Introduction

Human immunodeficiency virus (HIV) infection is one of the major public health challenges worldwide. According to most recent estimates, approximately 40 million persons are HIV-infected, among them 2.7 million children under 15 years of age [1]. An estimated 800,000 new HIV infections occurred among children in 2001, and the majority of these infections were among infants born to HIV-infected women and who acquired the virus in utero, during delivery, or through breast-feeding [1].

Since the start of the pandemic, HIV disease has been associated with protein-energy malnutrition in the form of ‘slim disease’ [2]. Later work showed that micronutrient deficiencies are also common in HIV infection, in particular deficiencies of β-carotene, vitamins A, B, C, and E and the minerals zinc, iron, and selenium [3]. Research among HIV-uninfected populations indicates that nutrient deficiencies and infection often accompany and aggravate each other [4]. Nutrient deficiencies also increase the risk of adverse pregnancy outcomes and may impair childhood development [5, 6]. While knowledge from HIV-uninfected populations is a useful starting point to understand the role of micronutrients in HIV disease, specific research among HIV-infected populations is needed to account for the physiological alterations imposed by the viral infection and to assess the risk of vertical transmission of HIV from mother to child. In this chapter, we will review the role of micronutrients for HIV-infected populations, with a focus on fetal outcomes and child health.
Maternal Micronutrient Status and Fetal and Child Health

In this section, we will describe how a woman’s micronutrient status during pregnancy and during the breast-feeding period affects infant health and risk of infant HIV infection.

Vitamin A deficiency (serum vitamin A levels <1.05 μmol/l) is common among HIV-infected pregnant women in developing and developed countries. The prevalence of vitamin A deficiency among HIV-infected pregnant women participating in a longitudinal study was 58% in Malawi and ranged from 13 to 31% in studies from the US [7–9]. In the Malawi study and one of the US studies, severe maternal vitamin A deficiency (serum vitamin A levels <0.70 μmol/l) was associated with a four- to fivefold increased risk of mother-to-child transmission (MTCT) of HIV after adjustment for several potential confounding factors [7, 9]. However, no such association was found in other studies from the US [8, 10] and the Ivory Coast and Burkina Faso [11]. Maternal vitamin A deficiency during pregnancy, as indicated by low serum retinol levels, was also a risk factor for infant HIV disease progression [12], infant mortality in the first 12 months of life [13], and slowed child growth independent of a child’s HIV status [14].

These observational studies have several limitations. The associations between vitamin A deficiency and adverse health outcomes may be due to reverse causality, i.e. HIV infection may have led to impaired absorption and increased excretion of vitamin A, which may have accounted for the biochemical deficiency. In addition, serum vitamin A levels may have been reduced due to the acute phase response to infection even if liver stores, and therefore vitamin A nutritional status, is adequate [15]. In this case, low serum vitamin A levels would be a marker of, rather than a causal factor for, an advanced stage of HIV disease. In an attempt to adjust for the underlying immunological status of the study participants, which is a potentially important confounding factor, surrogate markers of disease were in some cases adjusted for, such as CD4 cell counts and clinical signs. Since these surrogate markers may inadequately capture the true immunological status, residual confounding remains possible.

Given the limitations of these observational studies, more weight should be given to results from randomized controlled trials in determining whether maternal vitamin A status during pregnancy and the breast-feeding period is a causal factor for MTCT of HIV and child health. In a study from South Africa, small daily doses of vitamin A and β-carotene during the antenatal period and a large dose of vitamin A at delivery had no effect on the risk of MTCT of HIV (table 1) [16]. Similar results were obtained in a study from Malawi, in which HIV-infected pregnant women received vitamin A or placebo during the antenatal period [17]. A trial from Tanzania extended findings from the above trials as it administered vitamin A not only during the antenatal but also during the breast-feeding periods [18]. Vitamin A increased the total risk
Table 1. Randomized, placebo-controlled trials of vitamins administered antenatally and during the breast-feeding period to HIV+ women in relation to child health

<table>
<thead>
<tr>
<th>Study site [Ref.]</th>
<th>Intervention groups</th>
<th>Population</th>
<th>Endpoint</th>
<th>Measure of effect</th>
<th>p value</th>
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<tbody>
<tr>
<td>South Africa [16]</td>
<td>Vitamin A (5,000 IU) and β-carotene (30 mg) or placebo daily during the antenatal period + vitamin A (200,000 IU) or placebo at delivery</td>
<td>750 HIV+ pregnant women</td>
<td>Infant HIV infection by 3 months of age</td>
<td>Vitamin A</td>
<td>20.3% (15.7–24.9)a</td>
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<td></td>
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<td></td>
<td>Preterm delivery ≤37 weeks</td>
<td>Placebo</td>
<td>22.3% (17.5–27.1)a</td>
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<td></td>
<td>Preterm delivery 11.4% a</td>
<td>17.4%a</td>
<td>0.03</td>
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<tr>
<td>Malawi [17]</td>
<td>Vitamin A (10,000 IU) or placebo daily during the antenatal period</td>
<td>697 HIV+ pregnant women</td>
<td>Infant HIV infection status at: 6 weeks</td>
<td>Vitamin A&lt;sup&gt;b&lt;/sup&gt;</td>
<td>62 (26.6)</td>
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<td></td>
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<td></td>
<td>12 months</td>
<td>Placebo&lt;sup&gt;b&lt;/sup&gt;</td>
<td>66 (27.8)</td>
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<td></td>
<td></td>
<td></td>
<td>Proportion of low-birth weight infants</td>
<td>65 (27.3)</td>
<td>80 (32.0)</td>
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<tr>
<td>Tanzania [18–20]</td>
<td>2×2 factorial design: vitamin A (5,000 IU) and β-carotene (30 mg); multivitamins (20 mg B&lt;sub&gt;1&lt;/sub&gt;, 20 mg B&lt;sub&gt;2&lt;/sub&gt;, 25 mg B&lt;sub&gt;6&lt;/sub&gt;, 100 mg niacin, 50 μg B&lt;sub&gt;12&lt;/sub&gt;, 500 mg C, 30 mg E, and 0.8 mg folate); multivitamins and vitamin A; or placebo during the antenatal and breast-feeding periods. Supplements were provided daily</td>
<td>1,078 HIV+ pregnant women</td>
<td>Fetal loss</td>
<td>Multivitamins&lt;sup&gt;b&lt;/sup&gt;</td>
<td>31 (5.9)</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>HIV+ at birth</td>
<td>No multivitamins&lt;sup&gt;b&lt;/sup&gt;</td>
<td>52 (10.0)</td>
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<td></td>
<td></td>
<td></td>
<td>HIV+ at 6 weeks&lt;sup&gt;c&lt;/sup&gt;</td>
<td>38 (10.1)</td>
<td>24 (6.6)</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>Breast-feeding transmission</td>
<td>31 (16.2)</td>
<td>28 (15.6)</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>Total HIV infection</td>
<td>0.85 (0.61–1.19)&lt;sup&gt;d&lt;/sup&gt;</td>
<td>140 (31.0)</td>
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<td></td>
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<td>Total mortality by 24 months</td>
<td>149 (29.0)</td>
<td>173 (33.0)</td>
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<thead>
<tr>
<th>Study site</th>
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<th>p value</th>
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<tbody>
<tr>
<td></td>
<td></td>
<td>Vitamin A^b</td>
<td>No vitamin A^b</td>
<td></td>
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<tr>
<td></td>
<td>Fetal loss</td>
<td>37 (7.0)</td>
<td>46 (8.9)</td>
<td>0.25</td>
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<tr>
<td></td>
<td>HIV+ at birth</td>
<td>38 (10.0)</td>
<td>24 (6.7)</td>
<td>0.11</td>
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<tr>
<td></td>
<td>HIV+ at 6 weeks^c</td>
<td>35 (17.9)</td>
<td>24 (13.8)</td>
<td>0.29</td>
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<td></td>
<td>Breast-feeding transmission</td>
<td>1.33 (0.95–1.86)^d</td>
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<td></td>
<td>Total HIV infection</td>
<td>155 (34.0)</td>
<td>113 (25.0)</td>
<td>0.009</td>
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<tr>
<td></td>
<td>Total mortality by 24 months</td>
<td>163 (31.0)</td>
<td>159 (31.0)</td>
<td>0.97</td>
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^a Risk (95% CI).
^b Number (n) with percent in parentheses.
^c Assessed among those infants who were HIV-negative at birth.
^d RR (95% CI).
of HIV transmission (relative risk, RR, = 1.38, 95% confidence interval, CI, = 1.09–1.76), as defined by transmission occurring during the in utero, intrapartum, and breast-feeding periods [18]. These trials from Africa also examined the effect of vitamin A on other pregnancy outcomes. In the Tanzania study, there was no effect on risk of low birth weight, preterm birth, or small size for gestational age [19]. In the Malawi study, vitamin A reduced the risk of low birth weight but not preterm birth [17], while in the South Africa study, a benefit on prematurity was noted, yet not on low birth weight [16].

The Tanzania study also examined the effect of multivitamins (vitamins B, C, and E) at dosages of several times the recommended dietary allowance during the antenatal and breast-feeding periods on fetal outcomes and child health. First findings of the trial showed that multivitamin supplements resulted in significant reductions (~40%) in the risks of fetal death, low birth weight, and severe prematurity [19]. In subsequent analyses, multivitamins were not related to risk of MTCT of HIV during the in utero, intrapartum, and breast-feeding periods [20]. Subgroup analyses provided results consistent with the fact that the effect of multivitamins on child health was modified by a woman’s immunological and nutritional status during pregnancy [18]. Multivitamins (not including vitamin A) significantly reduced the risk of breast-feeding transmission among children born to women with proxies for poor nutritional status or advanced HIV disease yet showed no or little effect among infants born to women with better proxy values [18]. For example, among children born to women with low lymphocyte counts (<1,340/mm³) at enrollment, the risk of breast-feeding transmission was 0.37 (95% CI = 0.16–0.85) in the multivitamin group relative to the no-multivitamin group; among children born to women with high lymphocyte counts (≥1,340/mm³), the risk was 0.99 (0.68–1.45). Multivitamins also reduced death and prolonged HIV-free survival among children born to nutritionally or immunologically compromised women.

Micronutrients and Child Health

The prevalence of micronutrient malnutrition among HIV-positive children varies considerably, which may be due to differences in diet, age, stage of HIV disease, child care, and availability of antiretroviral therapy. For instance, Eley et al. [21] reported a high prevalence of micronutrient deficiencies in a cohort of HIV-positive South African children with median age of 25 months from an economically deprived setting, with 62% of the children having two or more vitamin or trace element deficiencies. Analyses from the US and France among HIV-infected children aged 2–11 years with access to antiretroviral therapy showed that deficiencies in vitamin A, zinc, and selenium were not common [22, 23]. Another small study from the US
Micronutrients and Child Health in HIV Infection

evaluated the contribution of zinc, selenium, and iron deficiency on disease progression and survival among perinatally infected children. In a multivariate regression model, low plasma selenium was the only nutritional indicator that was significantly related to mortality [24]. In addition, among the children who died, those with low selenium levels died at a younger age, which suggests a more rapid disease progression.

The association between infant micronutrient status and growth has been of interest, as growth failure is a strong prognostic indicator of mortality in pediatric HIV infection [25]. A prospective cohort study with 194 HIV-infected children from Uganda found that plasma vitamin A levels and low concentrations of provitamin and non-provitamin A carotenoids were related to decreased weight and height velocity [26]. When the relation between micronutrient levels and risk of mortality was examined, only low plasma β-carotene remained a significant predictor (RR = 3.16, 95% CI = 1.38–7.21) [26]. In the study by Eley et al. [21], no correlation was observed between micronutrient status and weight-for-age or height-for-age z scores. Likewise, there was no association between baseline vitamin A levels and their change over time with regard to mortality in a study among HIV-positive children in North America [27]. This lack of association may have been due to the infrequency of low vitamin A levels in this cohort.

Vitamin A supplementation reduced childhood mortality in some, but not all, community-based trials among HIV-uninfected children [28]. There is clear benefit of vitamin A supplementation for children who have measles [29]. Vitamin A supplements may also be beneficial in reducing the severity of disease in some cases of diarrhea. With regard to lower respiratory infections, hospital and community-based studies do not support a beneficial role of vitamin A supplements; some studies even suggest a possible adverse effect [29].

Several randomized-controlled trials of vitamin A have been carried out among HIV-infected children (table 2). Two small studies evaluated the effect of vitamin A on immunological and virologic markers of HIV disease. In a study from the US among HIV-infected children who received influenza vaccination, vitamin A therapy resulted in a significant decrease in viral load 14 days after supplementation but did not improve vaccine serologic response [30]. In a study from South Africa, an increase in CD4 cell count, as compared to baseline, was observed in the vitamin A group [31]. However, the clinical significance of the small short-term changes in viral load and CD4 cell count observed in these two studies remains unclear. A trial from South Africa among children born to HIV-infected women demonstrated a significant reduction in diarrheal morbidity among HIV-infected but not among HIV-uninfected children [32]. A modification of the effect of vitamin A by HIV status on child health outcomes was also noted in a Tanzanian study among HIV-infected and uninfected children. The reduction in mortality in the vitamin A group was apparently stronger among those who were HIV-infected, who experienced a significant 63% decreased risk of mortality.
Table 2. Randomized, placebo-controlled trials of vitamins among child populations with HIV infection

<table>
<thead>
<tr>
<th>Study site [Ref.]</th>
<th>Intervention groups</th>
<th>Population</th>
<th>Endpoint</th>
<th>Measure of effect</th>
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<tbody>
<tr>
<td>United States [30]</td>
<td>Vitamin A (200,000 IU) or placebo at days 0 and 1. Both groups received inactivated influenza vaccine at day 14</td>
<td>59 HIV+ children</td>
<td>14-day change in viral load (log_{10}/ml)</td>
<td>Vitamin A group: -0.13 (-0.04, -0.22)^a Placebo group: 0.14 (0.06, 0.22)</td>
</tr>
<tr>
<td>South Africa [31]</td>
<td>Vitamin A (200,000 IU) or placebo on 2 consecutive days</td>
<td>75 HIV+ children</td>
<td>CD4 cell count</td>
<td>Increase (p = 0.03)^b</td>
</tr>
<tr>
<td>South Africa [32]</td>
<td>Vitamin A (50,000 IU) or placebo at 1 and 3 months, vitamin A (100,000 IU) or placebo at 6 and 9 months, and vitamin A (200,000 IU) or placebo at 12 and 15 months</td>
<td>118 children born to HIV+ women (28 were HIV+)</td>
<td>HIV+ children: Diarrheal morbidity 0.51 (0.27–0.99)^c Total morbidity 0.69 (0.36–1.31)^c HIV- children: Diarrheal morbidity 0.89 (0.37–2.10)^c Total morbidity 0.74 (0.34–1.61)^c</td>
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<tr>
<td>Tanzania [33–35]</td>
<td>Vitamin A (200,000 IU/100,000 IU for infants) or placebo on day 1 and 2 of hospitalization, and at 4 and 8 months after discharge</td>
<td>648 children admitted to the hospital with pneumonia (58 were HIV+)</td>
<td>Mortality: Overall 0.51 (0.29–0.90)^c HIV+ children 0.37 (0.14–0.95)^c HIV- children 0.58 (0.28–1.19)^c Acute diarrhea HIV+ children 1.55 (0.75–3.17)^c HIV- children 1.21 (0.98–1.49)^c Cough and rapid respiratory rate HIV+ children 0.54 (0.24–1.20)^c HIV- children 1.47 (1.16–1.86)^c</td>
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<th>Study site [Ref.]</th>
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<tr>
<td></td>
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<td>Growth: HIV+ children and age &lt;18 months</td>
<td>2.8 cm (1.0–4.6)&lt;sup&gt;d&lt;/sup&gt;</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>HIV– children and age &lt;18 months</td>
<td>−0.2 cm (−0.8–0.5)&lt;sup&gt;d&lt;/sup&gt;</td>
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<sup>a</sup> p value for mean change in viral load between vitamin A and placebo groups = 0.02.

<sup>b</sup> From comparison of baseline to 4-week CD4 cell count within the vitamin A group.

<sup>c</sup> RR (95% CI).

<sup>d</sup> Difference in linear growth (95% CI).
(RR = 0.37, 95% CI = 0.14–0.95), compared to a 42% decreased risk among HIV-uninfected children (RR = 0.58, 95% CI = 0.28–1.19) [33]. There was no significant effect of vitamin A on acute diarrhea regardless of HIV status, and the risk of experiencing episodes of cough and rapid respiratory rate was lowered among HIV-infected children in the vitamin A group, but the association was only marginally significant [34]. Vitamin A supplements also improved growth among HIV-infected children by nearly 3 cm but had no effect among HIV-uninfected children [35]. The greater benefits of the supplements among the HIV-infected children may have been due to the fact that they were more undernourished compared to uninfected ones.

Potential Mechanisms

In this section, we will describe how micronutrients may influence fetal and child outcomes through their potential effects on general immunity, viral replication, HIV disease progression, epithelial integrity and mucosal immunity, and pregnancy outcomes.

General Immunity

Nutritional deficiencies are associated with impaired functioning of both the innate and adaptive arms of the immune system. Knowledge of such impairments has motivated vitamin supplementation studies, with the aim to prevent the occurrence of disease or limit its severity once disease has occurred.

Vitamin A has historically been known as the ‘anti-infective’ vitamin and deficiency in this vitamin appears to impair immunity at several levels. Neutrophils constitute the first line of defense against infections through their phagocytic function and production of cytotoxic metabolites. In animal studies, vitamin A deficiency leads to lowered ability of neutrophils to phagocytose infectious organisms and produce active oxidant molecules [36]. Animal models also show an inhibitory effect of vitamin A deficiency on antigen-specific antibody production such as tetanus toxoid. However, studies in humans on vitamin A and antibody response have provided conflicting results [37]. Impairments in cell-mediated immunity have been described in several animal models, such as reduced delayed-type hypersensitivity response in vitamin A-deficient mice and lowered function of cytotoxic T lymphocytes in chicks [38, 39]. In a study among Indonesian children, the researchers noted abnormalities of T-cell subsets among children with clinical vitamin A deficiency as compared to those without, such as a lower proportion of CD4 naïve cells and a lower CD4/CD8 ratio [40]. Upon vitamin A supplementation, the vitamin A group showed increases in the proportion of CD4 naïve cells and the CD4/CD8 ratio, which points to a reversal of the T-cell abnormalities [40]. Increases in CD4 cell count were also
noted in a study with healthy human volunteers who received daily doses of β-carotene for 2 weeks [41].

Vitamin E is a lipid-soluble antioxidant and a modulator of immune response. In animal studies, supplemental vitamin E increased CD4 cell counts and interleukin-2 production [42, 43]. Among healthy ethnic Chinese men and women, vitamin E supplements enhanced indexes of cell-mediated immunity, including increases in the CD4/CD8 ratio and improvements in T-cell proliferation [44]. Vitamin C may be important for immunity by reducing damage mediated by reactive oxygen intermediates, by decreasing T-cell death and enhancing natural killer cell activity [45]. Vitamin B₆ is essential for the maintenance of lymphoid tissues and their immunological functions. As a consequence, vitamin B₆ deficiency is associated with atrophy of lymphoid tissue, lowered lymphocyte counts, depressed response to antibody production after immunization, and lowered delayed dermal hypersensitivity [46]. In clinical studies, patients with low levels of serum vitamin B₁₂ had impaired neutrophil function [47]. Data from in vitro and animal studies indicate that vitamin B₁₂ supplementation is associated with enhanced antibody function and mitogenic responses [47].

Zinc deficiency affects various aspects of the immune system including phagocytic action, cell-mediated immunity, and decreased antibody production [48]. Zinc is indispensable for the action of the hormone thymulin, which regulates proper differentiation and maturation of CD4 cells [49]. Zinc may also prevent the programmed cell death (apoptosis) of precursor T-cell populations and mature CD4 cells through various enzymatic mechanisms and through a chronic production of glucocorticoids [50]. It has been proposed that an imbalance in the Th1-type and Th2-type responses contributes to the immune dysregulation associated with HIV infection [51]. In HIV-uninfected volunteers, an imbalance in the Th1-type and Th2-type responses has been demonstrated following depressed zinc intakes under experimental conditions [52, 53]. The imbalance was corrected by zinc repletion [52].

Selenium deficiency in animal studies has been shown to inhibit nonspecific immune function, humoral immunity, cellular immunity (such as cytotoxicity of T lymphocytes and natural killer cells), and resistance to infection; selenium supplementation, in contrast, enhances these immune functions, as well as resistance to infection [54]. Even when administered to documented ‘selenium-replete’ HIV-uninfected individuals, selenium improved T lymphocyte-mediated immune responses [55].

**Viral Replication**

In vitro studies have documented interactions between HIV and micronutrients. For vitamin A, the evidence of such an interaction has been conflicting. Vitamin A was associated with increased HIV replication in two in vitro studies [56, 57] whereas in three other studies, vitamin A was associated
with decreased HIV replication [58–60]. It is thus unclear how this evidence relates to findings obtained in epidemiological studies.

Vitamin E may slow replication of HIV by means of its neutralizing effect on reactive oxygen species, which can stimulate HIV replication through activation of the nuclear transcription factor-κB (NF-κB) [61]. Vitamin C has been shown to decrease HIV-1 replication in chronically infected T lymphocytes by inhibiting the reverse transcriptase enzyme [62].

Zinc exhibits anti-HIV activity in vitro [63, 64]. As a structural component of the enzyme Cu-Zn superoxide dismutase, zinc contributes to a reduction of HIV-1 replication in tumor necrosis factor-α-activated cell lines [65]. However, HIV is a zinc-dependent retrovirus and heightened availability of zinc may facilitate HIV replication in some cases. Zinc stimulates the activity of the viral enzyme integrase, which integrates viral DNA into host DNA [66]. Likewise, zinc is essential for the HIV-nucleocapsid protein p7 and the HIV-Tat protein that are involved in HIV replication [56, 57].

Selenium exhibits important antioxidant functions in the body as part of the glutathione peroxidase system. High glutathione peroxidase activity may decrease HIV replication mediated by reactive oxygen species [61]. However, there is also evidence that such beneficial effects of high glutathione peroxidase activity may be limited to the late phase of the HIV life cycle, which occurs as the result of transcription of the provirus, and that high glutathione peroxidase activity may be detrimental during the early phase of the viral cycle [67]. In experiments with HIV-infected cell lines, increased glutathione peroxidase activity was followed by a dramatic decline in viability and syncytia formation, accompanied by an enhancement of viral replication [68]. It is thought that these results were brought about by a suppression of the host cell apoptotic response to viral infection [67]. Hence, the role of selenium in viral replication may be contradictory.

**HIV Disease Progression**

There is a limited amount of direct evidence from studies among children on how micronutrient status may affect child health in the presence of HIV infection and account for reductions in mortality and morbidity observed in several child trials. A larger body of evidence exists on the role of micronutrients in HIV disease progression among adults and it may help explain findings from studies among children. HIV disease stage during pregnancy and breast-feeding is also a key determinant for the risk of vertical transmission of HIV, so that factors influencing disease progression may also be relevant for this outcome.

Observational Studies

The relationship between vitamin status and HIV disease progression among adults has been examined in several longitudinal studies. In the San Francisco Men’s Health (SFMH) study [69] and the Multicenter AIDS cohort
study (MACS) [70, 71], two studies among HIV-infected homosexual and bisexual males, a higher intake of vitamins was associated with slower progression of the disease. In the SFMH study, CD4 counts at baseline were positively related to intake vitamins B₁, B₂, and niacin. Risk of disease progression was inversely related to intake of vitamins A, E, C, B₁ and B₂, in addition to multivitamin use [69]. In the MACS, vitamin A intake had a U-shaped relationship with the risk of progression to AIDS [70] and the risk of death [71]. A higher intake of niacin, vitamin C, and vitamins B₁, B₂, and B₆ was associated with slower progression to AIDS, and all of these with the exception of vitamin C were also associated with a lower risk of mortality in the same studies. Therapeutic use of B vitamins was positively related to delay of progression to AIDS and death among a cohort of black HIV-infected individuals in South Africa [72].

Other research related serum vitamin levels to clinical outcomes. In the AIDS Linked to Intravenous Experiences study, serum retinol levels were inversely associated with the risk of mortality and wasting among individuals infected with HIV [73, 74]. In a third study among HIV-infected homosexual men, those study participants who developed biochemical deficiency of vitamin B₁₂ or vitamin A during an 18-month period experienced a decline in CD4 cell count, while higher cell counts were noted among those whose vitamin B₁₂ and vitamin A levels normalized over the same period [75]. In the MACS study, men with lower serum vitamin E or vitamin B₁₂ levels were more likely to progress to AIDS compared to those with higher levels; however, no such relation was observed with serum vitamin A levels [76].

The association between selenium and zinc status and HIV disease progression has also been examined in longitudinal studies. In the MACS cohort, higher intake of dietary zinc was associated with an increased risk of developing AIDS [70] and poorer survival [71]. Any intake of zinc supplements was also significantly related to poorer survival. No association between increased dietary zinc intake (from food and supplements) and time to progression to AIDS was observed in the SFMH cohort [69]. In studies that used biochemical markers to assess zinc status, low serum zinc was a significant predictor for progression to AIDS in one study [77] and normalization of plasma zinc was associated with a significant increase in CD4 cell counts in a second one [75]. In a third study, low levels of plasma zinc were predictive of mortality in single nutrient analyses that controlled for CD4 cell counts but not in analyses that also considered other nutrient deficiencies [78]. Serum selenium levels were a significant predictor of opportunistic infections and death in a 1-year study among HIV-infected adults [79]. Among intravenous drug users, selenium deficiency was associated with an exceptionally high risk of mortality (RR = 10.8, 95% CI = 2.31–49.2) even after adjusting for CD4 cell count and other nutrient deficiencies [78].

Anemia is a common hematological complication during HIV disease in both adults and children. The pathogenesis of anemia during HIV disease is
often multifactorial and contributing factors include ineffective hematopoiesis, infectious and neoplastic diseases, drug therapy, and iron deficiency [80, 81]. Anemia is associated with lowered quality of life, advanced disease progression, and early death [80]. Among children, anemia is also associated with slowed psychomotor development and lowered cognitive behavior [82]. In a recent study from Uganda, anemia (hemoglobin <110 g/l) was present among nearly 91% of HIV-infected infants and 44.3% of these infants also displayed signs of iron deficiency anemia (hemoglobin <110 g/l and ferritin <12 μg/l) [81]. It is unknown how well indicators of iron deficiency anemia perform during HIV disease, and the responsiveness of anemia to iron supplementation is thus not clear. Nevertheless, the supplementation of HIV-infected individuals living in areas of the world with a high incidence of iron-deficiency anemia has been recommended [83].

Several reports have implicated iron loading with adverse HIV-related outcomes. For example, thalassemia patients who received the chelating drug desferrioxamine had improved survival while patients who received the iron-containing drug dapsone as part of a secondary prophylaxis trial of Pneumocystis carinii pneumonia had lower survival [84, 85]. However, the relevance of these findings for HIV-infected populations from developing countries with high levels of anemia is not clear; in fact, a cross-sectional study among pregnant women from Malawi did not support a positive association between iron status and HIV disease severity [86].

Trials

A number of randomized, controlled trials have been conducted to investigate the effect of micronutrient supplements on immunological, virological, and clinical outcomes in HIV disease. In a cross-over study among 21 patients, those who received 180 mg of β-carotene daily for 4 weeks experienced a small increase in total white blood cell count, change in CD4 cell count, and percent change in CD4:CD8 ratio compared with subjects on placebo; these parameters decreased when the subjects in the β-carotene arm were switched to placebo (table 3) [87]. However, in a larger study with longer follow-up, no effect of β-carotene on these outcomes was observed [88]. In two studies from the US, a single large dose of vitamin A had no effect on CD4 cell measurements and viral load [89, 90]. An effect of vitamin A supplements on CD4 cell count was also absent among a cohort of HIV-positive women in Kenya who received vitamin A or placebo on a daily basis for 6 weeks [91]. Small daily doses of vitamin A and β-carotene administered to pregnant women did not affect the mean change in viral load in a placebo-controlled study from South Africa [92]. In a Canadian study, the effect of daily high-dose vitamin E and C supplements on oxidative stress and viral load was examined [93]. Markers of oxidative stress were improved, as indicated by reductions in breath pentane, plasma lipid peroxides, and malondialdehyde. The results were also suggestive of a reduction in viral load [93].
<table>
<thead>
<tr>
<th>Study site [Ref.]</th>
<th>Intervention groups</th>
<th>Population</th>
<th>Endpoint</th>
<th>Measure of effect</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>United States [87]</td>
<td>β-Carotene (180 mg) or placebo daily for 4 weeks</td>
<td>21 HIV+ subjects</td>
<td>CD4 cell count (/mm³)</td>
<td>Baseline mean (range)</td>
<td>0.11a</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>4 weeks mean (range)</td>
<td>339.5 (12–882)</td>
</tr>
<tr>
<td>United States [88]</td>
<td>β-Carotene (180 mg) or placebo daily for 3 months. Both groups received multivitamins</td>
<td>72 HIV+ subjects</td>
<td>CD4 cell count (/mm³)</td>
<td>Baseline mean (SD)</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>3 months mean (SD)</td>
<td>335.4 (29.5)</td>
</tr>
<tr>
<td>United States [89]</td>
<td>Vitamin A (200,000 IU) or placebo, single dose</td>
<td>120 HIV+ injection drug users</td>
<td>CD4 cell count (/mm³)</td>
<td>HIV viral load (log₁₀/ml)</td>
<td>0.17</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>β = 0.0045b</td>
<td>0.96</td>
</tr>
<tr>
<td>United States [90]</td>
<td>Vitamin A (300,000 IU) or placebo, single dose</td>
<td>40 HIV+ women</td>
<td>CD4 cell percentage</td>
<td>HIV viral load (log₁₀/ml)</td>
<td>0.56</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>–</td>
<td>0.66</td>
</tr>
<tr>
<td>Kenya [91]</td>
<td>Vitamin A (10,000 IU) or placebo daily for 6 weeks</td>
<td>400 HIV+ women</td>
<td>CD4 cell count (/mm³)</td>
<td>–</td>
<td>0.2</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>β = 0.34c</td>
<td>0.2</td>
</tr>
<tr>
<td>South Africa [92]</td>
<td>Vitamin A (5,000 IU) and β-carotene (30 mg) or placebo daily during the antenatal period + vitamin A (200,000 IU) or placebo at delivery</td>
<td>24 HIV+ pregnant women</td>
<td>HIV viral load (log₁₀/ml)</td>
<td>Baseline mean (SD)</td>
<td>0.21</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1 week postdelivery mean (SD)</td>
<td>3.58 (0.98)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Mean change</td>
<td>0.31 (0.41)</td>
</tr>
<tr>
<td>Country</td>
<td>Intervention/Placebo</td>
<td>Description</td>
<td>Baseline mean (SD)</td>
<td>Week 12 mean (SD)</td>
<td>Mean change</td>
</tr>
<tr>
<td>-----------</td>
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<td>-----------------------------------------------------------------------------</td>
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</tr>
<tr>
<td>Canada</td>
<td></td>
<td>Vitamin E (800 IU) and vitamin C (1,000 mg) or placebo daily for 3 months</td>
<td>49 HIV+ subjects</td>
<td>HIV viral load (log_{10}/ml)</td>
<td>Intervention</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Baseline mean (SD)</td>
<td>4.13 (0.27)</td>
<td>4.42 (0.39)</td>
<td>&lt;0.25</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Week 12 mean (SD)</td>
<td>3.67 (0.40)</td>
<td>4.92 (0.19)</td>
<td>&lt;0.025</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Mean change</td>
<td>–0.45 (0.39)</td>
<td>0.50 (0.40)</td>
<td>&lt;0.10</td>
</tr>
<tr>
<td>Tanzania</td>
<td></td>
<td>2 × 2 factorial design: Vitamin A (5,000 IU) and β-carotene (30 mg);</td>
<td>1,078 HIV+</td>
<td>CD4 cell count (/mm³)</td>
<td>Intervention</td>
</tr>
<tr>
<td></td>
<td></td>
<td>multivitamins (20 mg B₁, 20 mg B₂, 25 mg B₆, 100 mg niacin, 50 μg B₁₂, 500 mg</td>
<td>pregnant women</td>
<td></td>
<td></td>
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<tr>
<td></td>
<td></td>
<td>C, 30 mg E, and 0.8 mg folate); multivitamins and vitamin A; placebo during</td>
<td></td>
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<tr>
<td></td>
<td></td>
<td>the antenatal and breast-feeding periods. Supplements were provided daily</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Baseline mean (SD)</td>
<td>424 (207)</td>
<td>423 (211)</td>
<td>0.72</td>
</tr>
<tr>
<td></td>
<td></td>
<td>6 weeks postpartum mean (SD)</td>
<td>596 (312)</td>
<td>520 (339)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td></td>
<td>30 weeks postpartum mean (SD)</td>
<td>522 (278)</td>
<td>482 (268)</td>
<td>0.05</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Mean difference 1st (SD)</td>
<td>167 (210)</td>
<td>112 (268)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Mean difference 2nd (SD)</td>
<td>99 (208)</td>
<td>59 (167)</td>
<td>0.003</td>
</tr>
<tr>
<td>South</td>
<td></td>
<td>Vitamin A (5,000 IU) and β-carotene (30 mg); or placebo daily during the</td>
<td>312 HIV+ pregnant</td>
<td>HIV-related</td>
<td>Intervention</td>
</tr>
<tr>
<td>Africa</td>
<td></td>
<td>antenatal period + vitamin A (200,000 IU) or placebo at delivery</td>
<td>women</td>
<td>symptoms:</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Prenatal period</td>
<td>RR (95% CI) 1.21 (0.42–3.44)</td>
<td>RR (95% CI) 0.73 (0.27–1.94)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Postnatal period</td>
<td>RR (95% CI) 1.41 (0.73–2.72)</td>
<td>RR (95% CI) 1.42 (0.49–4.17)</td>
<td></td>
</tr>
</tbody>
</table>

(continued overleaf)
### Table 3. (continued)

<table>
<thead>
<tr>
<th>Study site</th>
<th>Intervention groups</th>
<th>Population</th>
<th>Endpoint</th>
<th>Measure of effect</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Zambia [95]</td>
<td>Vitamin A (10,500 IU), vitamin C (300 mg), vitamin E (300 mg), selenium (150 mg), and zinc (200 mg) or placebo daily for 14 days. Both groups received albendazole</td>
<td>106 HIV+ adults with persistent diarrhea</td>
<td>Diarrheal morbidity (time with diarrhea)</td>
<td>RR 0.99</td>
<td>0.98</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Mortality</td>
<td>RR 1.06</td>
<td>0.87</td>
</tr>
</tbody>
</table>

*a Denotes p value for difference between mean baseline and mean 4-week value.  
*b Regression coefficient obtained from multivariate linear regression model.  
*c Regression coefficient obtained from multivariate linear regression model using the square root of CD4 cell count as the dependent variable to approximate normality.  
*d Mean differences 1 and 2 are changes between baseline measurements and measurements at 6 weeks and 30 weeks postpartum, respectively.
A lack of effect of vitamin A on immunologic parameters was confirmed in the Tanzania study among pregnant women [19]. On the other hand, multivitamins (i.e. vitamins B, C, and E) in the same study resulted in improvements in CD4, CD8, and CD3 cell counts. However, as in previous studies, the clinical significance of these findings is not yet known. In a South African cohort of pregnant women, vitamin A and β-carotene supplements did not affect HIV-related symptoms during pregnancy, including fever, weight loss, diarrhea, and loss of appetite. In addition, there was no effect on pregnancy-related symptoms, as defined by lower abdominal pain and nausea and vomiting [94]. Finally, in a study from Zambia among adults with persistent diarrhea receiving albendazole, multivitamins plus selenium and zinc provided over a 2-week period did not offer any advantage over placebo with regard to diarrheal morbidity (measured as time with diarrhea after completion of treatment) or mortality [95].

**Epithelial Integrity and Mucosal Immunity**

**Genital Immunity**

A potential relationship between micronutrient status and integrity of the lower genital tract mucosa was described in early studies among rats, in which vitamin A deficiency was associated with cornification of the epithelium of the lower genital tract [96, 97]. This may have implications for MTCT of HIV, as a weakened lining of the lower genital tract may increase the risk of injury and bleeding during the process of delivery and increase the possibility that the baby becomes exposed to infectious maternal material.

Humoral and cellular immune function in the lower genital tract may prevent the shedding of HIV in lower genital tract secretions. There is evidence that micronutrient deficiency may result in weaker genital mucosal immunity and increase the risk of vertical transmission due to increased HIV shedding in genital secretions. In studies from Kenya, low serum levels of vitamin A [98, 99] and selenium [100] were positively related to the risk of HIV shedding in the female genital tract.

In another Kenyan study, subjects were randomized into three experimental arms to receive vitamin A alone; vitamins B, C, E and selenium, or placebo (table 4) [91, 101]. The study was designed to examine the effect of the micronutrient supplements on risk of HIV shedding in genital secretions. Vitamin A had no effect on HIV shedding as measured by the prevalence of HIV DNA in vaginal swabs or median vaginal HIV RNA concentration [91]. Selenium and multivitamins increased the prevalence of both quantifiable HIV-infected cells in vaginal secretions and prevalence of quantifiable vaginal HIV RNA [101]. However, selenium and multivitamins did not affect the prevalence of cervical HIV-1-infected cells and cervical HIV-1 RNA. Findings from the Tanzanian study among HIV-infected pregnant women [19] indicate that multivitamins (vitamins B, C, and E) did not have an effect on viral shedding in cervicovaginal lavage specimens (Fawzi et al., unpublished data).
<table>
<thead>
<tr>
<th>Study site [Ref.]</th>
<th>Intervention groups</th>
<th>Population</th>
<th>Endpoint</th>
<th>Measure of effect</th>
<th>p value</th>
</tr>
</thead>
</table>
| Kenya [91]        | Vitamin A (10,000 IU) daily for 6 weeks | 400 HIV+ women | Prevalence of HIV-1 DNA in vaginal swabs | Vitamin A 31 (18)
Vaginal HIV-1 RNA concentration (log_{10} copies/swab) | 0.4 |
| Kenya [101]       | Multivitamins (20 mg B\(_1\), 20 mg B\(_2\), 25 mg B\(_6\), 100 mg niacin, 50 \(\mu\)g B\(_{12}\), 500 mg vitamin C, 30 mg vitamin E, and 0.8 mg folate) and 200 \(\mu\)g selenium | 400 HIV+ women | Prevalence of quantifiable vaginal HIV-1 RNA | Intervention 133 (76)
Placebo 113 (64) | 0.02 |

\(^a\) Number (n) with percent in parentheses.

\(^b\) Median.

\(^c\) There was no effect on cervical HIV-1-infected cells or cervical HIV-1 RNA.
It is not clear which factors accounted for the differences in results between the Kenyan and Tanzania studies, given that the multivitamin regimens used in the studies were identical. Important differences may be that the study participants in the Kenyan study were not pregnant, at a more advanced disease stage, and received selenium in addition to the multivitamin regimen. Differences in the collection of specimens, i.e. vaginal and cervical swabs versus cervicovaginal lavage, may also account for some of the differences.

Breast Immunity

Breast-feeding transmission of HIV accounts for an additional 14% risk of MTCT of HIV from prevalent maternal infections [102]. Micronutrients may play a role in breast-feeding transmission by affecting the risk of mastitis. Mastitis is an inflammatory process in the breast that is accompanied by local tenderness as well infiltration of leukocytes and extracellular fluid into the breast milk [103]. Mastitis is a strong risk factor for MTCT during the entire breast-feeding period yet is of particular concern during the first 3 months following delivery, when a large proportion of HIV transmission through breast milk occurs [104]. In studies among cattle, mastitis or subclinical mastitis was associated with deficient levels of selenium and vitamins A, D, and E [105]. In supplementation studies in cattle, vitamins A, D, and E decreased the incidence of mastitis [106, 107]. In an observational study among HIV-infected pregnant women in Kenya, low plasma vitamin A concentrations were associated with a higher risk of viral shedding in breast milk [108]. A Tanzanian study among individuals whose HIV status was not determined suggests that antioxidants may result in a reduced risk of subclinical mastitis, as vitamin E-rich sunflower oil (but not provitamin A-containing palm oil) decreased milk Na/K ratio, which is used an indicator of subclinical mastitis [109]. Vitamin A supplements alone may not reduce the risk of subclinical mastitis, as illustrated by findings from a trial conducted in Bangladesh [110].

Gastrointestinal Immunity

There is evidence that micronutrient deficiencies facilitate injury of the gut mucosa and disruption of gut immunological barriers. This may be of relevance for vertical transmission of HIV, since the virus may be more likely to penetrate the compromised gastrointestinal mucosa of infants. Vitamin A deficiency leads to reduced intestinal cell division and differentiation, as well as a reduction in the amount of luminal mucus and the number of goblet cells. In two trials among infants in India, large doses of vitamin A improved the barrier function of the gut as measured by the lactulose-mannitol permeability test [111]. Vitamin A, in addition to protein deficiency, has also been associated with reduced secretory IgA, natural killer cell activity,
and intraepithelial count [112]. In a trial from South Africa, vitamin A and β-carotene supplementation of HIV-infected pregnant women improved markers of gut integrity among infants who themselves became HIV-infected [113]. An improvement in gut integrity may have important implications for gastrointestinal morbidity.

Adequate zinc status plays a role in intestinal mucosal repair and zinc deficiency leads to ultrastructural changes in the small intestine [114]. Zinc supplementation may improve intestinal permeability during childhood diarrhea, which may be suggestive of enhanced mucosal recovery [115].

**Pregnancy Outcomes**

Prematurity and low birth weight are risk factors for child morbidity and mortality, and have been associated with an increased risk of MTCT of HIV [116]. Evidence suggests that maternal micronutrient deficiency is implicated in the etiology of prematurity and low birth weight. In an observational study among presumably HIV-uninfected poor urban women from the US, prenatal micronutrient supplement use was associated with significant reductions in the risk of preterm and low birth weight delivery [117]. Despite adjustment for potential confounding factors, residual confounding cannot be excluded as an alternative explanation of these findings. In observational studies among HIV-uninfected populations, deficiency of zinc during pregnancy has been associated with low birth weight, intrauterine growth retardation, preterm delivery, premature rupture of the membranes, and the need for assisted or operative delivery [118]. However, causality is difficult to establish from these observational designs due to the possibility of unmeasured confounding.

A placebo-controlled trial conducted in Nepal examined the effect of small weekly doses of vitamin A or β-carotene on pregnancy outcomes among HIV-uninfected women who showed moderate levels of vitamin A deficiency [119]. The supplements, which were administered before conception, during pregnancy, and through 24 weeks postpartum did not have an effect on fetal loss or infant mortality in the first 6 months of life. Placebo-controlled trials of zinc during pregnancy indicate that zinc supplementation may be beneficial only for certain subgroups, such as those who are zinc deficient and in whom other micronutrient deficiencies do not limit the utilization and metabolism of zinc. The presence or absence of zinc deficiency may explain why zinc supplementation resulted in a significant increase in birth weight among low-income women with low plasma zinc levels in the southeastern US [120] but not among health middle-class women in Denmark [121]. The presence of other micronutrient deficiencies may help account for the lack of improvements in birth outcomes among nutritionally deprived urban poor in Bangladesh [122]. Protective associations with pregnancy outcomes also exist for other nutrients including iron, folate, calcium, and vitamin C. However, more research is needed to confirm these associations.
Conclusion

We have reviewed published reports on the role of micronutrient status in modulating fetal and child health in the presence of HIV infection. Several conclusions can be drawn from the existing body of evidence. With regard to child mortality, vitamin A has important benefits for HIV-infected children in developing countries. The effect of vitamin A on morbidity among HIV-positive children is less clear, but risk reductions for diarrheal morbidity, cough and rapid respiratory rate and improvements in child growth have become apparent. The effects of other nutrients on health of HIV-infected children have not been examined.

An elevated risk of vertical transmission was associated with maternal vitamin A deficiency, as determined by low levels of serum vitamin A, in some but not all observational studies. However, randomized, placebo-controlled trials have shown no protective effects of vitamin A given during pregnancy on vertical transmission. In one trial, vitamin A supplements administered during the prenatal and breast-feeding periods resulted in an increased risk of vertical transmission. Multivitamins (vitamins B, C, and E) may not have an effect on vertical transmission, except for breast-feeding transmission among women with impaired immunological and nutritional status.

The risk of fetal outcomes such as low birth weight and prematurity was lowered in some vitamin A supplementation studies but not in others. It is difficult to identify factors that account for these contradictory findings. Multivitamins (vitamins B, C, and E) led to important reductions in the risk of fetal death, low birth weight, and severe prematurity in a study from Tanzania. These results are promising for the value of nutrition in HIV disease. It will be important to examine whether dosages at several times the recommended dietary allowance, as used in the Tanzanian study, are needed to observe these effects or whether lower dosages are sufficient. If dosages closer to the recommended intakes are sufficient, this would be further support for an approach that promotes the use of whole foods, which is likely to be more sustainable. Nevertheless, nutrition therapy may meet the need for cheap and simple interventions that can be universally implemented in developing countries and that provide public health benefits regardless of HIV-1 status.

References

Micronutrients and Child Health in HIV Infection

Micronutrients and Child Health in HIV Infection


Sprietsma JE: Cysteine, glutathione (GSH) and zinc and copper ions together are effective, natural, intracellular inhibitors of (AIDS) viruses. Med Hypotheses 1999;52:529–538.


Micronutrients and Child Health in HIV Infection

Micronutrients and Child Health in HIV Infection


Discussion

Dr. Sazawal: Have you any kind of hypothesis for the mechanism of the effects observed in the B vitamin group? The reason I am asking this question is because these women were given iron and folate routinely and then vitamin C. Do you have any immunological data? Could it have been through the improvement in iron absorption and the iron status? Do you have any indicators to prove or disprove that?

Dr. Fawzi: It is possible that it could have acted through enhanced absorption of iron particularly since the multivitamins included a large dose of vitamin C. It is hard to know for sure. We did look at hemoglobin levels at 6 weeks postpartum and the women who received vitamins B, C and E during pregnancy had significantly higher hemoglobin levels compared to those who did not. Whether that is due to a beneficial effect of the vitamins themselves or enhanced absorption of iron is obviously hard to know for sure. We also observed a significant improvement in the immune status of the mothers, at least through 6 months after delivery, and that might also explain the beneficial effect as far as pregnancy outcomes or child health outcomes are concerned.

Dr. Pettifor: You showed a reduction in infant mortality in the first 2 years of life in those that received the multivitamins, is that correct?

Dr. Fawzi: That is right.

Dr. Pettifor: And a reduction in HIV transmission?

Dr. Fawzi: Within children born to women who were immunologically or nutritionally compromised.

Dr. Pettifor: If you look at those infants who were HIV-positive, was there any reduction in mortality in that group specifically as opposed to the total group?

Dr. Fawzi: We stratified by HIV status and the beneficial effect tended to focus more among HIV uninfected children, so those who were infected did not really benefit from the supplements.

Dr. Verhoef: Can I ask what the basis for the selection of the multinutrients was? Why didn’t you include things that might be expected from a mechanistic point of view to enhance the effect of vitamin A, for example zinc? Perhaps you could answer that question.

Dr. Fawzi: It is hard to be really sure what the best mix of supplements is. We looked at vitamin A separately because there was more evidence based on observational data that vitamin A might be beneficial. The studies looked at plasma levels of vitamin A with respect to disease progression as well as mother-to-child transmission, so that was worth looking at by itself. With respect to the other nutrients there was some concern about zinc, largely from observational data, that a higher intake of zinc might be associated with increased disease progression, so we tended to stay away from that at least in this initial trial. There was a lot of evidence in favor of the other vitamins particularly B, C and E, but it was hard to look at them individually. Another nutrient that could have been included is selenium. At the time we started the trial, which was in 1995, there was not really much evidence in favor of selenium. More recently, over the last few years, there have been some data [1, 2], still observational, that suggested that low plasma selenium is also harmful. So the way we have gone about it is to look separately at vitamins B, C and E and vitamin A. We found that vitamins B, C and E are very clearly beneficial with respect to adverse pregnancy outcomes, and that has become part of the standard of care in our setting for
HIV-positive women. Among HIV-negative women it is still a question of investigation that we are pursuing with separate trials. But for HIV-positive women we are about to complete a trial that looks specifically at zinc by itself. These are HIV-positive pregnant women who receive iron folate and vitamins B, C and E which has become a part of standard of care, and on top of that they are randomized to zinc versus placebo. Hopefully in the next year we will start another trial on selenium.

*Dr. Christian:* I have two questions. What is the mechanism that you are postulating for the adverse effect of vitamin A supplementation and is the dose and the length of supplementation with vitamin A a potential issue with regard to the adverse effect? And the second question: your vitamin A arm had β-carotene (30 mg), and do you think that β-carotene was doing something differently? You are calling it vitamin A, but there is a huge amount of β-carotene.

*Dr. Fawzi:* Yes you are absolutely right. Obviously it is impossible to disentangle the potential effects of β-carotene from vitamin A. There has been a lot of evidence from observational data that vitamin A might be beneficial. These were pregnant women and we did not want to go up to higher doses so we limited the dose to 5,000 IU which is safe as far as potentially teratogenic effects are concerned. We wanted to potentiate that dose as much as possible through the use of β-carotene, which might be converted to vitamin A. Alternatively, β-carotene may act as an antioxidant; there is evidence in the literature about potential beneficial effects of antioxidants in HIV disease. But it is hard disentangle the two. There are studies that have looked at β-carotene in lung cancer and have found that β-carotene is associated with an increased risk of lung cancer. Whether the particular adverse effect in the Tanzania trial is due to β-carotene or the preformed vitamin A is hard to be sure of. But there are studies, limited as they are, in the literature that raise concerns about preformed vitamin A. Some in vitro studies suggest that vitamin A is associated with increased replication of the virus [3, 4]. The mechanism postulated is that vitamin A might increase the differentiation of lymphoid and myeloid cells which is how it might be beneficial in reducing mortality and morbidity in general, and through that increased differentiation it leads to an increased expression of CCR5 receptors that are important for attachment of the virus and eventually replication of the virus. A study in Kenya looked at serum retinol levels in an observational way and heterosexual transmission, and they found that those who had high plasma levels of vitamin A tended to transmit the virus more to their HIV-negative partners [5]. The other data that can be brought here is the U-shaped relationship that has been observed in the MACS cohort that suggest that perhaps high levels of intake, which were not really very high, could be potentially harmful in terms of faster disease progression [6] and risk of mortality [7].

*Dr. Christian:* What is the duration?

*Dr. Fawzi:* The supplements were given during pregnancy (starting on average at about 20 weeks gestation), until delivery and through breastfeeding.

*Dr. Young:* HIV infection has a profound effect on the partitioning of energy substrate utilization on protein turnover, on the synthesis of specific proteins, retinol-binding protein for example, and on the partitioning of protein metabolism within the body. To what extent has this understanding and knowledge being taken into account in the design of your studies and in the interpretation thereof?

*Dr. Fawzi:* I am not sure if I am answering your question directly, but part of the concern about the prior studies, that were mainly observational and used plasma levels, is that within the context of HIV infection plasma levels of vitamin A are not good markers of vitamin A status. Even in the presence of adequate liver stores of vitamin A there could be a reduction in plasma levels. The associations that were observed showing that low plasma levels are associated with increased transmission
or increased mortality could alternatively be interpreted as an association with advanced disease stage, for which low plasma retinal may be a proxy. So that was necessary therefore to move to the next level of study design and look at it in a randomized controlled fashion.

**Dr. West:** It is a very important study so it deserves all these great questions. I really tried to look and see a J-shape in that curve. I could not find it. The one that you showed was a U-shape. Was there a J-shape there?

**Dr. Fawzi:** I think you are referring to the paper by Tang et al. [6]. They reported that the risk of HIV progression was smallest in the second and third quartiles, as compared to the lowest and highest quartiles. The risk in the lowest and highest quartiles was also about the same, pointing to a U-shaped relationship.

**Dr. West:** In terms of the amount of β-carotene, at 30 mg as you pointed out, there has been evidence of increased chronic disease with about that same dosage for a long time. Have you looked at indicators of oxidative stress in these 2 groups and if so, is there any evidence that there was a difference in total antioxidant capacity? Is it different and could it have been higher in the β-carotene-supplemented group? The third question relates to the N that was used for the analysis of the transmission data. The transmission was based on 580 infants at 2 years of age while the mortality data were based on 998 or 989. Therefore, you were able to pretty much ascertain vital status for the full cohort, but the transmission data were based on an N that was 40% lower than that. Were the treatment groups really balanced in terms of those infants for whom you did not have transmission data?

**Dr. Fawzi:** On the issue of oxidative stress we did not look at that but we would like to do so at some point when time and funds allow it. We are in the process of looking at HIV viral load and will be able to see whether that is one of the potential mechanisms how vitamin A had the detrimental effect and vitamins B, C, and E had the beneficial effect. On the sample size, we describe this point more completely in the paper [8]. Briefly, it was 580 that had final HIV status, which is defined as HIV status after the cessation of breast-feeding, and 318 were HIV negative at the last specimen that was examined but obviously we did not have it at the time that they stopped breastfeeding, either because the child died or the mother moved out of the city, and a large part of that was due to mortality of the mother or child, probably more of the child. So the transmission analysis was based on much more than 580, namely 898 and obviously some were truncated the last time we knew their HIV status, our mid point between the last negative and the first positive for those individuals.

**Dr. West:** Did you do the analysis just for the 580, or whatever, for whom you have the 2-year status, and eliminate the others for whom you had to impute their 2nd birthday status, just to see that those lines remain the same even for the subgroup for whom you have got complete follow-up?

**Dr. Fawzi:** We did not impute 2nd birthday status but rather censored observations once they were lost to follow-up. We did a lot of analysis to try to make sure that this is indeed the correct message, and unfortunately it is, at least with respect to vitamin A.

**References**


