# Micronutrients: current issues for HIV care providers

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## Introduction

Malnutrition, often accompanied by low serum levels of micronutrients, was common in HIV-infection prior to the introduction of highly active antiretroviral therapy (HAART), and is still common in much of the world that has limited access to antiretroviral therapy (ART). Chronic diarrhea, anorexia, malabsorption, impaired nutrient storage, increased energy demands and altered metabolism were the primary contributors to these nutritional deficiencies. Today, macronutrient deficiencies are less common and less severe in HIV-infected populations living in resource-sufficient countries. However, following the introduction of HAART, new questions about the importance of micronutrients in HIV infection are emerging.

One of the most vexing issues facing HIV-positive individuals in the developed world today is fat redistribution or lipodystrophy syndrome, characterized by subcutaneous fat wasting, visceral fat accumulation, lipid abnormalities, and insulin resistance or glucose intolerance. Certain aspects of this syndrome may be associated with oxidative stress, thereby increasing the body's demand for certain antioxidants and potentially the need for micronutrient supplementation. HAART medications may increase oxidative stress levels above and beyond levels caused by the virus itself. The potential for HIV infection and its treatment to be associated with an increased risk for cardiovascular disease is real and there may also be a role for antioxidants in ameliorating this risk.

Other important issues that still need to be addressed are the role of micronutrients in vertical HIV transmission, disease progression, and bone health in the presence of HAART. As ART becomes more available to HIV-infected populations, these issues will recur throughout the world, particularly in populations where the ongoing potential for macronutrient and micronutrient deficiencies remains high.

If a need for supplementation is found, then appropriate dosages of micronutrients need to be established. Current nutrient recommendations exist for healthy populations [1–3], however there are few guidelines for people with compromised health. With the widespread and often costly use of micronutrient supplements among HIV-positive individuals, there is a need to know whether pharmacologic dosing of micronutrients is safe, beneficial or contra-indicated.

The objectives of this review are, therefore, as follows: (1) to summarize the data that has been generated on micronutrients and their impact on HIV infection in the pre-HAART era; (2) to determine the critical questions that have been answered and questions that need further research; and (3) to identify new issues dealing with the

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role of micronutrients in the face of a changing epidemic. This review will not focus on the proposed mechanisms of action for each particular micronutrient.

## Pre-HAART

### Prevalence of micronutrient abnormalities

Micronutrients play a critical role in the proper functioning of the immune system (Table 1). Therefore, it is not surprising that at the beginning of the HIV epidemic, researchers began to notice micronutrient abnormalities in AIDS patients. Several review articles have been published examining the prevalence and consequences of micronutrient abnormalities in HIV-infection during the pre- and early HAART eras [4−9]. In brief, several studies documented lower serum levels of many specific micronutrients among HIV-infected individuals [10−13]. Blood selenium levels and gluta-thione peroxidase activity (a marker of selenium activity) were found to be significantly lower in individuals with HIV infection than in individuals without HIV infection [14,15] Similarly, vitamin $B_{12}$ [16,17] and vitamin C levels [18,19] were lower in HIV-infected individuals than in healthy controls. Low vitamin A levels were reported in various populations with HIV infection, including 2−11% of homosexual men [10,20], 15% of injection drug users [21], and 30−60% of pregnant women in developing countries [22−24]. Low plasma carotenoid levels (beta-carotene and/or total carotenoids) [12,23,25−27] as well as low zinc levels [28−30] were also

found to be highly prevalent abnormalities in HIV infection.

In laboratory, animal, and human studies, both iron deficiency and iron overload have been shown to have deleterious effects on the immune system [31−33]. In more recent studies, iron deficiency and iron deficiency anemia were found to be common in HIV-infected patients, particularly among female injection drug users [34,35]. In a cross-sectional study, the prevalence of anemia was significantly higher among HIV-infected than uninfected women (44 versus 26%; $P < 0.02$), with approximately 40% of both groups having low plasma ferritin levels ($< 30$ $\mu$g/l) [35]. Iron-deficiency anemia accounted for approximately half of the total anemia observed in these women. While some of these women may have been on HAART or other ARTs, no information on HIV medications was given.

In the majority of studies published during this time, low serum micronutrient levels were equated with micro-nutrient deficiencies. However, the interpretation of serum micronutrient levels is complicated. Many trace elements and vitamins are acute phase reactants and shift between various body compartments during active infection [36−38]. For example, serum zinc and retinol levels decrease during an acute phase response, whereas serum levels of copper and ferritin increase. Since many of the participants in these earlier studies presented with opportunistic infections and AIDS, or no information about concurrent infections was given, serum levels of micronutrients may not have accurately reflected true

**Table 1. Selected micronutrients and their functions.**

| | |
|---|---|
| Vitamin A, Carotenoids | Vitamin A refers to three types of compounds that exhibit biologic activity: the alcohol (retinol), the aldehyde (retinal or retinaldehyde), and the acid (retinoic acid). Plants contain a group of compounds called 'carotenoids' which are converted to retinol in the body. Beta-carotene is the most biologically active carotenoid. |
| | Has essential roles in vision and various systemic functions, including normal cell differentiation and cell recognition, growth and development, bone development, immune functions, and reproduction |
| Vitamin $B_6$ | Coenzyme in numerous enzyme reactions particularly amino acid transport and metabolism |
| | Direct effect on immune system through its role in protein and nucleic acid synthesis |
| | Deficiency leads to a reduction in nucleic acid synthesis which restricts proliferation of lymphocytes |
| Vitamin $B_{12}$ | Coenzyme involved in transmethylation from methylfolate to homocysteine |
| | Released unmethylated folate becomes available for nucleic acid synthesis |
| Vitamin E | Most important lipid-soluble antioxidant in cell membranes |
| | Protects unsaturated phospholipids of the membrane from oxidative degradation from ROS by donating a hydrogen (called free radical scavenging) |
| | Is important component of the cellular antioxidant defense system, which involves other enzymes (e.g. superoxide dismutases, glutathione peroxidases, glutathione reductase, catalase, etc.) many of which depend upon adequate levels of other antioxidants. Therefore the antioxidant function of vitamin E can be affected by the levels of other nutrients (zinc, selenium, copper, vitamin C) |
| Selenium | Active form functions as selenoenzyme |
| | Major function as part of glutathione peroxidase which reduces cellular peroxides to $H_2O$ and alcohol and prevents oxidative damage to proteins, lipid, lipoproteins and DNA |
| Zinc | Zinc binds to protein, forming zinc fingers that are involved in DNA transcription factors, hormone receptors, and enzymes |
| | Zinc deficiency has been shown to impair a variety of immune function: ↓ lymphocyte counts, loss of T-helper cell function, ↓ T-lymphocyte killer activities, delayed dermal hypersensitivity responses, depressed humoral and cell-mediated immunity |
| | Excess levels of zinc intake can have toxic effects on the immune system and may promote viral replication |

micronutrient status. Furthermore, many of these early pre-HAART studies were cross-sectional, and thus the causes and timing of low micronutrient levels are unclear. Nevertheless the consistency of the numerous published studies at the time suggested some degree of micronutrient abnormality among HIV-infected patients who were not treated with antiretroviral drugs.

## Micronutrients and HIV disease progression

In the mid- to late-1990s, researchers began to examine the associations between micronutrient abnormalities and HIV disease progression. Decreasing vitamin E levels over time were associated with significant declines in CD4+ cell counts and increased progression to AIDS [39]. Likewise, low vitamin $B_{12}$ levels were found to be associated with more rapid disease progression as defined by time to first AIDS diagnosis or CD4+ cell decline to $< 200 \times 10^6$ cells/l [40]. Low serum selenium levels increased the risk of HIV-related mortality by more than ten-fold in one study [41]. Low vitamin A levels were associated with increased risk of mortality in HIV-infected injection drug users [21,42].

Although zinc is essential for proper immune functioning and for the integrity of mucosal surfaces, increased zinc intake was shown to have potentially adverse effects on HIV progression in early observational studies [43,44]. Zinc binds to viral nucleocapsid protein Ncp7 and forms zinc fingers, which are essential in pro-viral DNA synthesis and the activity of reverse transcriptase. Thus there was some concern that zinc supplementation might promote, rather than inhibit, viral replication [45,46].

High iron status, like zinc, may also accelerate the course of HIV infection. Among HIV-positive individuals, those with a certain haptoglobin phenotype (Hp2-2) were more likely to have higher levels of serum ferritin, oxidative stress, and viral replication, and worse prognosis than those with two other haptoglobin phenotypes [47]. Infections with *Candida* spp., *Pneumocystis carinii*, and *Mycobacterium* spp. were significantly more common among HIV-infected patients with high bone marrow macrophage iron stores than in those with low to normal stores [48]. Furthermore, those with higher macrophage iron stores had an increased risk of death.

## Oxidative stress

In HIV infection, reactive oxygen species may enhance viral replication by activating nuclear transcription factors, such as NF-κB, which ultimately lead to viral gene expression. In the laboratory, antioxidants have been shown to inhibit the activation of HIV transcription [49]. Further, in HIV-infected adults, zidovudine was shown to promote oxidative damage to DNA, a process that was reversible with vitamin C and vitamin E supplementation [50]. Several pre-HAART studies found that both asymptomatic HIV-infected individuals and AIDS patients had higher levels of oxidative stress, as indicated by increased plasma metabolites of lipid peroxidation and/or reduced antioxidant levels, compared with healthy controls [19,51–53]. More comprehensive reviews on this topic have been published previously [54,55].

## Micronutrients and HIV transmission

There has been concern of increased risk of HIV transmission to sexual partners, or from mother to child, among HIV-infected individuals with particular nutrient deficiencies. Low serum selenium levels in HIV-positive women in Kenya were associated with a three-fold increased risk of genital mucosal shedding of HIV, suggesting the possibility of increased sexual transmission in selenium-deficient women [56]. In two observational studies, low serum levels of vitamin A in HIV-infected pregnant women were significantly associated with a higher risk of vertical transmission. Among 338 HIV-infected pregnant women in Malawi, 32% of those with serum retinol $< 0.70$ μmol/l transmitted the virus to their offspring, compared with only 7% of women with serum retinol levels $> 1.04$ μmol/l ($P < 0.0001$) [57]. Among HIV-infected pregnant women in two US cities, those with serum retinol $< 0.70$ μmol/l were five times more likely to transmit HIV to their infants than those with adequate retinol levels, after controlling for percentage CD4+ cells, gestational age, duration of membrane rupture, and mode of delivery [58].

While data from these early observational studies suggested strong associations between inadequate micronutrient levels and disease progression or vertical transmission, clinical trials examining this issue have revealed somewhat mixed results. The following section outlines the results of clinical trials of various micronutrient supplements that have been conducted to date.

## Clinical trials of micronutrients and HIV

Studies of micronutrient supplementation among HAART-naive populations consist primarily of early trials in the United States (pre-HAART) and larger, ongoing trials in Africa and other resource-poor countries that have little or no access to antiretroviral medications. Results of earlier trials among HIV-infected adults and children have been recently reviewed [9].

Over the last decade, several small clinical trials of micronutrient supplementation have been conducted in the US with less than remarkable results. The results of these trials have been reviewed previously [4]. Briefly, three small trials in HIV-positive patients on zidovudine therapy showed no beneficial effect of $B_{12}$ injections on zidovudine-related toxicity [59–61]. An early clinical trial of beta-carotene supplementation showed promising results for improving immune function [62], however, an extended evaluation published 3 years later revealed no significant differences in T-cell subsets, natural killer cells, p24 antigens, or body weight between the supplemented

and placebo groups [63]. Similarly, a single, high-dose vitamin A supplement had no effect on HIV viral load or CD4+ cell counts in a placebo-controlled, randomized trial conducted among injection drug users in Baltimore, Maryland [64]. However, promising results were published from a randomized placebo-controlled trial of vitamin C (1000 mg daily) and vitamin E (800 IU daily) supplementation [65]. The authors reported significantly reduced levels of several markers of oxidative stress and a trend toward a reduction in HIV viral load after only 3 months.

Several larger clinical trials of micronutrient supplementation have been conducted among HIV-infected populations in international settings with little or no access to antiretroviral medications (Table 2). A series of trials conducted in Durban, South Africa examined the effects of vitamin A supplementation on mother-to-child transmission (MTCT), pregnancy outcomes, and the morbidity and mortality of HIV-infected mothers and their offspring. In 1995, 118 infants of HIV-infected women were randomized to receive either vitamin A or placebo every 3 months up to 15 months of age [66]. HIV status of the infants was determined at 15 months of age. Among all children, the supplemented group had a 30% lower overall morbidity (diarrhea, thrush, lower and upper respiratory tract infections, rash) than the placebo group ($P < 0.05$). Among the children with known HIV infection, those supplemented with vitamin A had a significant reduction in diarrhea [odds ratio (OR), 0.51; 95% confidence interval (CI), 0.27−0.99], but not overall morbidity, compared to the placebo group. There was no effect of supplementation among children known to be uninfected with HIV. In a larger clinical trial, the investigators randomized more than 700 HIV-infected pregnant women to receive either a placebo or 5000 IU of retinyl palmitate and 30 mg of beta-carotene daily during pregnancy and 200 000 IU of retinyl palmitate at delivery. The investigators found no difference in MTCT rates by 3 months of age between the vitamin A and placebo groups (20.3 versus 22.3%) [68]. In addition, there were no differences in rates of infant or fetal mortality between the two groups. However, women in the vitamin A group were significantly less likely to have a pre-term delivery than women in the placebo group (11.4 versus 17.4%; $P = 0.03$). Among a subset of 312 women from this clinical trial, there was no effect of vitamin A supplementation on HIV- or pregnancy-related symptoms during the pre- or post-natal periods [69], or on pre-partum weight gain among these women [70]. However, there was a benefit of vitamin A supplementation on maintenance of post-partum weight, particularly among women with low serum retinol levels or with CD4+ cell counts $< 200 \times 10^6$ cells/l at baseline [70].

Between 1995 and 1997, over 1000 HIV-infected pregnant women in Tanzania were enrolled into a double-blinded, placebo-controlled micronutrient supplementation trial that continued through lactation. These women were randomized into one of four study arms: vitamin A, multivitamins without vitamin A, multivitamins with vitamin A, or placebo. At delivery, women in the vitamin A groups received an additional oral dose of vitamin A, whereas women in the placebo and multivitamin only groups received a placebo. At 6 months of age, all infants born to the mothers in the trial received 100 000 IU of vitamin A, and were given twice that amount every 6 months thereafter. Multivitamin supplementation, but not vitamin A, was significantly associated with improved birth outcomes and improvements in CD4+, CD8+, and CD3+ cell counts among these women [71]. Multivitamin supplements were also found to improve weight gain during the third trimester of pregnancy [72]. In 2000 and 2002, results were published on the effects of supplementation on vertical transmission and early childhood mortality [73,74]. There appeared to be no effect of either vitamin A or multivitamin supplementation on infant mortality rates or risk of vertical transmission up to 6 weeks post-partum [73]. After 6 weeks of age, however, multivitamin supplementation alone reduced the risk of mortality among children who were HIV negative at birth and reduced the risk of vertical transmission among children born to mothers who were immunologically and nutritionally compromised [74].

Vitamin A supplementation had no effect on child mortality, but was unexpectedly associated with a significant increase in risk of vertical transmission through breastfeeding. Multivitamin supplementation of mothers appeared to provide additional benefits to their offspring. During their first 24 months of life, children of women in the multivitamin arm experienced significantly less diarrhea and had higher mean CD4+ cell counts than children of women who did not take multivitamins [75]. While maternal intake of vitamin A had no effect on the children's diarrhea or CD4+ cell counts, it did reduce the risk for a symptom of pneumonia (cough with a rapid respiratory rate) [75].

After 4 years of follow-up, results have recently been published on the effects of vitamin supplementation on HIV disease progression and mortality in these women [76]. Multivitamin supplements were found to reduce the risk of progression to late-stage disease and death by approximately 30% compared to placebo (relative risk, 0.71; 95% CI, 0.51−0.98). Vitamin A supplementation, however, appeared to have no such benefit.

A placebo-controlled trial conducted in Zambia examined the effects of micronutrient supplementation as an adjunct to antiprotozoal therapy among HIV-infected patients with persistent diarrhea [77]. Subjects were randomly assigned to receive albendazole plus placebo or albendzole plus a multivitamin (vitamin A, vitamin C, vitamin E, selenium, and zinc). Although

**Table 2. Main results from clinical trials of micronutrient supplementation in resource-poor settings.**

| Authors | Place of study | Study population | Study arms | Negative results | Positive results |
|---|---|---|---|---|---|
| Coutsoudis et al. 1995 [66] | Durban, South Africa | 118 offspring of HIV+ women | Vit A (50 000 IU at 1 and 3 months; 100 000 IU at 6 and 9 months; 200 000 IU at 12 and 15 months.) Placebo | No effect of Vit A on diarrhea in HIV-negative children | Lower overall morbidity in Vit A group; Lower diarrhea-associated morbidity in Vit A group among HIV-infected children. |
| Coutsoudis et al. 1997 [67] | Same as above | 24 HIV+ pregnant women | Vit A (5000 IU retinyl palmitate (RP) and 30 mg beta-carotene (BC) daily during pregnancy. At delivery: 200 000 IU RP. Placebo | | No increase in viral load in Vit A group. |
| Coutsoudis et al. 1999 [68] | Same as above | 728 HIV positive pregnant women, third trimester; 632 infants born followed for 3 months | Same as above | No difference in vertical transmission by 3 months; No difference in fetal or infant mortality rates | Vit A group less likely to have a pre-term delivery |
| Kennedy et al. 2000 [69] | Same as above | 312 HIV positive pregnant women, 28–32 weeks gestation | Same as above | No benefit of Vit A on pre- or post-natal symptoms | |
| Kennedy-Oji et al. 2001 [70] | Same as above | Same as above | Same as above | No benefit of Vit A on pre-partum weight gain | Benefit of Vit A on maintenance of postnatal weight among women with low retinol levels or CD4 cell count $< 200 \times 106$ cells/l at baseline. |
| Fawzi et al. 1998 [71] | Dar es Salaam, Tanzania | 1075 HIV+ pregnant women, 12–27 weeks gestation | Vit A (30 mg BC and 5000 IU preformed Vit A) Multivitamin (MV) (B1, B2, B6, niacin, B12, folate, C, and E) MV + Vit A Placebo At delivery, women in groups 1 and 3 received 200 000 IU Vit A; Groups 2 and 4 received placebo | No effect of Vit A on birth outcomes or T-cell subsets. | MV associated with lower risk of: (a) fetal deaths, (b) low birthweight, (c) severe preterm birth, and (d) small size at birth. MV associated with increase in CD4+, CD8+ and CD3+ cell counts. |
| Fawzi et al. 2000 [73] | Same as above | 1083 HIV+ pregnant women, 12–27 weeks gestation; Infants born followed for 6 weeks post-partum | Same as above; Additionally, all infants received 100 000 IU Vit A at 6 months and 200 000 IU Vit A every 6 months thereafter. | No effect of Vit A or MVs on risk of vertical transmission up to 6 weeks postpartum. | MVs associated with higher birthweight among babies who were HIV-negative at birth. |
| Fawzi et al. 2002 [74] | Same as above | 1078 HIV+ pregnant women, 12–27 weeks gestation; Infants born followed for 24 months | Same as above | No effect of MVs on overall risk of vertical transmission; Vit A associated with significantly increased risk of vertical transmission; No effect of Vit A on child mortality. | MVs reduced mortality among children who were HIV negative at birth; MVs reduced vertical transmission among immunologically or nutritionally compromised mothers. |

(continued)

**Table 2. (continued).**

| Authors | Place of study | Study population | Study arms | Negative results | Positive results |
|---|---|---|---|---|---|
| Villamor et al. 2002 [72] | Same as above | 1075 HIV+ pregnant women, 12–27 weeks gestation | Same as above | No effect of Vit A on overall weight gain or third trimester weight gain. | MVs increased weight gain in third trimester; MVs reduced risk of low total weight gain, weight loss, and low rate of weight gain in third trimester; MVs + Vit A resulted in lower risk of low total weight gain than MV alone. |
| Fawzi et al. 2003 [75] | Same as above | Same as above; 788 infants born followed from 6 weeks to 24 months | Same as above | No effect of Vit A on infant diarrhea or CD4+ cell counts | MVs decreased risk of infant diarrhea MVs increased CD4+ cell counts among infants Vit A decreased risk of cough with rapid respiratory rate |
| Fawzi et al. 2004 [76] | Same as above | 1078 HIV+ pregnant women; Subgroup of 297 women for viral load endpoint | Same as above | No effect of Vit A on disease progression or death | MVs decreased risk of progression to late-stage disease or death MVs reduced risk of oral, GI and other symptoms MVs associated with higher CD4+ cell counts and lower viral load |
| Kelly et al. 1999 [77] | Zambia | 135 HIV-positive patients with persistent diarrhea | Albendazole + MV (vitamins A, C, and E, selenium and zinc) Albendazole + placebo | No effect of MVs on mortality, time with diarrhea, CD4+ cell counts, or serum levels of vitamins A and E after 1 month | Low serum levels of vitamins A and E at baseline associated with increased mortality |
| Jiamton et al. 2003 [78] | Bangkok, Thailand | 481 HIV-positive patients with CD4+ cell counts between 50 and 550 × 106 cells/l. | MV (vitamins A, B1, B2, B6, B12, C, D3, E, and K, beta carotene, folacin, pantothenic acid, iron, magnesium, manganese, zinc, iodine, copper, selenium, chromium, and cysteine) Placebo | MVs had no effect on CD4+ cell counts or viral load. | MVs reduced death rates among participants with CD4+ cell counts < 200 × 106 cells/l. |

low serum levels of vitamins A and E at baseline were associated with a higher mortality, multivitamin supplementation did not increase serum levels, improve CD4+ cell counts, reduce time with diarrhea, or reduce mortality during the first month following treatment.

Finally, a clinical trial in Thailand examined the effect of a high-dose multivitamin supplement on survival and disease progression among HIV-positive patients [78]. Participants were randomized to take, twice daily, a placebo or a multivitamin. The investigators found that the multivitamin supplement significantly reduced death rates after 48 weeks, but only among the subgroup of participants with CD4+ cell counts $< 100 \times 10^6$ cells/l ($n = 40$ supplemented; $n = 41$ placebo). There was also some benefit of micronutrient supplementation on death rates among those with CD4+ cell counts $< 200 \times 10^6$ cells/l, although this result was only border-line significant. Multivitamin supplements had no effect on CD4+ cell counts or viral load.

## Summary of pre-HAART studies

As with other issues of importance in HIV medicine, general descriptive studies were the first to appear in the literature. Studies measuring serum micronutrient levels were common, and levels of vitamins A, $B_6$, $B_{12}$, C, and E, carotenoids, selenium, and zinc were often found to be low. Many early studies associated these low micronutrient levels with adverse clinical outcomes, including progression to AIDS and death. It is widely recognized, however, that assessments of micronutrient status are often imperfect. In the presence of acute and chronic infection, nutrient metabolism is altered and a redistribution of certain circulating nutrients occurs, clouding the interpretation of low serum micronutrient levels.

Taken collectively, the results of randomized clinical trials of micronutrient supplements have been somewhat mixed. Early supplementation trials in the US found little or no benefit of beta-carotene or vitamin A supplements on immune function or viral load. Studies among HIV-infected mothers in South Africa found a benefit of vitamin A supplementation in reducing diarrhea among their children who were also infected with HIV. However, studies from Tanzania found little effect of vitamin A on episodes of diarrhea among HIV-infected children. Both groups (South Africa and Tanzania) found little effect of vitamin A or multivitamin supplementation on vertical transmission. Investigators in Tanzania actually found a significant *increase* in risk of HIV transmission through breastfeeding among mothers given vitamin A. The reason for this increase in risk remains unclear. Vitamin A supplementation may enhance the differentiation of myeloid and lymphoid cells, which have been associated with an increased expression of CCR5, leading to an increase in susceptibility to HIV infection.

Alternatively, the increased risk of transmission through breastfeeding may be the result of prolonged survival of babies born HIV-negative, thus exposing them to HIV-infected breast milk for longer periods. The implications of this finding should be explored further, particularly in countries with ongoing vitamin A supplementation programs for children.

Multivitamin supplementation, but not vitamin A, did appear to confer some benefits on HIV-infected women and their offspring. Multivitamin supplementation was associated with increased CD4+ cell counts, more weight gain during the third trimester of pregnancy, and improved birth outcomes (including higher birth weights and lower infant mortality). In Thailand, multivitamin supplements reduced mortality among HIV-positive subjects with more advanced disease. Yet, in Zambia, multivitamin supplements had no effect on CD4+ cell counts, time with diarrhea, or mortality. To conclude, it appears that a combination of vitamins may afford some benefits to undernourished HIV-infected populations, particularly those with more advanced disease. However, the role of individual nutrients (vitamin A and beta-carotene, in particular) is less clear.

## HAART era

Since HAART has become the standard of care in the treatment of HIV infection in the resource-sufficient world, the incidence of opportunistic infections and death has significantly declined in these regions [79]. A few recent investigations have examined the effects of HAART on micronutrient status to determine if the clinical benefits of HAART have extended to improvements in micronutrient levels (see Table 3).

In one study, the micronutrient status of 44 patients, mostly drug users, was examined over a 3-year period, before and after the initiation of HAART [80]. In 1995, before HAART was widely available, 77% had low plasma selenium levels ($< 60$ μg/l), 23% had low plasma zinc levels ($< 75$ μmol/l), and 19% had low iron levels ($< 11$ μmol/l). Low selenium levels were significantly more prevalent among patients with CD4+ cell counts $< 250$ than $> 250 \times 10^6$ cells/l. In 1998, follow-up data were obtained on 30 of the 44 patients, 77% of whom had initiated HAART. At that time, 10% had low selenium levels, 27% had low zinc levels, 13% had low iron levels, 82% had low vitamin A levels ($< 1.5$ μmol/l), and 30% had low vitamin E levels ($< 6$ mg/l). Vitamins A and E were not measured in 1995. Among patients who were on HAART in 1998, there were no significant differences in mean levels of any of the micronutrients examined compared to 1995. Within person changes in micronutrient levels over the 3 years of follow-up were not examined in this study.

**Table 3. Observational studies of micronutrient status in the highly active antiretroviral therapy (HAART) era.**

| Nutrient | Author, year | Study design | Study population | Results |
|---|---|---|---|---|
| Vitamin $B_{12}$ | Remacha et al. 2003 [81] | Cross-sectional with historical controls | 126 HIV+ patients on HAART, 109 HIV+ non-HAART historical controls (Barcelona, Spain) | HAART patients had significantly lower prevalence of low serum $B_{12}$ levels than historical controls not on HAART (8.7 vs. 27%). |
| Vitamin $B_{12}$ | Hepburn et al. 2004 [82] | Retrospective cohort study | 251 HIV+ patients from Brooke Army Medical Center, 1990–2001. 38 with $B_{12}$ levels pre- and post-HAART. (Houston, Texas, USA) | 13% had at least one low $B_{12}$ level during the course of their HIV infection. In a subgroup of patients, $B_{12}$ levels significantly increased after initiating antiretroviral therapy (416 vs. 535 pg/ml, $P = 0.04$). |
| Vitamin $B_{12}$ | Woods et al. 2003 [83] | Cross-sectional with repeated measures | 412 HIV-pos participants in the NFHL cohort (Boston, Massachusetts, USA) | During intervals with no PI use, increased $B_{12}$ intake was significantly associated with increased serum $B_{12}$ levels. No association between intake and serum levels found among intervals with PI use. |
| Antioxidants | Tang et al. 2000 [89] | Cross-sectional | 175 HIV+ and 210 HIV– IDUs in the ALIVE study (Baltimore, Maryland, USA) | Serum antioxidant levels (vitamin E, beta-carotene, betacryptoxanthin) were significantly higher among HIV+ IDUs on PI-based HAART than on HIV+ IDUs not on HAART |
| Multiple micronutrients | Rousseau et al. 2000 [80] | Cross-sectional at two time points | 44 HIV+ patients in 1995, 30 with data available in 1998 (Marseille, France) | In 1995: 0% on HAART. Significantly lower selenium levels in those with CD4+ cell counts $<250$ (vs. $>250$) $\times 10^6$ cells/l. No differences for zinc, copper, or iron. In 1998: 77% on HAART. No differences by CD4+ cell counts for any micronutrient. No difference in micronutrient values between 1995 and 1998 in patients on HAART in 1998. |
| Zinc | Wellinghausen et al. 2000 [84] | Cross-sectional | 79 HIV+ patients, 66% on ART (Ulm, Germany) | No significant difference in prevalence of low zinc levels between patients not on ART and those on triple therapy (22 vs. 25%) |

ART, antiretroviral therapy; IDU, injecting drug user; PI, protease inhibitor.

A few studies have examined the effect of HAART use specifically on vitamin $B_{12}$ and folate levels. In a cross-sectional study, the prevalence of low vitamin $B_{12}$ levels was significantly lower in patients receiving HAART than in historical controls who were not treated with HAART (8.7 versus 27%) [81]. However, the authors did not adjust for other factors that may have differed between the two time periods that could have confounded the results. In a longitudinal study examining $B_{12}$ and folate levels before and after initiation of ART, there was a significant increase in $B_{12}$, but not folate, levels after initiation of ART [82]. Taking any HIV medication was associated with increased levels of $B_{12}$ ($P = 0.08$), after adjusting for multivitamin use and other potential confounders. No independent effects of either zidovudine or protease inhibitors (PIs) were found in this study. In a third study, mean serum $B_{12}$ levels in a cohort of HIV-positive individuals were significantly higher during periods in which PI use was reported [83]. However, the prevalence of low $B_{12}$ levels ($< 350$ pg/ml) was similar between periods of PI use and non-use (approximately 20%). Additionally, PI users were significantly less likely to increase their serum $B_{12}$ levels through dietary intake alone compared to non-PI users, suggesting possible interference of $B_{12}$ absorption due to PI use.

Two small cross-sectional studies reported that plasma zinc and retinol levels were lower in HIV-positive patients on HAART than among untreated patients [84,85]. Taken together, these initial studies do not overwhelmingly show improvements in serum micronutrient levels after HAART use. However, the majority of these studies have been cross-sectional with small sample sizes. Most of the studies compared mean levels among a group of non- or pre-HAART users with mean levels among HAART users, without looking at within person changes. It could be assumed that the nutritional status of patients would improve with HAART use since the relief of clinical symptoms would likely result in improved dietary intake, lower prevalence of severe and chronic diarrhea, and better absorption. However, the evidence for this is still lacking. Further studies of vitamin $B_{12}$ need to be undertaken to determine if PIs may actually hinder absorption of dietary $B_{12}$ intake as suggested previously [83]. One of the studies demonstrated that very few HIV-infected patients with low serum $B_{12}$ levels were actually $B_{12}$ deficient, as measured by homocysteine levels which increase in true $B_{12}$ and folate deficiencies [81]. Instead, abnormalities in $B_{12}$ binding proteins, increased oxidative stress levels, and/or increased concentrations of immune system activation markers are hypothesized to be the cause of low serum $B_{12}$ levels. These mechanisms would be less amenable to $B_{12}$ supplementation or $B_{12}$ injections.

More longitudinal studies are needed to determine the clinical benefits of increased serum micronutrient levels after HAART, if found to be the case. This question is important since research from the US, Europe, and Canada show that many HIV-infected patients are still taking vitamin supplements, some at very high doses, in conjunction with HAART [86–88]. If low serum levels are not a marker of actual deficiencies then the benefits of high levels of supplementation remain questionable.

## Oxidative stress in the HAART era
In populations of HIV-infected patients treated with HAART, the role of oxidative stress in disease progression has become more complicated. Whereas HIV itself increases oxidative stress levels through replication, control of the virus with antiretroviral therapy may not, as one might expect, reduce oxidative stress levels, as the medications themselves may increase oxidative stress. In one study, serum levels of vitamin E, beta-carotene, and beta-cryptoxanthin were significantly higher among HIV-positive IDUs on PI-based HAART than among HIV-positive IDUs taking non-HAART regimens or no antiretroviral therapies, suggesting that oxidative stress levels might be lower among individuals taking HAART [89]. This result held up even after adjusting for gender, drug use, dietary intake, use of vitamin supplements, and alcohol intake. More recently, a cross-sectional study examined $F_2$-isoprostane levels as a marker of oxidative stress in HIV-infected individuals, 74% of whom were taking antiretroviral medications [90]. Higher $F_2$-isoprostane levels were significantly associated with lower HIV viral load and use of the antiretroviral, efavirenz. Oxidant stress levels were not increased in subjects with uncontrolled viral replication. These results suggest that the effect of viral replication on oxidative stress is outweighed by the stronger effect of the medication on oxidative stress. In a third cross-sectional study, no differences in plasma malondialdehyde (MDA) or peripheral blood mononuclear cell (PBMC) glutathione (GSH) levels were found between HIV-positive patients receiving and not receiving HAART [91]. In light of the inconsistent results from these cross-sectional studies, longitudinal studies on the role of antiretroviral therapies on oxidative stress need to be conducted.

Thus far, two small clinical trials have been published examining the effects of antioxidant supplementation on oxidative stress in the era of HAART. In an earlier placebo-controlled trial, the effects of daily supplementation with vitamins E (800 IU) and C (1000 mg) on oxidative stress levels (measured by breath pentane, plasma lipid peroxides, and MDA) were examined in HIV-infected patients, of whom approximately 75% were on a combination antiretroviral therapy [65]. After 3 months, the supplemented group had lower oxidative stress levels, by all three measures, than the placebo group (all differences were statistically significant). In a later study, investigators randomly assigned 30 HIV-infected patients (all on HAART) to receive either a placebo or a daily multivitamin supplement containing 5000 IU vitamin A, 100 IU vitamin E, and 50 mg vitamin C) for 6 months

[92]. The supplemented group experienced significant declines in levels of modified DNA bases and thiobarbituric acid reactive substances (TBARS), as well as an improvement in the activity of antioxidant enzymes. The HIV-infected subjects as a whole had lower antioxidant enzyme activity and higher amounts of modified DNA bases and TBARS levels than a group of HIV-negative controls, indicating higher levels of oxidative stress. Although these two clinical trials were not designed to determine the effects of HAART use on oxidative stress, they do demonstrate that daily antioxidant supplementation can reduce oxidative stress levels regardless of the cause. However, the clinical implications of this reduction still need to be determined.

The effects of selenium supplementation on clinical outcomes (CD4+ cell decline and hospital admissions) were examined in a clinical trial conducted among HIV-infected drug users in Florida [93]. Over 200 HIV-infected IDUs (none of whom had selenium levels < 85 μg/l) were randomized to daily selenium supplementation (200 μg/day) or placebo for 2 years. Approximately half the participants reported HAART use. Selenium supplementation was shown to reduce the risk of hospitalization rates, regardless of antiretroviral therapy, age, CD4+ cell count, and viral load at baseline. Hospital admissions were mostly due to opportunistic infections (41%), other HIV-related conditions (lymphoma, thrombocytopenia, and cervical cancer), psychiatric diagnoses, and drug abuse-related problems. The placebo group also had a higher proportion of individuals with a decline in CD4+ cells greater than $50 \times 10^6$ cells/l compared with the selenium-supplemented group (46 versus 25%; $P = 0.01$).

## Micronutrients and other complications of HIV and its therapy

One of the most vexing problems that has appeared in the HAART era is the occurrence of the HIV-associated lipodystrophy syndrome, the hallmarks of which are body shape and metabolic abnormalities that are likely to be multifactorial in origin. The body shape abnormalities include subcutaneous fat atrophy and visceral or central fat accumulation. The metabolic abnormalities include elevations in serum triglycerides and total cholesterol, suppression of serum high-density lipoprotein cholesterol levels, and hyperinsulinemia in normoglycemic individuals. There are also reports of chronic asymptomatic elevations in serum lactate (probably associated with the use of nucleoside anti-retroviral agents) and decreases in bone density. Studies on the role that micronutrient deficiencies may play in the development of these abnormalities have, thus far, been sparse.

### Glucose metabolism and plasma lipids

The use of nucleoside reverse transcriptase inhibitors (NRTIs) has been associated with peripheral/subcutaneous fat atrophy [94,95]. A small pilot trial of antioxidant supplementation was conducted among 10 HIV-infected patients who were treated with NRTIs. Patients had either lipoatrophy (defined as a decrease in fat in at least two of the following areas: face, arms, legs, or buttocks) by self-report and confirmed by the investigator and an experienced registered dietitian ($n = 9$), or sustained hyperlactatemia for 4 months prior to study entry ($n = 1$) [96]. All subjects were given a daily vitamin supplement containing vitamin E (800 IU), vitamin C (1000 mg), and a twice-daily supplement of N-acetyl cysteine (NAC) (600 mg). After 24 weeks, there were no changes in fat atrophy. Fasting glucose and measures of insulin resistance significantly increased over the course of the study. Due to the small sample size and lack of a placebo control group, however, it is unclear whether these changes were due to the natural progression of metabolic complications or to the antioxidant supplementation.

### Hyperlactatemia

Mitochondrial toxicity, induced by NRTI therapy, may lead to the development of asymptomatic hyperlactatemia or, in rare cases, lactic acidosis [97]. In a non-randomized study design, two groups of HIV-infected patients on NRTI therapy were compared [98]. One group was taking various antioxidant and multivitamin supplements; the other group was taking none. Both groups were assessed for lipoatrophy, central fat accumulation, markers of oxidative stress, plasma micronutrients and plasma lipids. The supplemented group had significantly lower venous lactate levels compared to the non-supplemented group ($1.37 \pm 0.10$ mmol/l versus $1.82 \pm 0.19$ mmol/l; $p = 0.04$). However, selection bias may have been a problem in this study as subjects taking supplements were selected from a special clinic that prescribed nutritional supplementation as part of its treatment plan. In addition, the supplements were not standardized, but administered according to individual clinical findings and plasma oxidant stress markers.

### Osteoporosis

There is emerging evidence that bone metabolism in HIV infection is altered. Early studies (pre-HAART) suggested that HIV infection itself might be involved in bone loss [99–101]. However, the evidence is less clear from recent studies as to whether HIV itself or antiretroviral therapy is responsible for bone loss. In one study, 50% of osteopenic HIV-infected individuals were treated with a PI-based HAART regimen [102]. In another study, a high prevalence of reduced bone mineral density (BMD) was found among HIV-infected individuals, with even further reduced BMD among those on PI-containing HAART regimens [103]. Longer duration of HAART use was associated with a small, but statistically significant, loss of bone mineral content among HIV-positive men [104]. Similarly, longer duration of stavudine therapy was independently associated with low BMD [105]. In contrast, in one cross-sectional study, BMD was

significantly lower in HIV-positive subjects than in HIV-negative subjects; however, no difference in BMD was found between HIV-positive subjects on various types of antiretroviral therapies, indicating that bone loss was more likely due to HIV itself [106]. Another study reported improvement of the bone remodeling process, as measured by serum markers of bone formation (osteocalcin) and bone resorption (C-telopeptide) during 24 months of antiretroviral therapy [107]. Levels of the active form of vitamin D (1,25-dihydroxyvitamin D$_3$) were found to be severely deficient among HIV-infected subjects (53% had serum levels below the normal range and 33% had undetectable levels), with the lowest levels found among those with the most clinically advanced HIV disease [108].

## Conclusions

Micronutrients play a critical role in the maintenance of immune function (including mucosal immunity) and overall metabolism. With the complexity of HIV infection and treatment, and the pace of clinical research, it is not surprising that sophisticated studies of the role of micronutrients in HIV have lagged somewhat behind other areas of inquiry. Much credit is due to researchers who have been asking, and continue to ask, questions about micronutrient status and the progression of HIV infection. As HIV becomes a more chronic, manageable disease, and treatment becomes available to more of those infected throughout the world, it may be possible to begin to more precisely define the areas in which micronutrients may help to maximize the clinical status of HIV-infected patients. Studies to date reveal that this is a complex topic, fraught with pitfalls. For example, it is difficult to ascertain true micronutrient status, measures of intake are imprecise, recommendations for desired intakes vary, and surrogate markers of oxidative stress may not be reproducible. Table 4 summarizes some of the outstanding questions that remain in this area of research.

As more HIV infected individuals around the world are initiated on effective anti-HIV therapy, the need to maximize durability of viral suppression will become increasingly important. Data are needed on the role that micronutrient status may play on low-level viral replication among subjects on therapy. For HIV-infected individuals with adequate viral suppression, but inadequate CD4+ cell counts, micronutrients could play a role in boosting the immune response.

As HIV-infected individuals co-exist with chronic viral infection and chronic antiretroviral therapy, the need to examine creative interventions to minimize long-term complications such as fat atrophy, insulin resistance, lipid abnormalities are becoming increasingly important. The role that micronutrients, particularly the antioxidants, may play in modulating these toxicities is not yet clear and should be studied. In addition, for the patient with multiple chronic viral infections, such as HIV and hepatitis B and/or C, micronutrient supplementation may also be beneficial in minimizing the co-morbidities associated with these co-infections. As the HIV-infected population ages, and the risk of cardiovascular disease increases, antioxidants may again play a role in diminishing progression of such disease. There is also some evidence for the role of antioxidants in modulating subtle cognitive defects in HIV and this area needs further study as well [109].

Table 4. Summary of future research needs.

| Area of research | Questions remaining |
|---|---|
| HAART-treated patients | Is there a role for micronutrients in patients with low-level viral replication? <br> Can micronutrients enhance durability of viral suppression? <br> Can micronutrients improve CD4+ cell responses in patients with adequate viral suppression |
| HAART-naive patients | Will the beneficial effects of multivitamin supplements found in Africa (decreased HIV progression, morbidity in mortality in certain subgroups) hold true in HIV-infected populations in other parts of the world with differing baseline nutritional status and/or viral subtypes? |
| Oxidative stress | Do ARTs increase oxidative stress through their direct mechanisms of action? <br> Do ARTs decrease oxidative stress through viral suppression? <br> Will antioxidant supplementation counteract the effects of ARTs or HIV on oxidative stress? <br> Is there a role for antioxidant supplementation in lipodystrophy? <br> Is there a role for antioxidant supplementation in preventing or reducing the severity of cognitive defects associated with HIV-infection? |
| Bone loss | Is bone loss in HIV-positive patients due to HIV-infection itself or to the effects of ARTs? <br> Can some ARTs reduce bone loss? <br> Will Vitamin D supplementation help to reduce bone loss? |
| Co-infections | Will micronutrient supplementation help to minimize the morbidity associated with co-infections, such as hepatitis B, hepatitis C, and tuberculosis? <br> If so, which micronutrients and at what dose? |

HAART, highly active antiretroviral therapy; ART, antiretroviral treatment.

Research is also needed to address the appropriate dosages of micronutrient supplementation among HIV-infected individuals at various stages of disease and treatment. Little consistency has been found in the studies done to date on dose or duration of administrations, making comparisons between studies and interpretations of results difficult.

The intention of clinicians and clinical researchers in HIV in 2005 should be to maximize the quality and duration of survival for HIV-infected individuals throughout the world. This objective could entail the study and use of interventions, such as micronutrients, that may not be part of the traditional care or thought process of the HIV care provider. Attempts to improve dietary quality and micronutrient status may play an overall role in maximizing health for the HIV-infected individual, particularly in undernourished populations, and may also play a role in the more subtle management of HIV infection in the future.

## Acknowledgements

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