among subjects in seleniferous areas in South Dakota and Wyoming with intakes up to 724 μg/d. With the newly published Dietary Reference Intake, a daily intake of 400 μg was suggested as the safe upper level.

**HOST DEFENSE AND INFECTIONS**

Several laboratory animal studies have shown that selenium is essential to both cell-mediated immunity and B-cell function. Selenium supplementation trials have documented marked immunostimulant effects of selenium on clonal expansion of activated T-cells, cytotoxic T-cells cytotoxicity, and natural killer cell activity. This stimulatory effect seems to be closely linked to the ability of selenium to upregulate the interleukin-2 receptor on activated T-lymphocytes and natural killer cells.

Although little is known about the role of selenium in specific infections, work on Keshan disease by Chinese researchers and recent studies by Beck and Levander have opened new avenues in the field of nutrition-infection interactions.

**Keshan Disease**

Keshan disease was first discovered in 1935 when an epidemic outbreak of a myocarditis among young children and women of reproductive age occurred in Keshan County, Heilongjiang Province in northeastern China. The disease, characterized by a multifocal myocardial necrosis and fibrosis leading to congestive cardiomyopathy, became a serious public health problem.

The fascinating Chinese research into the complex etiology of Keshan disease was first published in English in 1979, and was later reviewed by Ge and Yang. Although Keshan disease had features of an infectious disease, it only occurred in a belt running diagonally northeast to southwest through China. It was notable that the selenium content of soil and grain, as well as the selenium content of blood and hair of the inhabitants in the affected areas, was considerably lower than in the unaffected areas. Several selenium supplementation trials were conducted that convincingly demonstrated that selenium deficiency was a necessary co-factor of Keshan disease. For example, a randomized, placebo-controlled selenium trial was conducted from 1974 to 1977 among more than 12,000 children between 1
and 9 years of age in 169 production teams. The experimental intervention was the administration, by a barefoot doctor, of 0.5 to 2.0 mg (depending on age) of selenium as sodium selenite once a week. During 1974 and 1975, the number of new cases of Keshan disease was 17, with one death among the children given sodium selenite, while it was 106 with 53 deaths among the children given a placebo. Due to the convincing effect, all children were given selenium in 1976 and 1977, and no new cases occurred in 1977. However, although selenium supplementation proved adequate to prevent Keshan disease, the prior presence of epidemic outbreaks and the seasonal variation in incidence suggested the presence of an infectious, probably viral, co-factor. Later culture and genetic sequencing based on samples from confirmed cases of Keshan disease have suggested that coxsackievirus B or a coxsackie B-like virus is involved.

The Coxsackievirus-Mouse Model

Beck and Levander ran a series of elegant experiments in laboratory mice fed normal or selenium-deficient diets and subsequently exposed to coxsackievirus (CV) B3, either as the virulent B3/20 or the benign B3/0 strain, demonstrating that in a selenium-deficient host, an otherwise harmless virus can acquire virulent traits. That work has been reviewed by Beck and Levander.

The virulent strain B3/20, known to cause myocarditis in normally fed mice, was found to result in higher viral titers and to cause more heart damage in selenium-deficient mice. As expected, the benign strain B3/0 did not cause any heart damage in selenium-adequate mice. However, when inoculated into mice on selenium-deficient diets, it was found to cause heart damage with similarities to that seen in humans with Keshan disease. Furthermore, when the virus was passed on to selenium-adequate mice, it retained the virulence it had acquired in the selenium-deficient mice. Genetic sequencing of the virus showed that it had mutated at six of the seven nucleotide positions in which the benign B3/0 differed from the virulent B3/20. This is the first example of the ability of the host micronutrient status to alter pathogen genes.

Deficiency of the antioxidant vitamin E in combination with either fish oils or iron, both increasing the requirement for antioxidants, had similar effects as selenium deficiency, which could be negated by administration of the synthetic antioxidant N,N′-diphenyl-p-phenylenediamine. Beck and Levander, therefore, concluded that the effects were mediated by increased cellular oxidative stress. Interestingly, it appears that supplementation with iron, known to have prooxidant properties, had effects similar to deficiencies of selenium and vitamin E. Thus, the nutritional interventions apparently led to oxidative stress, which changed the genome of
the virus. Analogously, an experiment involving GSHPx1-knock-out mice, i.e., mice unable to make glutathione peroxidase, demonstrated that the enzyme was essential in the avoidance of oxidative damage to the RNA-viral genome that resulted in the myocarditis-inducing mutations.\textsuperscript{48} It is currently unknown whether the oxidative stress damaged the viral RNA, thereby leading to mutations, or led to a selection of viral quasispecies similar to the virulent CVB3/20 strain.\textsuperscript{40}

Beck and Levander went on with intriguing speculations about whether selenium deficiency could have played a role in the precipitation of previous influenza pandemics in China, and the crossing over of HIV from monkey to man in selenium-deficient areas in Africa.\textsuperscript{40,44} Understanding the role of selenium in these and other RNA virus infections, e.g., polio and hepatitis B and C, may be of public health importance.

Other Infections

In Qidong County, China, the prevalence of hepatitis B virus (HBV) carrier state and the incidence of primary liver cancer (probably mainly caused by HBV) were high, especially in areas with low soil selenium. A study was therefore conducted, where table salt fortified with selenium was administered to the 20,000 inhabitants of one township, while the inhabitants of the four surrounding townships received plain table salt. Prior to the study, the prevalence of HBV carrier state and the mean annual incidence of primary liver cancer were similar in the five townships, but during the 8 years of intervention both were reduced among those supplied with selenium-fortified table salt to almost 50% of the control populations.\textsuperscript{49} Similar effects on the incidence of infectious hepatitis from the same study have previously been reported.\textsuperscript{50} A controlled trial from China showed that infants hospitalized with respiratory syncytial virus (RSV) infections recovered faster if they received selenium the day after admission.\textsuperscript{51}

**HIV INFECTION**

Selenium Status in HIV Infection

Low selenium status in HIV infection is to be expected, because HIV, like other generalized infections, leads to reduced intake and absorption and increased utilization and excretion of nutrients. In viral infections, the production of reactive oxygen species (ROS) is increased (directly or through production of prooxidant cytokines) considerably above what is part of normal aerobic metabolism.\textsuperscript{52} This increased production of ROS is followed by an increased consumption of exogenous antioxidants such as vitamins
C and E, and sequestration of selenium and other minerals essential to antioxidant enzymes. It has been shown that HIV has the potential to encode for selenoproteins, and it has been postulated that the synthesis of viral selenoproteins sequesters the host’s selenium and further contributes to selenium depletion of the host.53 Accordingly, several studies have reported low selenium status in patients with AIDS,54-59 and even in people with asymptomatic HIV infection.60,61 Interestingly, a congestive cardiomyopathy, similar to that known as Keshan disease, is not infrequently reported in patients with HIV infection who often have low selenium concentrations in cardiac tissue.62,63 In fact, the annual incidence rate was found to be 15.9 per 1000 asymptomatic HIV-infected patients.64 Although the etiology is complex,65 HIV and the cardiotropic virus64 may interact with selenium deficiency to cause cardiomyopathy. A case of cardiomyopathy and very low serum selenium in a child with AIDS has been reported in which the cardiac function improved following selenium supplementation.66

**Selenium and HIV Progression**

*Laboratory Studies*

If the intake of the antioxidant vitamins and minerals is not increased among HIV-infected individuals, then antioxidant deficiencies and oxidative stress will ensue, as suggested by the increased levels of malondialdehyde and other markers of oxidative damage.52,67 The oxidative stress may impair immune functions and induce apoptosis (programmed cell death) of CD4 and other immune cells, as well as lead to increased viral replication. The mechanism whereby oxidative stress increases viral replication involves the nuclear transcription factor NF-κB. In the cytosol, NF-κB is complexed to an inhibitory factor called I-κB. Oxidative stimuli lead to activation of NF-κB by dissociating it from I-κB. Upon release, the NF-κB transcription factor then moves to the nucleus, where it activates the HIV gene expression.68 Conversely, the major cellular antioxidant glutathione (GSH), and even N-acetyl-L-cysteine (NAC), known to increase the intracellular level of GSH, may block the activation of NF-κB.

Similarly, selenium supplementation of latently HIV-infected T-lymphocytes in vitro not only increased the GSHPx activity, but also prevented hydrogen peroxide (H$_2$O$_2$) from activating NF-κB and thus increasing viral replication by transactivation of the HIV-1 long terminal repeat region.69 Another in vitro study found no effect of selenium on the spontaneous HIV replication in acutely infected cells, but found that selenium reduced the tumor necrosis factor-α (TNF-α) induced HIV replication in latently infected
Thus, there is evidence that HIV-infected individuals often have low selenium status, and that low selenium status impairs immune functions. Whether increased intake of selenium could serve to maintain a state of viral latency, and thus reduce HIV progression and maybe transmission, remains to be elucidated.

Theoretical evidence from Taylor’s group shows that the potential to make viral selenoproteins (like GSHPx) is present in HIV-1 as well as in HIV-2, coxsackievirus B3, hepatitis B and C, and measles virus, and *in vitro* experiments demonstrate activity of an HIV-1 encoded glutathione peroxidase. Furthermore, it has been demonstrated that the pox virus, *molluscum contagiosum*, can incorporate host selenium into a viral selenoprotein, a homolog of GSHPx. Thus, viruses might reduce the ability of the host to mount an effective immune response by depleting the host’s selenium supply. Sufficient selenium host reservoirs may protect against viral disease progression by maintaining host immune competence and appropriate redox control. Taylor hypothesized that as long as selenium levels are adequate, cellular immunity will be high and the host cell less likely to die. Under those conditions, the best viral strategy is to replicate at low levels and establish a persistent infection. However, when selenium levels are low, increased oxidative stress and apoptosis activate the virus, which must replicate at higher rates to escape from a dying cell, leading to more pronounced pathogenic effects.

**Clinical Studies**

Only a few human selenium supplementation studies have been conducted among HIV-infected individuals, and most have used antioxidant status rather than viral load or clinical outcome as an effect parameter. A study was conducted among 45 French HIV-infected patients divided into three groups. Two of the groups were given daily supplements, containing 100 μg selenium as selenomethionine or 60 mg β-carotene, for 12 months. In the 14 patients allocated to daily selenium supplements, the erythrocyte GSHPx increased from 47 to 67 U/g Hb, while it declined from 63 to 36 in the 18 HIV-infected patients not receiving supplements. Allegedly, there was a decline in oxidative stress markers in the selenium group and an increase in the nonsupplemented group, while there was no effect of selenium on CD4 counts or the incidence of opportunistic infections. The effect of combined 600 mg N-acetylcysteine thrice a day and 500 μg selenium (as sodium selenite) once a day were evaluated among 25 patients with early stages of HIV infection (CDC-I and II). Serum selenium increased, but erythrocyte GSHPx did not. The CD4/CD8 ratio increased, but there was no change in the viral load.
Published studies using clinical endpoints, such as the development of AIDS or AIDS-related deaths, have all been observational. Data from a correlational study of states in the United States found an inverse correlation between selenium in the soil, as assessed by the content in the plant alfalfa, and AIDS mortality.78 Obviously, it is not possible to draw causal inferences from a study relating data for populations rather than individuals. Furthermore, the incidence of HIV is the major determinant of AIDS mortality, and although this may indeed be affected by selenium intake, the complex of cultural, socio-economic, and behavioral determinants of primary HIV infections should not be neglected.

A prospective study was conducted in which selenium status was assessed in 95 HIV-infected patients, who were followed up for 1 year.75 Low serum selenium was found to be associated with progression of HIV to AIDS or death, even when the CD4 count was controlled for in the analysis. Similarly, Baum and colleagues conducted three independent cohort studies of HIV-infected individuals in the United States in which they assessed the relationship between low levels of various nutrients at recruitment and progression of HIV-infection to AIDS or death, while controlling for CD4 counts. In a study among 125 HIV-infected drug users, 21 died of HIV-related causes during the 3.5-year follow-up period.79 Low serum levels of vitamin A, B12, zinc, and selenium were all associated with death, but in the final multivariate analysis, only low serum selenium (<85 μg/L) was a predictor, with a relative risk of more than 10. Similarly, a prospective cohort study was conducted among 24 HIV-infected symptomatic children, of whom 12 died from HIV-related causes during the 5-year follow-up.80 Children with low serum selenium had six times greater risk of mortality, and among those who died, low serum selenium was associated with death at a younger age. However, the only other micronutrient status parameters included were serum zinc and iron, both considered invalid as measures of status. Similar effects of low selenium on mortality were found in a cohort of homosexual men.81

As for other observational studies, the association found between low serum selenium and HIV progression could be spurious rather than reflecting a cause-and-effect relationship. For example, low selenium may reflect low intake or status of another nutrient not assessed for, and that had a true effect on HIV progression. Of particular concern is the decline in serum selenium during infections. Thus, the association between low selenium and HIV progression could be due to the confounding effect of an acute phase response. The problem of micronutrients (e.g., zinc) or micronutrient indicators (e.g., ferritin) that behave as acute phase reactants is well-known in observational studies of effects of zinc, vitamin A, and iron.82 Although the decline in serum selenium may reflect an influx of selenium into cells in need, thus being beneficial to the host,18 the validity of serum selenium and other measures of status during HIV and other infections
needs to be studied. Adjusting for the acute phase response using an acute phase protein such as $\alpha_1$-antichymotrypsin or $\alpha_1$-acid glycoprotein in the analyses may be useful. These problems notwithstanding, the promising findings from laboratory as well as epidemiological studies should justify the conduct of randomized, placebo-controlled, double-blind selenium intervention trials using viral load or mortality as the endpoint. Such studies are in progress.

CONCLUSION

Selenium has only recently been recognized as an essential micronutrient. Selenium deficiency has been shown to impair the antioxidant defense and immune system, and coxsackievirus-mouse studies have demonstrated that host selenium deficiency may lead to changes in the viral genome that are associated with severe disease outcome. Selenium status is often low in HIV infection, and low status has been shown to be a strong predictor of mortality in several independent cohort studies. Accordingly, improving selenium status among HIV-infected individuals may prove beneficial in terms of reducing the progression of disease. Clarifying the role of selenium in HIV progression and transmission, as well as viral evolution, should be a research priority.

REFERENCES


MICRONUTRIENTS IN THE CASE MANAGEMENT OF HIV INFECTION

Heloise Buys and Gregory Hussey

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INTRODUCTION

In industrialized countries, HIV disease has become a chronic disorder that has been modified through the prevention and treatment of invasive opportunistic infections, and by treatment with highly active antiretroviral
therapy. The situation is quite different in the developing world where HIV disease is a relentless, progressive disorder that culminates in the death of the affected individual and the disruption of the family unit. Disease progression varies and tends to be faster in children.

While viral load and lymphocyte count are important predictors of disease progression, other factors such as malnutrition, early exposure to pathogens, overcrowding, poor access to water and sanitation, and poverty contribute to morbidity and mortality. In addition, budgetary constraints in many of these resource-strapped countries have limited the development of specific medical interventions that may significantly impact on HIV and its complications. A major challenge in developing countries is to identify low-cost interventions to reduce the burden of HIV-related disease. This chapter discusses the importance and role of micronutrients in the case management of HIV disease.

In considering the role of micronutrients in the case management of the HIV-infected individual, it is essential to consider the overall nutritional status of that individual and the contribution of different micronutrients to HIV disease progression. The contribution of malnutrition to childhood mortality cannot be overemphasized. Malnutrition is coupled to over 50% of the world’s mortality of children under 5 years of age. The contribution of HIV disease to this group’s mortality is thought to be significant but not yet documented accurately. Malnutrition and wasting eventually become characteristic features in the progression of HIV disease in both adults and children. Synergistically, they contribute to immune dysfunction in affected individuals.

MICRONUTRIENT DEFICIENCIES IN HIV INFECTION

Prevalence

Even in the face of normal nutrition and asymptomatic HIV disease state, marginal and subclinical micronutrient deficiencies occur even in developed countries. Deficiencies become more pronounced in patients with advanced disease and in developing communities where diets are inadequate in meeting recommended daily allowances (RDA) of protein and energy, let alone micronutrients. While permanent stunting can result from chronic malnutrition in infancy and childhood; micronutrient deficiencies, too, can result in permanent deficits such as blindness with vitamin A deficiency, mental retardation with iodine deficiency, and cognitive deficits with iron deficiency. These specific micronutrient deficiencies constitute major public health problems worldwide. HIV-infected children from sub-Saharan Africa have documented multiple micronutrient deficiencies. Vitamin A deficiency was most notable in 80% of stable children
in one study. Even in children from developed countries who were generally well nourished, deficiencies of vitamin A, E, and lycopene have been documented. In adults, marginal micronutrient levels were noticeable in HIV-positive homosexual men as compared to matched noninfected controls. Other adult studies document multiple micronutrient deficiencies in 36% of subjects taking adequate diets. In a further study 87% of adults had at least one micronutrient deficiency. The prevalence of micronutrient deficiencies in adults from developing countries is not well documented; however, a study from Zambia documented vitamin A deficiency in 63% and vitamin E deficiency in 55% of ill, HIV-positive adults.

**Contributing Factors**

HIV disease is more prevalent in disadvantaged communities where poverty, adverse socio-economic circumstances, and malnutrition abound. The staple diets tend to be deficient in terms of providing adequate micronutrients as well as protein and energy requirements. Malnutrition per se has been associated with severity of micronutrient deficiencies such as vitamin A, and is a potent co-factor in the HIV disease process potentiating further deficiencies and disease progression.

Poor intake may be independently linked to herpetic and candidal oropharyngeal and esophageal infection. HIV-associated encephalopathy may cause inanition and upper gastrointestinal dysmotility. Nutrient absorption may be affected adversely by several related factors. The gut is a target organ for HIV replication leading to HIV enteropathy in the absence of infectious agents. Associated malnutrition and micronutrient deficiencies may induce and aggravate gut lining atrophy and lead to diarrheal disease, which may further be recurrent or prolonged in the presence or absence of other infectious agents. Immunity may thereby be further depressed, exposing the individual to increased susceptibility to infections that may be life threatening or even subclinical. For example, parasitic infestations and subtle mucosal lining breaches lead to further micronutrient losses. Intestinal parasites adversely affect host growth and nutrition and, in particular, micronutrient status. Vitamin A malabsorption has been observed with *Ascaris lumbricoides* and iron deficiency anemia with hookworm. Elimination of *Ascaris* infestation has been shown to improve vitamin A absorption in adults and children.

In situations where both mother and infant are affected by HIV infection, illness in one may impact negatively on the other. Infants whose mothers are not well may not meet their requirements in terms of nutrition and psycho-social stimulation. This is further aggravated at those times when the infant falls ill and places increased demands on the mother. Mothers from families where the father figure may have died or deserted may be forced
to seek employment, placing the infants in less than ideal childcare facilities. Infants of mothers who have died of AIDS are also vulnerable.28

**Impact of Other Infections**

Infections are common complications of the HIV disease burden, and they increase demand for micronutrients. For example, vitamin A deficiency has been documented during measles.29-31 Increased zinc losses are recorded in patients with diarrheal disease32 and, as with iron, zinc tends to be sequestered to the liver during episodes of infection.33 Deficiencies of both vitamin A and zinc are associated with poor gut lining integrity and malabsorption, which interfere with both macronutrient and micronutrient absorption. Anorexia and inanition are common accompaniments of both simple and more complicated childhood infections, leading to further reduction in intake.51 Untreated opportunistic infections often lead to wasting, even in those individuals whose nutrient intake is being supplemented with energy- and protein-dense nutrition supplements.35 During infective episodes, cytokine-mediated inflammatory pathways are activated and antioxidant micronutrients are consumed. The HIV disease process per se can lead to cytokine activation.36,37

**Impact on HIV Morbidity and Mortality**

HIV-infected mothers and children are prone to a more severe vitamin A deficiency and have a higher risk of dying as compared to uninfected mothers and children.38-40 The mechanism of this negative impact appears to be related to the essential role of vitamin A in maintaining epithelial and immune system integrity.41 Independent of general nutritional status, lower vitamin A levels have been associated with severe immune suppression and lower CD4 counts.42 Because vitamin A deficiency is associated with increased risk of diarrheal disease and other infections, by inference, this impacts negatively on nutritional status.

In Africa, maternal vitamin A deficiency has been associated with an increased rate of mother-to-child transmission of HIV.40 It is postulated that, due to mucosal breaching, transmission of HIV from mother to child occurs via viral shedding in the birth canal and breast milk,43 but data from the United States conflict with those from Africa.44

A number of micronutrient deficiencies have been associated with increased disease progression. Vitamin A, vitamin B\textsubscript{12}, and zinc deficiency have been associated with HIV disease progression in adults.45 Selenium deficiency has been linked to increased mortality in adult and pediatric studies,47 and increased viral replication and load in children with reduced glutathione levels.48
MICRONUTRIENT INTERVENTIONS

While micronutrient deficiencies occur in both adult and pediatric HIV-infected individuals, and are exacerbated by coexisting malnutrition, there is, however, a paucity of published scientific data on micronutrient interventions for HIV-positive persons (Table 10.1). In particular, there are few well-conducted, randomized, placebo-controlled clinical trials evaluating the efficacy of micronutrient interventions.

Single Micronutrient Interventions

Intervention trials in children and pregnant mothers have shown that vitamin A is beneficial to mothers and children with HIV disease. In children, high-dose vitamin A has been shown to reduce AIDS-related mortality\(^49\) and diarrheal disease morbidity,\(^50\) and to improve CD4 lymphocyte counts.\(^42\) There is also a suggestion that vitamin A supplementation in pregnant women may reduce mother-to-child transmission of HIV in preterm infants,\(^51\) although this has not been the case with term infants. Viral loads did not increase following influenza vaccination of HIV-infected children who were pretreated with vitamin A supplementation.\(^52\)

The data on zinc supplementation are incomplete and inconclusive for HIV-infected children. However, there is a suggestion that body weight and lymphocyte counts improve,\(^53\) and the incidences of certain opportunistic infections (\textit{Candida} and \textit{Pneumocystis carinii}) decrease with zinc supplementation in adults.\(^54,55\) The role of zinc supplementation in HIV-positive persons requires further study. This is vitally important since efficacy trials have shown that zinc supplementation can reduce the incidence of diarrheal disease and pneumonia in HIV-negative children from developing countries.\(^56\) Selenium deficiency is linked to increased mortality in HIV disease; supplementation improves antioxidant enzyme levels,\(^57\) and has benefited adults who were selenium deficient and had HIV-related dilated cardiomyopathy by improving left ventricular function.\(^58\) Intervention trials with the antioxidants \(\beta\)-carotene, vitamin E, and vitamin C suggest that they may have a role in improving immune function and reducing viral replication by reducing oxidative stress.\(^59-62\)

Multimicronutrient Interventions

Applying single micronutrient supplements to each HIV-infected patient would not be a sustainable strategy. Various study groups have looked at providing multivitamin and mineral supplements because micronutrient deficiencies tend to be multiple rather than single. Abrams and colleagues prospectively studied the development of AIDS in HIV-positive men in
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<td>Vitamin A</td>
<td>2 doses 200,000 IU on days 1 and 2 to 36 children</td>
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<td>100,000–200,000 IU on days 1 and 2, and months 4 and 8 to 58 infants</td>
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<td>50,000–200,000 IU at months 1, 3, 6, 9, 12, and 15 to 58 infants</td>
<td>Increased vitamin A levels, decreased diarrheal disease by 49%, and decreased hospital admissions by 77%</td>
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<td>Vitamin A and immunization for 60 children 200,000 IU to 120 IV drug users</td>
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<td>Zinc</td>
<td>1.8–2.2 mg/kg/day for 4 weeks to 13 children</td>
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<td>8 adults</td>
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<td>β-Carotene</td>
<td>120 mg weekly for 6 months to 5 children and 7 adults</td>
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<td>135 adults with advanced disease and diarrhea</td>
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<td>Daily multivitamins; cohort study with 296 adults</td>
<td>No observed benefit</td>
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<td>Decreased hazard of AIDS</td>
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relation to dietary intake. They concluded that daily multivitamin use was associated with a decreased hazard of developing AIDS and a decreased risk for low CD4 counts. Multivitamins have been found to have an impact when combined with nutritional intervention in malnourished HIV-infected men by halting catabolism and loss of lean body mass, but without alteration of CD4 counts. In HIV-positive pregnant mothers, multivitamin and vitamin A supplements failed to reduce maternal-to-child-transmission. However, multivitamin supplementation had other important benefits, including improvement of mothers’ CD4 lymphocyte counts and infant birth weights, and there were reductions in the risk of growth retardation and severe prematurity.

Micronutrient supplementation also benefits the mother–fetus relationship during pregnancy by reducing anemia and maternal mortality, as well as the risks of congenital abnormalities and fetal mortality. A study from Zimbabwe indicated that regular use of a commercial substitute that provided protein, energy, and micronutrient supplements resulted in appreciable weight gain in HIV-infected children. The importance of nutritional counseling has been evaluated and found to benefit malnourished HIV-infected individuals by improving energy intakes and certain aspects of cognitive function. In addition, nutritional interventions can reduce the characteristic loss of lean body mass when combined with nutritional counseling. In Zambia, adult patients who were suffering from persistent diarrheal disease did not appear to benefit from short-term multivitamin and trace element supplementation. However, the patients all had advanced disease, were malnourished, and had had diarrheal disease for at least a month, suggesting, also, that absorptive processes were not optimal.

Adverse Events

It would seem logical to correct micronutrient deficiencies based on evidence of the impact of their deficiencies on the morbidity and mortality of HIV-infected individuals. Whether these dosages should be at pharmacological levels to achieve supranormal tissue levels or at physiological levels simply to restore nutrient concentrations to within the normal range is not yet clear, as reference standards for HIV-infected individuals do not exist. There are concerns that megadosing may be harmful. Documented side effects of vitamin A supplementation include teratogenicity and bulging fontanelles in infants without deficits in cognitive outcome. There are data to suggest that vitamin A increases HIV viral replication in vitro, and increases the rate of disease progression in individuals consuming regular high-dose vitamin A supplements. However, two randomized, controlled studies have shown no impact on the viral loads of subjects following vitamin A treatment. Zinc supplementation has been linked to increased mortality in HIV-infected adults, and studies in
malnourished HIV-negative children document increased mortality in those receiving high-dose supplementation during the acute phase of the illness. There are concerns, too, that iron and zinc supplementation may be harmful during infective episodes; microbes require these elements for their metabolism, and they may increase microbial virulence.

Other important issues to consider are the possible positive and negative consequences of multiple micronutrient interactions. It is known that the divalent metal ions share the same absorptive pathway and, therefore, nondietary imbalances in their concentrations may lead to competitive inhibition of absorption. Zinc supplementation can lead to copper deficiency if given in sufficient dosage long term. On the other hand, certain micronutrients interact favorably to improve bioavailability; for example, vitamin C improves iron absorption and vitamin A improves iron absorption. An important practical issue regarding toxicity involves the storage of micronutrient supplements in childproof containers in the home. These, in general, are not available at most primary healthcare centers, and preventive measures would lie in the education of caregivers. Accidental iron overdose is a recognized cause of childhood mortality.

**CASE MANAGEMENT STRATEGIES**

Realistically, efforts aimed at reducing viral loads are the best methods in modifying the impact of the disease on host immune function and its course. In an ideal world this would be done by applying specific highly active antiretroviral therapy. In this situation, nutritional support and micronutrient supplementation complement this strategy. This is not so in developing countries, where micronutrient supplementation is viewed as an alternative strategy to alter the progression of the disease and improve the individual’s quality of life.

Current knowledge, even though the evidence base is limited, suggest that

- Micronutrient deficiencies are common in HIV disease and are more pronounced in malnourished states.
- Micronutrients may be involved in the pathogenesis of HIV disease, and their deficiencies can enhance disease progression.
- Micronutrient supplementation can correct micronutrient deficiency states, even in malnourished individuals.

It is, therefore, reasonable to suggest that HIV-infected persons be given micronutrient supplements. However, to date, no clear recommendations have emerged. Optimal formulations and dosage regimens have not been defined. In children, it may be prudent to follow the WHO guidelines for
micronutrient supplementation in children with severe malnutrition. They suggest a standard multivitamin and mineral mixture that includes zinc, copper, manganese, iodine, and selenium. In addition, high-dose vitamin A supplementation should be provided. Supplemental iron should not be given to acutely ill children.

There are no established guidelines for micronutrient supplementation in adults with HIV disease. In situations where individuals are mildly affected by HIV and may be receiving antiretroviral therapy, it is suggested that the dose of the micronutrient supplementation does not exceed 1 RDA. In individuals with severe disease, supplements containing at least 2 RDAs would be appropriate.

Micronutrient deficiencies can be prevented by ensuring that basic case management principles, applicable in all situations, are adhered to. These include the following:

- Provision of basic medical, psycho-social, and emotional support to all infected persons. Many of the conditions associated with advanced HIV infection are difficult to treat and can have adverse psychological effects.
- Education of clients about the need to maintain good nutrition and how to maintain a healthy lifestyle.
- Regular monitoring of nutritional status should include documentation of specific indicators such as weight and height, particularly in children.
- Ensuring adequate nutrient intake and, where necessary, providing supplementary feeds. In children, the energy content of food may be increased by adding a teaspoon of vegetable oil or a teaspoon of sugar to the milk feed or cereal.
- Prevention of opportunistic infections is important to counteract the negative impact that infections have on nutrition. Cotrimoxazole prophylaxis has been shown to prevent not only *Pneumocystis carinii* pneumonia, but also severe enteric and other bacterial infections. Isoniazid prophylaxis for HIV-infected persons with positive tuberculin skin tests delays the onset of tuberculosis, another important contributor to severe malnutrition.
- Prompt recognition and treatment of common opportunistic infections such as oro-pharyngeal candidiasis and gastrointestinal parasitic infestations will limit the negative nutritional impact that such conditions may have.
- Regular deworming at 6-month intervals with albendazole will prevent parasitic infestations and promote and improve growth, and may improve development in treated children.
UNRESOLVED ISSUES

While there is evidence to suggest that micronutrient deficiencies are common in HIV infection, and that micronutrient supplements may be beneficial, there are a number of unresolved issues requiring further research. These include

- Sensitive indicators of deficient micronutrient status, particularly marginal states.
- The impact of micronutrient deficiency on immune function, survival, and mortality of HIV-infected persons in developing countries.
- Understanding the mechanisms of micronutrient deficiency in HIV disease.
- The molecular functions and interactions of specific micronutrients in HIV disease.
- Determining the correct supplementation dosages suited to the requirements of individuals at various disease stages and with various disease complications such as infection and malabsorption.

In conclusion, micronutrient deficiencies are common in HIV-infected persons, contribute to morbidity, and should be corrected. Dosages should concur with age-related RDA to avoid causing harm until further information possibly advocating larger doses becomes available. Single-nutrient interventions are probably not justified. Food-based recommendations to provide the full spectrum of macronutrients and micronutrients would be ideal but not yet realistic in the developing world. Therefore, short-term strategies like micronutrient supplements are justified while longer-term sustainable interventions are implemented.

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# 11

## MICRONUTRIENT INTERVENTIONS AND THE HIV PANDEMIC

*Henrik Friis, Exnevia Gomo, and Kim F. Michaelsen*

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INTRODUCTION

“Most African countries are carrying a burden of HIV/AIDS that is 100 times heavier than that of industrialized countries. Yet, their ability to fund their effort against AIDS is often several hundred times less. While limited resources impede action against the epidemic, they do not make action impossible.”

UNAIDS & UNICEF, 1996

Although primary prevention is the most important response to the HIV epidemic, the current lack of access to care for most of the 36.1 million people already living with HIV cannot be neglected. However, this is one of the most complex development problems, raising ethical, political, economic, and social issues. The dramatic advances in the treatment of HIV infection have not benefited HIV-infected individuals in developing countries. Globally, antiretroviral drugs are used only by 1% of those with HIV infection. Despite the growing international commitment to provide antiretroviral drugs to developing countries, there are serious barriers to its widespread availability, including lack of access to health centers with specialized staff and advanced laboratory facilities. Even if antiretroviral drugs were introduced within the regular health services, treatment may not only be ineffective due to lack of specialized staff, but severe side-effects may occur together with rapid development of drug resistance.

Furthermore, even with the recent reductions in the price of HIV drugs in developing countries to one fifth of its price in the United States, and the prospect of local production of generic drugs, the cost of the drugs for lifelong therapy will remain unaffordable for the vast majority of those in need, and will be prohibitive for the public sector. Many developing countries have health budgets around US$10 per capita per year. Most of this is tied up in salaries, and only the remaining US$2 to 4 are available for diagnostics, vaccines, drugs, and other consumables. This is necessary to
prevent the otherwise considerable infant and child morbidity and mortality from trivial childhood infections, the high risk of disability and mortality among women in relation to pregnancy, as well as to treat other common diseases, injuries, etc. It has been argued that policy-makers should invest these limited resources in improving the existing health services in order to cope with tuberculosis, pneumonia, and other opportunistic infections, rather than introducing expensive antiretroviral treatment.5

Although it seems unlikely that lifelong triple-combination therapy will be affordable and feasible for more than a minority in developing countries, a similar scepticism with regard to the use of antiretroviral drugs to prevent mother-to-child HIV transmission has been put to shame. It has been discovered recently that a simple (single dose to mother and infant) and cheap (US$4) regimen of nevirapine may reduce transmission by 50%.7,8 Therefore, efforts should continue to develop cheap, effective, and safe drugs, and to strengthen the health service infrastructure for the benefit of individuals with HIV, thus bridging the unacceptable gap in healthcare between individuals living in different parts of the world.

At the same time, interventions against risk factors of HIV transmission and progression should be developed, assessed, and implemented. Micronutrient deficiencies could be such risk factors fueling the HIV pandemic. Among clinicians, micronutrient deficiencies are generally seen as simply manifestations of advanced HIV infection, as shown in Figure 11.1A. According to this simple model, the only rational intervention is antiretroviral treatment, and attempts to address micronutrient deficiencies are characterized as “too little, too late.” However, as reviewed in detail in Chapters 4 to 9, emerging data demonstrate the existence of a more complex two-way relationship between micronutrient deficiencies and HIV infection. Micronutrient deficiencies develop early in HIV infection. They may impair immune functions and affect viral replication and, as such, influence the transmission and progression of HIV infection, as well as the risk of secondary (i.e., common and opportunistic) infections. According to this complex model (Figure 11.1B), micronutrients could play a role in efforts to combat the HIV pandemic and its impact.

**MICRONUTRIENTS OF IMPORTANCE TO HIV INFECTION**

Despite the scarcity of available data on micronutrients and HIV infection, there is compelling evidence that micronutrient deficiencies seriously impair immune functions and the antioxidant defense system, thereby contributing to infectious disease morbidity and mortality.9 This is a particular problem among people in developing countries, but is also a concern in underprivileged groups in developed countries. Importantly, these are the
populations most intensively exposed to or infected with HIV, and with the least access to treatment, care, and support. It is firmly documented that multiple micronutrient deficiencies develop early in HIV infection, and that people with HIV have much higher requirements of most micronutrients due to reduced intake and absorption and increased utilization and loss. This, alone, should justify interventions to increase the micronutrient intake

Figure 11.1 Models showing the relationship between HIV and micronutrient deficiencies. A. Simple model: HIV infection will impair the immune functions and lead to opportunistic infections. Due to reduced intake and absorption of micronutrients and increased utilization and loss, micronutrient deficiencies will eventually develop. B. Complex model: micronutrient deficiencies, preexisting or due to HIV infection, affect the course of HIV infection via impaired immunity or viral replication.

among individuals with HIV infection in order to meet the increased requirements and to ensure optimal body functions and quality of life. Into the bargain, improved micronutrient nutrition also may serve to strengthen host defense and reduce morbidity from diarrhea and respiratory tract infections caused by common or opportunistic pathogens, and even reduce HIV progression and transmission. Nevertheless, detrimental effects of increased intake of some micronutrients cannot be excluded.

The current knowledge on the role of individual micronutrients in host defense and HIV infection is summarized below, as is our conclusion about their potential as well as research priorities. A condensed summary is given in Table 11.1.

**Vitamin A**

As reviewed in Chapter 4 by Semba, vitamin A deficiency impairs both non-specific and specific immune functions. HIV-infected individuals have increased vitamin A requirements and low status. While in vitro studies have implied both positive and negative effects on HIV replication, several intervention trials have found no effect of vitamin A on viral load. Nevertheless, regular megadose vitamin A supplements were found to reduce AIDS-specific mortality in Tanzanian children. Studies in the United States found high intakes to be associated with reduced progression, but very high intakes were associated with increased progression. Daily supplements to pregnant women were found to reduce mother-to-child HIV transmission among preterm but not term babies. Given early findings from observational studies, the lack of effect of vitamin A supplements on mother-to-child HIV transmission to term babies has been disappointing. Effects on sexual transmission seem plausible, as vitamin A is essential to the integrity of mucosal membranes, but there are no data to support this. Good vitamin A nutrition is important in general, and among HIV-infected individuals in particular. Although high doses do not affect viral load in individuals with low status, it should probably be avoided in vitamin A replete populations.

**B Vitamins**

Vitamins B<sub>6</sub> and B<sub>12</sub>, and to a lesser extent other B vitamins, seem to be of importance to immune functions, as reviewed in Chapter 2 by Beisel, and Chapter 5 by Garland and Fawzi. Low serum B<sub>12</sub> may be associated with progression to AIDS, and high dietary or supplemental intakes of several B-vitamins associated with increased survival. Vitamin B<sub>12</sub> deficiency may also lead to impaired cognitive function and drug toxicity in patients with HIV infection. High doses of B vitamins, together with vitamins C and
<table>
<thead>
<tr>
<th>Vitamin</th>
<th>Immune Functions</th>
<th>Antioxidant Capacity</th>
<th>Resistance to Infections</th>
<th>Replication</th>
<th>Viral Load</th>
<th>Progression/Mortality</th>
<th>Transmission</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vitamin A</td>
<td>↑↑↑</td>
<td>↑</td>
<td>↑↑↑</td>
<td>↑</td>
<td>↓</td>
<td>-</td>
<td>↓ (↓)</td>
</tr>
<tr>
<td>B vitamins</td>
<td>↑↑</td>
<td>↑</td>
<td>↑</td>
<td>?</td>
<td>?</td>
<td>(↓)</td>
<td>?</td>
</tr>
<tr>
<td>Vitamin C</td>
<td>↑</td>
<td>↑↑↑</td>
<td>?</td>
<td>↓ (↓)</td>
<td>?</td>
<td>?</td>
<td></td>
</tr>
<tr>
<td>Vitamin E</td>
<td>↑↑</td>
<td>↑↑↑</td>
<td>?</td>
<td>↓ (↓)</td>
<td>?</td>
<td>?</td>
<td></td>
</tr>
<tr>
<td>Iron</td>
<td>↑</td>
<td>↓</td>
<td>↓</td>
<td>↑</td>
<td>?</td>
<td>(↑)</td>
<td>?</td>
</tr>
</tbody>
</table>

**Summary**

- **Vitamin A**: Increase intake to reduce AIDS-mortality in children, and vertical transmission to pre-term infants. May reduce progression and sexual transmission. Caveat high intake.
- **B vitamins**: Increase intake to reduce adverse pregnancy outcome and increase CD4 in HIV infected pregnant women. May reduce impairment of cognition and drug toxicity.
- **Vitamin C**: Increase intake to reduce viral load and HIV progression. May have prooxidant properties in those with high iron stores.
- **Vitamin E**: Increase intake to reduce viral load and HIV progression. May reduce subclinical mastitis and postnatal progression.
- **Iron**: Increase intake to increase viral replication and HIV progression. Clarifying the
Micronutrient Interventions and the HIV Pandemic

The role of iron in HIV infection is a research priority, as iron deficiency is a major global health problem.

Zinc

↑↑↑ ↑↑↑ ↑↑↑ ↓ ↑ ? (↑) ?

Reduce diarrheal and respiratory tract infection morbidity in HIV-uninfected infants. Clarifying the role of improved intake in HIV-infected individuals with low absorbable intake is a research priority. High absorbable intake should be avoided.

Selenium

↑ ↑↑↑ ↑ ↓ (↑) ?

May reduce viral load and HIV progression. Intervention trials are in progress. Conceivable that improved intake may reduce mutation rate and development of drug resistance.

Note: ↑, ↑↑, ↑↑↑: slight, moderate, considerable increase. ↓, ↓↓, ↓↓↓: slight, moderate, considerable decrease. †: increase or decrease.

~: no effect. ?: not known. (): only weak or observational data.
Vitamins C and E

As reviewed in Chapter 6 by Tang and Smit, vitamins C and E are important to antioxidant defense. In addition, vitamin E is involved in both cell-mediated and humoral immunity, while vitamin C is primarily of importance to phagocytic function. Although data are inconsistent, *in vitro* studies show that oxidative stress increase and vitamins C and E may suppress viral replication. In accordance with the finding that vitamins C and E status is often low in HIV-infected individuals, supplementation with vitamins C and E has been shown to reduce oxidative stress and probably reduce viral load. Micronutrient deficiencies may be involved in the pathogenesis of subclinical mastitis, a condition with elevated levels of milk sodium and cytokines, which is a strong risk factor of postnatal HIV transmission. In accordance with results of veterinary studies, vitamin E-rich sunflower oil was found to reduce subclinical mastitis in Tanzanian women. Thus, vitamins C and E are likely to be important in HIV infection.

Iron

The importance of iron to immune functions is modest, but iron is essential to pathogens. Iron may increase malarial and bacterial morbidity, in particular in malnourished infants and children. Nevertheless, oral doses are considered safe in semi-immune, malaria-endemic populations, and may even reduce susceptibility to helminth infections. However, as reviewed in Chapter 7 by Weinberg et al., iron seems to increase viral replication and morbidity from viral infections. Analogously, iron increases HIV replication *in vitro*, but there are no data from human studies on the effect of iron supplementation on viral load. Several retrospective studies have shown that high iron status is associated with, and iron supplementation may increase, progression and mortality in HIV patients. The circumstantial evidence pointing at a potentially detrimental effect of iron in HIV is a cause of concern, because iron supplements are routinely given to treat or prevent anemia.

Zinc

As mentioned in Chapter 8 by Friis and Sandström, zinc is of fundamental importance to the immune system, and plays a role in antioxidant defense and apoptosis. Zinc supplementation reduces morbidity from diarrhea, and
respiratory tract, malaria, and helminth infections in children with low zinc intake.\textsuperscript{33-35} \textit{In vitro} studies provide plausibility to both adverse and beneficial effects on HIV replication.\textsuperscript{33,36,37} A single cohort study found a high zinc intake to be associated with increased HIV progression,\textsuperscript{22} but this was not found in other studies,\textsuperscript{38} and was most likely attributable to very high supplemental intakes of zinc among people with diets with high bioavailability. Thus, this finding should not delay the assessment of the effect of zinc supplements on the course of HIV infection in populations with low intake of absorbable zinc. In fact, improved zinc intake may have beneficial effects on HIV infection per se and, in particular, on diarrheal, respiratory tract, and other secondary infections. High supplementary intakes should probably be avoided, however, especially where dietary zinc intake and bioavailability are high.

**Selenium**

Selenium is important to the immune and antioxidant defense systems. As reviewed in Chapter 9 by Friis et al., selenium has also been shown to affect the replication and virulence of coxsackievirus in animal models,\textsuperscript{39} and supplementation reduces the incidence of viral myocarditis and hepatitis.\textsuperscript{40,41} Similarly, HIV selenoproteins are involved in regulation of viral replication.\textsuperscript{42} Therefore, the consistent findings from four cohort studies that a low selenium status is strongly associated with increased HIV progression\textsuperscript{43-45} is biologically plausible. Thus, selenium is likely to be important in HIV and other viral infections.

**MULTIPLE MICRONUTRIENT DEFICIENCIES AND INTERACTIONS**

When interpreting data from observational and interventional studies, and when planning public health interventions, the widespread coexistence of micronutrient deficiencies and micronutrient–micronutrient interactions should be taken into consideration.

**Coexisting Micronutrient Deficiencies**

A range of micronutrient deficiencies usually coexist. This is so among underprivileged individuals in developed as well as developing countries, due to inadequate access to micronutrient-rich foods with high bioavailability. For example, a little fish in the diet would increase considerably the intake of vitamin A, zinc, and iron. Multiple micronutrient deficiencies also coexist in individuals with generalized or otherwise severe infectious diseases, because reduced intake and absorption and increased utilization and excretion affect a number of micronutrients.
Micronutrient–Micronutrient Interactions

The intake and status of one micronutrient may interfere with the absorption, metabolism, and effects of other micronutrients. For example, minerals may compete for absorptive pathways, so that a high intake of iron may impair absorption of zinc and copper. Copper deficiency, on the other hand, may lead to iron-deficiency anemia, because copper is essential to the activity of the enzyme ferroxidase I (ceruloplasmin), which is responsible for mobilization of iron from the stores and its incorporation into hemoglobin. However, copper deficiency is rarely a concern unless induced by a high intake of zinc. Zinc deficiency, on the other hand, impairs the mobilization of vitamin A from the stores, so functional vitamin A deficiency may occur, which responds to zinc, but not vitamin A. The antioxidant vitamin C in the diet increases the absorption of non-heme iron, and also serves to restore the radical-scavenging activity of vitamin E. The antioxidants selenium and vitamin E both serve to scavenge reactive oxygen species, and a low intake of one increases the requirements of the other. The prooxidant iron, on the other hand, increases the requirements of antioxidants. For example, iron supplements lead to an increased generation of free radicals in the intestinal tract at the site of absorption, but this may be prevented by prior intake of vitamins C and E. However, there is concern that vitamin C may have prooxidant properties in individuals with high iron stores. Finally, several micronutrients are required simultaneously for a physiological function such as growth. The response to inadequate intake of these so-called Type II nutrients is described in Chapter 8 on zinc by Friis and Sandström.

The complexity of these and other interactions between individual micronutrients needs to be considered when interpreting the results of clinical or epidemiological studies. It may even be difficult to interpret data from randomized, controlled trials; the finding of an effect of a single micronutrient intervention may not be generalizable to populations with different intakes of interacting micronutrients. For these reasons, and to maximize cost effectiveness, public health interventions aimed at improving the intake and status of multiple micronutrients are preferable to single micronutrient interventions.

DETERMINANTS OF MICRONUTRIENT DEFICIENCIES

An understanding of the determinants of micronutrient deficiencies is indispensable when translating current knowledge on micronutrient–HIV interactions into public health action. The United Nations Children’s Fund (UNICEF) has suggested a conceptual framework to facilitate the analysis and understanding of the determinants of child nutritional status. As seen
from the modified model presented in Figure 11.2, the determinants of micronutrient deficiencies are categorized according to their proximity to the individual in the causal web. The immediate determinants operate within the individual, the underlying determinants at household levels, and the basic determinants at community or national level. The immediate causes are inadequate dietary intake and infectious and other diseases. They determine the micronutrient requirements and interact with dietary intake, absorption, utilization, and excretion of nutrients. The underlying determinants include household food and nutrition insecurity, inadequate
care practices, inadequate health services, and unhealthy environment. Finally, the basic determinants are the resources made available by the political, cultural, religious, economic, and social systems to households, individuals, and health services (Figure 11.2).

MICRONUTRIENT INTERVENTIONS

In developed countries, improving micronutrient intake and status for the benefit of HIV-infected individuals are most appropriately dealt with as part of case management, as described in Chapter 10 by Buys and Hussey. In developing countries, micronutrient deficiencies are widespread even in the general population. Furthermore, in as many as 16 countries, all in sub-Saharan Africa, more than one tenth of the adult population is HIV-infected. In seven of these countries, the prevalence is above one fifth, reaching one third in the worst-hit country. The vast majority of those infected are unaware of their HIV status, as access to testing and counseling is limited. In such settings, case management is not an appropriate strategy to improve micronutrient intake in those with HIV infection. Rather, available public health interventions to improve micronutrient status among populations should be launched, expanded, or modified, where this may reduce the magnitude of the HIV epidemic or increase the quality of life of those living with HIV.

The General Population

Several interventions are at hand to improve the micronutrient status of whole populations or vulnerable groups. As seen in Table 11.2, the micronutrient interventions differ with respect to micronutrients that can be provided, potential target population, and requirements in terms of behavioral change and investment.

Dietary Diversification

A change to a more balanced diet comprising foods with a high content and bioavailability of micronutrients is considered the only sustainable solution and should be the backbone of any strategy to improve micronutrient status. In developing countries, having gardening and nutrition on the school syllabus could be instrumental, especially by involving future mothers and care providers. Health and nutrition education for pregnant women and mothers during antenatal care, and mother-child health services could have an impact on micronutrient intake of not only children, but also of other family members. However, in accordance with Figure 11.2, knowledge is not enough if people do not have access to the
Empowering them to get access to technologies, skill-building opportunities, and other basic resources will enable them to increase the appropriate production and processing of foods, which can be used for home consumption and sale. The cash then can be used to purchase foods and pay for health services.

Table 11.2  Interventions to Improve Micronutrient Status of Populations

<table>
<thead>
<tr>
<th>Intervention</th>
<th>Target Micronutrients</th>
<th>Target Population</th>
<th>Behavioral Change Needed</th>
<th>Investment Needed Short-Term/Long-Term</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Food Based</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dietary diversification</td>
<td>Multiple</td>
<td>All</td>
<td>High</td>
<td>No/No</td>
</tr>
<tr>
<td>Food modification</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Plant breeding</td>
<td>Few&lt;sup&gt;c&lt;/sup&gt;</td>
<td>All</td>
<td>No</td>
<td>High/No</td>
</tr>
<tr>
<td>Traditional food technologies&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Iron, zinc</td>
<td>All</td>
<td>High</td>
<td>Low/Low</td>
</tr>
<tr>
<td>Fertilization</td>
<td>Selenium</td>
<td>All</td>
<td>No</td>
<td>Low/Low</td>
</tr>
<tr>
<td>Fortification</td>
<td>Few&lt;sup&gt;c&lt;/sup&gt;</td>
<td>All</td>
<td>No</td>
<td>High/Low</td>
</tr>
<tr>
<td><strong>Non-Food Based</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Supplementation</td>
<td>Single or multiple</td>
<td>High-risk groups&lt;sup&gt;d&lt;/sup&gt;</td>
<td>Low</td>
<td>High/High</td>
</tr>
<tr>
<td><strong>Infection control&lt;sup&gt;b&lt;/sup&gt;</strong></td>
<td>Multiple</td>
<td>Children, adults</td>
<td>No to high</td>
<td>No to high</td>
</tr>
</tbody>
</table>

<sup>a</sup> Traditional food technologies, such as soaking, malting, germination, and fermentation.

<sup>b</sup> Infection control interventions include programs to promote: immunization, oral rehydration for diarrhea, breast feeding, growth monitoring, improved sanitation, and water supply, vector control, school-based anthelminthic treatment.

<sup>c</sup> Provitamin A, iron, zinc, for example.

<sup>d</sup> Pregnant women, children, for example.
Food Modification

Most people in developing countries subsist on wheat, maize, rice, cassava, beans, or other food staples. These foods are inexpensive sources of dietary energy, but are often poor sources of micronutrients. Modification of staple foods to increase their micronutrient content or bioavailability is potentially important, as it will benefit those most in need. Several techniques are available. Food processing technologies serving to preserve existing micronutrients or to increase absorption of micronutrients should be promoted. These include the traditional food technologies such as soaking, germination, and fermentation. Phytates in cereals bind and prevent the absorption of zinc and iron. However, phytates are water soluble and can be partly removed by soaking, or through the activation of endogenous phytases followed by hydrolysis of phytates through germination, fermentation of bread, or microbial fermentation of cereals. Furthermore, germination and fermentation lead to synthesis of vitamin C and organic acids, which also serve to increase the absorption of zinc and iron. Fermentation of weaning foods is particularly important, as it may prevent contamination, increase the energy and nutrient density, lead to beneficial colonization of the gut, and reduce the risk of diarrhea in infants.

Fertilization with a mineral like selenium may effectively increase the selenium content in staple and fodder crops, and subsequently increase the intake and status of the population, as exemplified by the Finnish experience. Plant breeding of food staples is a promising strategy to increase the mineral and vitamin content, and may even make the plants more resistant to diseases and drought. Wheat varieties with a higher zinc content and soybeans with a higher iron content have already been developed. In addition, breeding for crops with a lower content of anti-nutrients or a higher content of absorption enhancers serving to increase the bioavailability of minerals may be useful. Transgenic techniques also have been used to increase the iron or β-carotene content of rice.

Food Fortification

Fortification of foods (e.g., flour and margarine) or condiments (e.g., salt) with one or more micronutrients has been widely used in developed countries. For example, the elimination of rickets from several Western countries is attributed to the fortification of margarine with vitamin D; iodine deficiency disorders were eliminated with the universal iodization of table salt. Food fortification requires political commitment, legislation, and collaboration with the private sector, and food production has to be accessible with a limited number of production plants. It is, therefore, most efficiently exploited in industrialized countries. Nevertheless, if the technologies can
be transferred, then it may be well suited for developing countries since it is potentially a long-term, sustainable, cost-effective micronutrient intervention that does not require behavioral changes of the consumer. In Guatemala, the necessary technology has been developed and legislation put in place to fortify sugar with vitamin A. Among young children, 25% of the daily intake of vitamin A is from fortified sugar.64 This could be an important intervention in many more countries with widespread vitamin A deficiency. In contrast, countries with widespread HIV infection may have reasons to be cautious with iron fortification until the role of iron in HIV infection has been clarified.

**Supplementation**

Ideally, micronutrients should be obtained through a balanced diet, as this will also ensure an adequate intake of nutrients for which Recommended Dietary Allowances (RDA) have not been defined, as well as other beneficial food components.65 Nevertheless, supplementation with micronutrients may be a feasible, cost-effective, short-term intervention. An argument from opponents of supplementation as a public health intervention is that it is a pharmacological approach to a nutritional problem caused by poverty. On the other hand, the practice of supplementing the diet with minerals, especially during pregnancy, through eating soil from termite mounds66 seems to be deeply rooted in human evolution and culture, and may be seen as a vestige of paleonutrition.67

Micronutrient supplementation is a particularly cost-effective intervention when targeting groups of vulnerable individuals, such as children and pregnant women, who can be reached through the primary healthcare (e.g., antenatal delivery and care, immunization, etc.) or school systems. Periodic delivery of capsules with megadoses of vitamin A to 6-month to 5-year-old children has been shown to reduce all-cause mortality by 30%.68 Many countries where vitamin A deficiency has been identified as a public health problem have, therefore, adopted the policy recommended by UNICEF, the World Health Organization (WHO), and the International Vitamin A Consultative Group (IVACG) of high-dose vitamin A supplementation of children every 4 to 6 months or at annual immunization days.69 The cost of a capsule containing 200,000 IU is 0.02 US$ through UNICEF’s supply division. Consequently, vitamin A supplementation is one of the most cost-effective health interventions, with a gain as high as 1 DALY (disability adjusted life year) per U.S. dollar spent.70

It is recommended that pregnant women attending antenatal clinics receive a daily supplement containing 60 mg iron and 400 μg folic acid for 6 months of pregnancy.50 Although high doses of vitamin A may be teratogenic in early gestation, weekly vitamin A supplements were found not
only to reduce maternal mortality by half, but to even reduce the risk of malformations. Based on this and other studies, it has been realized that the diets of pregnant women are short in a wide range of micronutrients that may contribute to the high prevalence of adverse pregnancy outcome. The cost-effectiveness of a prenatal supplement could, therefore, be considerably increased if vitamin A and other micronutrients were put in the same tablet. UNICEF, WHO, and United Nations University have taken the initiative to develop a perinatal multimicronutrient supplement for daily administration, containing not only iron and folate, but also one RDA of the vitamins A, B₁, B₂, B₆, B₁₂, C, D, E and niacin, and the minerals zinc, iodine, selenium, and copper. Due to the enhancing effect of vitamin C on non-heme iron absorption, the amount of iron was reduced to 30 mg. The tablets should be taken for as much of the pregnancy as possible, and should be continued until 3 months postpartum.

**Infectious Disease Control**

Effective control of infectious diseases not only serves to reduce suffering, but also prevents micronutrient depletion due to diarrhea and malaria, as well as respiratory tract, helminth, and other infections. Since a synergistic relationship often exists between a micronutrient deficiency and a specific infectious disease, improving micronutrient status may itself serve to break the vicious circle. But in addition to the micronutrient interventions mentioned above, a wide range of measures are available to control the infections, and should be implemented simultaneously. The burden of infectious diseases is particularly heavy among infants and children in developing countries. Immunization, growth monitoring, and the development and promotion of adequate complementary diets, as well as the promotion of continued feeding during infections and oral rehydration for diarrheal diseases, are all essential interventions to reduce morbidity among children. Additionally, effective curative services through a well-functioning primary healthcare system, safe water and sanitation, improving personal hygiene, and controlling vector-borne diseases will benefit both children and adults.

**High-Prevalence Populations**

In the following we discuss the abovementioned interventions under a high-HIV prevalence scenario based on the data summarized in Table 11.1. Health and nutrition education and other interventions that increase dietary diversity are likely to be beneficial to those with HIV infection, inasmuch as the intake of vitamin A and other vitamins will increase. This is achievable among well-off, educated households with HIV-infected
members in both developed and developing countries. However, with the high micronutrient requirements of HIV-infected individuals, this may not suffice. Fortification of food vehicles with A and B vitamins could be considered in countries with high burdens of HIV and other infectious diseases, whereas fortification with iron should await the possible acquittal of iron with respect to being harmful to HIV-infected individuals. In contrast, if ongoing intervention studies can confirm the promising findings from observational studies that low selenium status increases HIV progression, then fertilization with selenium could be considered.

Nonetheless, the food-based interventions may not suffice, because HIV-infected individuals have micronutrient requirements in multiples of the RDA. Micronutrient supplements can be distributed through the primary healthcare system. Women and children are most easily reached, as they regularly attend antenatal clinics and mother-child services. The proposition by UNICEF to change the recommendation from daily administration of a prenatal iron-folate supplement to a perinatal multimicronutrient supplement should be of particular benefit for women with HIV infection. The new multimicronutrient supplement contains all potentially beneficial micronutrients, including vitamins A, B, C, and D, as well as zinc and selenium, whereas the content of iron has been reduced. However, while the adequacy of one RDA of each micronutrient, even for uninfected women, can be discussed, it will definitely be inadequate for most HIV-infected individuals.

On the basis of available data, it may be prudent to recommend that where the prevalence of HIV is known to be high, all pregnant women should be given 2 tablets a day, and women known to be HIV infected, or found HIV positive during antenatal care, should be given 3 tablets per day. Continuing the supplements up to 3 months postpartum, as recommended, will serve to keep the lactating mother and infant repleted. If, indeed, iron supplements prove to be unfavorable during HIV infection, and if this effect cannot be counteracted by simultaneous antioxidant intake, it may be necessary to formulate a multimicronutrient tablet without iron for use in areas with high HIV prevalence. Iron should then only be given to women known to be HIV negative or those with anemia. Women with access to antiretroviral therapy may have lower requirements, while those with more advanced disease may have higher needs.

It is important to note, however, that there is no solid documentation for the expected benefits of the recommended three-RDA regimen to HIV-infected women. For example, the multivitamin supplement found to halve adverse pregnancy outcome and increase CD4 counts among pregnant HIV-infected Tanzanians contained from 3 to 15 times the RDA. In this trial, no effects of vitamin A or multivitamins on mother-to-child HIV transmission were seen, and the effect on progression is still to be ascertained.
Nevertheless, when interpreting the effect of the high-dose multivitamin tablet used in the Tanzanian trial, it should be noted that all women received 120 mg of iron per day, which may have increased their requirements for antioxidants. Furthermore, the UNICEF tablet contains minerals, such as zinc and selenium, and vitamins that were not included in the Tanzanian trial. In addition to the anticipated effects on adverse pregnancy outcome and maternal and child mortality, it is conceivable, but not documented, that it may be beneficial in terms of reducing the transmission of HIV. The multimicronutrient supplement also may reduce HIV progression, but because fertility is reduced in HIV infections, administration through antenatal clinics will make it mainly available to women with early HIV infection.

It is particularly promising that supplementation may reduce postnatal transmission. As many as 20% of lactating women may have subclinical mastitis as defined by an elevated breast milk sodium level. Interestingly, HIV-infected women with subclinical mastitis have considerably higher breast milk HIV load and are more likely to transmit HIV to their infants than those without. Subclinical mastitis could be due to low-grade infections, but there is evidence to suggest that oxidative damage to the mammary epithelium is involved in the pathogenesis. In fact, vitamin E-rich sunflower oil was shown to reduce the prevalence of subclinical mastitis in Tanzanian women. That finding is in accordance with studies of dairy cows showing beneficial effects of both vitamin E and selenium supplementation. Furthermore, interventions increasing the breast milk content of micronutrients may serve to improve the integrity of the gut mucosa, thus making it less susceptible to HIV infection. Although this has not been shown, maternal vitamin A supplements seemed to prevent the deterioration of gut integrity in infants with HIV infection.

To both infected and uninfected children of HIV-infected mothers, the WHO/UNICEF/IVACG policy of regular administration of high-dose vitamin A capsules is particularly important, but it is questionable if a 4- to 6-month interval is sufficient. Supplementing HIV-infected children with other micronutrients is logistically more difficult, since zinc has to be taken on a daily basis to meet the requirements. Thus, multimicronutrient supplements, similar in composition but not in dose, to those for pregnant women could be developed for the benefit of HIV-infected children.

The infectious disease control measures mentioned above will serve to reduce the depleting effect of chronic or frequent infections on micronutrient status, thus potentially increasing resistance to infection among the HIV uninfected and reducing the progression among the HIV infected. Additionally, this will prevent the immune activation some of these infections cause, thus reducing HIV replication and progression.
HIV/AIDS Patients

Despite the considerably increased micronutrient requirements of HIV-infected individuals, only few authors have attempted to give recommendations. In Chapter 10 on micronutrients in the case management of HIV infection, Buys and Hussey recommend that individuals mildly affected by HIV or on antiretroviral therapy receive a supplement containing 1 RDA per day, whereas those with severe HIV disease should receive at least 2 RDA. Others have suggested the following provisional recommendations of antioxidants to counteract the increased oxidative stress associated with HIV infection: vitamin A (5000–10,000 IU/d), β-carotene (30–50 mg/d), vitamin E (200–400 IU/d), vitamin C (200–500 mg/d), and selenium (100–200 µg/d). However, it is recommended that the patient should be monitored by a physician and that serum levels should be determined yearly.

This approach is rarely possible in developing countries, where the majority of those with HIV infection will only be in contact with the health system when in need of treatment for opportunistic infections, or when receiving outreach visits by local health clinic staff during home-based care. If multimicronutrient supplements without iron are manufactured and distributed through UNICEF to pregnant women, then these supplements could be prescribed to those who are either hospitalized or under home-based care. Iron supplements, if needed, could be given when the secondary infections are under control.

RESEARCH PRIORITIES

Research is needed to elucidate the role of specific micronutrients in HIV infection. Micronutrients may affect: (1) progression of HIV infection, (2) sexual HIV transmission, (3) mother-to-child HIV transmission, and (4) morbidity due to common and opportunistic infections in HIV-infected individuals. Nevertheless, studies aimed at answering some of these research questions raise important ethical questions.

HIV Progression

Although clinical endpoints reflecting HIV progression, such as AIDS and HIV-related mortality, are preferable when assessing the effect of micronutrients on HIV progression, this requires large studies and long-term follow-up. Alternatively, since viral load has been shown to be a strong predictor of mortality, changes in viral load could be used as a proxy for progression. This will allow conducting small, short-term randomized, controlled micronutrient supplementation trials instead of or prior to trials using clinical endpoints. For example, a single study has suggested that supplements
containing vitamins C and E may reduce viral load.\textsuperscript{26} However, effects on clinical outcomes may be possible in the absence of effects on viral load. For example, vitamin A supplementation does not seem to have any short-term effect on viral load, but seems to reduce AIDS-related mortality.\textsuperscript{15} The effect of zinc on viral load should be studied in individuals with low intake of absorbable zinc. Results from studies assessing the effect of selenium supplementation on viral load and clinical outcomes are forthcoming. Of equal importance is the possible detrimental effect of iron on viral load. Clarifying this may cause ethical dilemmas, but so does not clarifying it, since a large number of pregnant women are currently receiving prenatal supplements containing iron. But it should be justifiable to compare the effect of iron-containing prenatal supplements with supplements without iron on viral load among pregnant women without severe anemia. Alternatively, analyses of repository sera from historical iron trials may be useful. However, a simplistic view of the role of iron should be avoided, because iron may only be harmful under conditions of low antioxidant capacity.

Finally, one may speculate that if selenium and other antioxidants affect the HIV mutation rate, then it may conceivably affect development of drug resistance. Similarly, effective drug treatment may even be conditional upon adequate micronutrient nutrition, as implied by a study on zinc and retrovir.\textsuperscript{83} These issues may be relevant among HIV-infected individuals in developed countries, and will be highly relevant in developing countries as antiretroviral drugs become increasingly available.

**Sexual HIV Transmission**

Conducting ethically sound and epidemiologically valid studies on the role of micronutrients in sexual transmission is difficult. Sexual transmission involves both infectiousness of the infected and susceptibility of the uninfected. Micronutrient interventions found to reduce HIV load in blood are likely to reduce infectiousness, but HIV shedding in semen and cervico-vaginal secretions may be better proxies of male-to-female and female-to-male transmission, respectively. Obtaining data on the role of micronutrient deficiencies in susceptibility to HIV infection would likewise be of tremendous importance. Such effects could be mediated through effects on integrity of the mucosa, local mucosal immunity, or indirectly through increased morbidity from other genital infections (e.g., sexually transmitted diseases or female genital schistosomiasis) that could be risk factors of HIV infection.

**Mother-to-Child HIV Transmission**

Although several trials have not demonstrated any effects of vitamin A supplements on mother-to-child HIV transmission,\textsuperscript{25} except in preterm infants,\textsuperscript{18}
other micronutrients could play a role. With the discovery that a 2-dose–$4 nevirapine regimen may halve the transmission during the first 14 to 16 weeks, future studies using infant HIV status as an endpoint will require large sample sizes. Thus, randomized, controlled micronutrient supplementation trials using viral shedding in cervico-vaginal secretions as a proxy for intrapartum transmission may be a more feasible option. Transmission of HIV through breast milk has become an important research topic, since the current nevirapine regimen mainly protects against transmission around the time of delivery. Given the fundamental importance of breast milk for infants and young children, it is crucial that all possible attempts are made to identify effect modifiers of postnatal transmission. This makes it possible to develop interventions that reduce postnatal transmission thus allowing continued vigorous promotion of breast feeding.

In addition to promotion of exclusive breast feeding, interventions to treat or prevent mastitis could be such an intervention. Clarifying the role of improved maternal micronutrient intake on breast milk HIV load and infant gut integrity should be given a high research priority. If research in this field leads to definition of the optimal composition and content of relevant micronutrients, this could be accommodated in the new UNICEF perinatal supplements.

Common and Opportunistic Infections

An important area of research is the role of micronutrients in morbidity from secondary infections among HIV-infected individuals. It seems likely that the beneficial effects demonstrated in children in developing countries of micronutrient supplementation on morbidity and mortality from specific infections, also could be expected in HIV-infected children and adults with low micronutrient status. For example, zinc supplementation has been shown to reduce considerably morbidity from both diarrheal and respiratory tract infections. Because these infections are common in HIV infection, and presumably contribute to disease progression, the effect of improved intake of zinc and other micronutrients should be assessed among individuals infected with HIV. The potentially detrimental effect of iron, reviewed in Chapter 7 on iron and HIV infection by Weinberg et al., on not only HIV per se, but also on tuberculosis and other bacterial, viral, and fungal infections in patients with HIV infection should be studied without delay.

CONCLUSIONS

The incompleteness of current knowledge about the nature of the two-way relationship between individual micronutrients and HIV infection is evident, but should not prevent action. There is a large body of evidence on
the importance of micronutrients in infectious disease morbidity. Given the feasibility and cost-effectiveness of micronutrient interventions, and the magnitude of the HIV pandemic, available knowledge should be translated into public health policies and programs for the benefit of the vast number of people exposed to or living with HIV, while continuously striving to fill the knowledge gaps through research.

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