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Vitamins in HIV Disease Progression and Vertical Transmission

Wafaie W. Fawzi¹ and David J. Hunter²

Human immunodeficiency virus (HIV) infection is a major public health problem worldwide, but particularly in sub-Saharan Africa and Asia. Numerous observational studies report inverse associations between vitamin status, measured biochemically or as levels of dietary intake, and the risk of disease progression or vertical transmission. Evidence to support these findings has been obtained from a few randomized placebo-controlled trials. In this paper, we review studies that examined the role of vitamins A, B, C, D, and E in HIV disease progression and transmission, and we discuss the potential mechanisms of action of these vitamins. Adequate vitamin

intake leads to enhancement of epithelial integrity and systemic immunity and could contribute to improved clinical condition among HIV-infected subjects and reduce vertical transmission by reducing the risk and severity of opportunistic infection and reducing viral load in blood. Adequate vitamin status may also reduce vertical transmission through the intrapartum and breastfeeding routes by reducing HIV viral load in lower genital secretions and breast milk, respectively. Vitamin supplements may be one of a few potential treatments that are inexpensive enough to be made available to HIV-infected persons in developing countries. (Epidemiology 1998;9:457-466)

Keywords: HIV infection, transmission, progression, vitamin A, multivitamins, micronutrients, United States, developing countries, Africa.

The World Health Organization estimates that approximately 22.6 million persons worldwide were living with human immunodeficiency virus 1 (HIV-1) infection or AIDS by December 1996.¹ In several urban centers in sub-Saharan Africa, more than 10% of asymptomatic adults and about 15-30% of women attending prenatal care clinics are infected. HIV infection has been spreading very fast in Asia, particularly in India, Thailand, Burma, and Cambodia. A large body of data suggests that inadequate intake of micronutrients decreases immune function and may accelerate disease progression and increase the risk of vertical transmission in HIV-infected persons. Interest in micronutrient supplementation has been accentuated recently because it may be an important prophylactic and treatment modality for HIV-seropositive persons, particularly in developing countries where specific antiretroviral and prophylactic drugs are virtually unavailable. The relation of vitamin status and heterosexual transmission of HIV has not been examined, probably because of the low rates of

seroconversion to HIV in many communities, and the relatively fewer cohorts that were formed to study factors associated with this endpoint. Here, we present a brief background on the natural history and vertical transmission of HIV infection and review the evidence on the relation between vitamins and these endpoints.

HIV Progression and Vertical Transmission

Progression among HIV-positive children and adults is faster in Africa than in Europe or the United States.² Whereas the median time from seroconversion to AIDS is more than 10 years in the United States,³ it was 45 months in Kenya.⁴ Several laboratory markers, such as declining count of CD4⁺ lymphocytes⁵ and HIV viral load,⁶ are markers of disease progression. Only a few modifiable risk factors for progression have been identified, however, including smoking,⁷ intravenous drug use,⁸ and parasitic and sexually transmitted infections.⁹⁻¹¹

The rates of vertical transmission of HIV in African studies are generally higher (30-45%) than in European and American studies (7-30%).² Transmission of HIV infection can occur *in utero*, near or at the time of delivery, or during breastfeeding. Predictors of vertical transmission have been reviewed recently.^{12,13} The mother's stage of HIV disease and humoral and cellular immune function probably play a role in determining the probability of transmission at all three times. CD4 cell count and viral load are associated with vertical transmission. Chorioamnionitis (inflammation of the chorion and amnion, possibly due to infectious agents)

From the Departments of ¹Nutrition and ²Epidemiology, Harvard School of Public Health, Boston, MA.

Address reprint requests to: Wafaie W. Fawzi, Department of Nutrition, Harvard School of Public Health, 665 Huntington Avenue, Boston, MA 02115.

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may increase placental permeability to HIV,¹⁴ leading to a higher risk of transmission.¹⁵ Neutralizing antibodies against specific domain gp120 of the virus are associated with a reduced risk of transmission in some but not all studies.² Prematurity and low birthweight are other possible risk factors for transmission during labor and during breastfeeding, although they may also be consequences of *in utero* transmission.

The higher rates of HIV disease progression and vertical transmission in developing countries coincide with similarly higher rates of malnutrition and vitamin deficiencies, indicating that HIV infection may be modified by nutritional status. Other possible explanations for this variation in transmission and natural history include differences in maturation of the epidemic; potential differences in viral clade; genetic differences in humans, such as mutations in the CCR5 gene, which codes for a receptor displayed on the surface of CD4 cells; and differences in behavior and in access to programs that can reduce the rate of disease progression, such as prophylaxis and treatment of opportunistic infections, antiretroviral treatment, and supportive care.

Here, we review the epidemiologic evidence that vitamin status may influence HIV progression or vertical transmission. The studies are presented in three parts: vitamin A and HIV disease progression, vitamin A and vertical transmission, and vitamins other than A and disease progression. Within each part, we present epidemiologic evidence in the form of experimental and non-experimental studies when the endpoint was clinical disease progression or its surrogate markers, HIV infection, or mortality in infancy. We discuss studies that shed light on the potential mechanisms of the role of vitamins in HIV infection, including *in vitro* and animal studies of nutrition and HIV infection and studies on nutrition and immune function. We identified the included studies by searching the MEDLINE database from 1980 to 1997 and by reading the reference lists of those articles reporting the relation of nutritional factors and HIV infection.

Vitamin A and HIV Disease Progression

EPIDEMIOLOGIC EVIDENCE

Vitamin A supplementation reduced child mortality in some, but not all, community-based trials among children who were not infected with HIV.¹⁶ Reduction in the duration and severity of diarrhea in these trials was reported; however, an effect on the incidence or severity of respiratory infections has not been reported consistently.¹⁷⁻²¹ With respect to diarrhea, vitamin A deficiency has been related to diarrhea caused by infectious agents, including rotavirus²² and *Salmonella*.²³ Vitamin A supplementation was associated with reduced mortality and reduced severity of diarrhea and respiratory infection among children hospitalized with measles.^{24,25}

In cross-sectional studies, vitamin A deficiency was more prevalent among HIV-infected persons compared with HIV-negative persons.²⁶⁻²⁹ Cross-sectional studies are limited, however, by the difficulty in determining

the temporal relation between HIV infection and vitamin deficiency. HIV infection could lead to nutritional deficiency through decreased food intake, malabsorption, and increased utilization and excretion of nutrients.³⁰ On the other hand, vitamin deficiencies may contribute to faster progression of HIV disease through an adverse impact on immune function.

Longitudinal studies are better suited to examine the relation of nutritional factors and HIV infection. In studies that examined this question, vitamin A status was evaluated either by using a biochemical marker (serum retinol measured using high-pressure liquid chromatography) or by assessment of dietary vitamin A using a food frequency questionnaire. In a case-control study nested within a longitudinal study of intravenous drug users, the risk of death among HIV-infected subjects with adequate serum vitamin A levels was 78% less, when compared with vitamin A-deficient subjects³¹ (Table 1). In another longitudinal study among HIV-positive intravenous drug users in Baltimore, low serum retinol was associated with an increased risk of mortality.³² In a study carried out among HIV-positive homosexual men, development of vitamin A deficiency over an 18-month period was associated with a decline in CD4 cell count, widely used as a marker of HIV immune impairment. Normalization of vitamin A was associated with higher CD4 cell counts.³³ Finally, lower serum levels of vitamin A were associated with a faster rate of progression among men who participated in the Multicenter AIDS Cohort Study (MACS).³⁴ These biochemical studies are limited by the fact that serum vitamin A levels may be reduced as a result of the acute phase response to infection even in the presence of adequate liver stores of vitamin A.³⁵ Hence, vitamin A deficiency may be a marker of, rather than a causal factor for, an advanced stage of HIV disease. Vitamin A is fat-soluble and stored in the liver; for a well nourished person, it takes a long time to develop a deficiency due to poor intake or severe illness. Low serum vitamin A levels may also be an indicator of general nutritional status, although this has not been adjusted for in all studies.

Measuring dietary intake of nutrients is preferable when biochemical indicators are affected by the acute phase response to infection. Among well nourished HIV-seropositive men who participated in the San Francisco Men's Health Study, high energy-adjusted vitamin A intake at baseline was associated with higher CD4 cell count at baseline, as well as with lower risk of developing AIDS during the 6 years of follow up.³⁶ In the MACS study, a U-shaped relation was observed between energy-adjusted vitamin A intake (from diet and supplements) and the risks of progression to AIDS³⁷ or death.³⁸ Levels of intake between 9,000 and 20,000 IU were apparently protective, whereas subjects with higher levels of intake experienced no decrease in risk. A number of limitations need to be considered in the interpretation of these biochemical and dietary prospective studies. First, reverse causality may provide an explanation for the positive association between vitamin deficiency and disease progression: HIV infection could lead to adverse effects

TABLE 1. Prospective Cohort Studies of Nutritional Status in Relation to Progression of HIV Disease

Study Site and Reference	Population	Endpoint	Vitamin	Relative Risk (95% CI)	Variables Adjusted for
United States ¹¹	50 deaths, 235 controls, nested in a longitudinal study of HIV ⁺ injection drug users	Mortality	Serum level: A ≥ 1.05 vs < 1.05 $\mu\text{mol/liter}$	0.22 (0.09–0.55)	CD4 cell count, wasting
United States ¹²	126 HIV ⁺ drug users	Mortality	Serum level: A ≥ 1.05 vs < 1.05 $\mu\text{mol/liter}$	0.23 (0.06–0.91)	CD4 cell count, hepatitis B surface antigen
United States ¹³	108 HIV ⁺ homosexual men	Change in CD4 cell count	Developing deficiency in: Serum vitamin A Serum vitamin B ₁₂ Normalization of: Serum vitamin A Serum vitamin B ₁₂	–176 cells/mm ³ * –112 cells/mm ³ * +61 cells/mm ³ * +95 cells/mm ³ *	AZT use, CD4 cell count
United States ^{14,82}	310 HIV ⁺ homosexual and bisexual men	Progression to AIDS	Serum level: A ≥ 3.15 vs 1.82 $\mu\text{mol/liter}$ E > 23.5 vs < 1.23 $\mu\text{mol/liter}$ B ₁₂ ≥ 305 vs < 105 pmol/liter	0.73 (0.46–1.16) 0.66 (0.41–1.06) 0.55 (0.35–0.86)	Age, symptoms, CD4 cell count, antiretroviral use, serum albumin and folate, alcohol consumption
United States ¹⁶	296 HIV ⁺ homosexual and bisexual men	Progression to AIDS	100% increase (doubling) in dietary intake: Carotenoids Retinol B ₁ B ₂ Folate Niacin C E Daily multivitamin use	0.93 (0.78–1.12) 0.98 (0.78–1.23) 0.92 (0.84–1.01) 0.90 (0.81–1.00) 0.89 (0.66–1.19) 0.83 (0.67–1.04) 0.90 (0.81–1.01) 0.91 (0.83–1.00) 0.69 (0.46–1.03)	Age, smoking, energy intake, symptoms, CD4 cell count
United States ¹⁷	281 HIV ⁺ homosexual and bisexual men	Progression to AIDS	Dietary intake: A 9,062–20,268 vs $< 9,062$ IU/day A $> 20,268$ vs $< 9,062$ IU/day B ₁ > 4.9 vs ≤ 2.4 mg/day B ₂ > 5.9 vs ≤ 5.9 mg/day B ₆ > 5.7 vs ≤ 2.0 mg/day C > 715 vs ≤ 715 mg/day Niacin > 61.0 vs ≤ 61 mg/day	0.55 (0.35–0.88) 0.94 (0.56–1.57) 0.60 (0.36–0.98) 0.61 (0.38–1.00) 0.60 (0.35–1.05) 0.55 (0.34–0.91) 0.52 (0.31–0.86)	Age, symptoms, CD4 cell count, energy intake, antiretroviral use, use of prophylaxis against <i>P. carinii</i>
United States ¹⁸	281 HIV ⁺ homosexual and bisexual men	Mortality	Dietary intake: A 9,098–12,221 vs $< 9,098$ IU/day A $> 13,221$ –20,762 vs $< 9,098$ IU/day A $> 20,762$ vs $< 9,098$ IU/day B ₁ > 5.3 vs ≤ 5.3 mg/day B ₂ > 6.3 vs ≤ 6.3 mg/day B ₆ > 5.9 vs ≤ 5.9 mg/day Niacin > 64.0 vs ≤ 64 mg/day	0.80 (0.48–1.34) 0.72 (0.43–1.21) 1.21 (0.74–1.99) 0.60 (0.38–0.95) 0.59 (0.38–0.93) 0.45 (0.28–0.73) 0.57 (0.36–0.91)	Age, symptoms, CD4 cell count, energy intake, antiretroviral use, use of prophylaxis against <i>P. carinii</i> ; for all nutrients except vitamin A, results are adjusted for beta-carotene and zinc intake

* Association was measured as change in CD4 cell count between baseline and follow-up times.

on appetite, leading to reduced intake, or on absorption and metabolism of nutrients, leading to biochemical deficiency. In the dietary studies, HIV infection may affect dietary habits, although it is unlikely to have a major impact since the subjects in the dietary studies did not know their HIV status at the time of dietary assessment. Also, given that most of individuals in these prospective studies were at an early stage of HIV disease, a major impact of illness on dietary intake is unlikely. Second, onset confounding provides another explanation for the findings, given that all of these studies used prevalent cohorts. This type of confounding occurs when the unknown calendar date of infection is associated both with the risk of disease progression and the exposure variable of interest.³⁹ Factors related to both risk of infection and disease progression, such as sexual behavior or drug abuse, cannot be adequately adjusted for in prevalent cohorts. Different lengths of follow-up time among vitamin-deficient and -sufficient groups could bias the association with disease progression. In an attempt to adjust for the stage of disease, surrogate markers of disease progression were adjusted for, including CD4 cell counts and clinical signs. The adjustment for these factors would not remove the potential bias if vitamin-deficient persons developed AIDS and were thus excluded from the prevalent cohort at the time of its formation.³⁹ Seroincidence cohorts are needed to assess these associations reliably. In some, but not all, of the studies, potential confounding by the use of antiretroviral drugs and prophylaxis for opportunistic infections was adjusted for.

A number of intervention studies, limited by their small sample size and the lack of a control group, provide support for the hypothesis that vitamin A and beta-carotene supplementation result in slower progression of HIV infection.⁴⁰⁻⁴³ Randomized placebo-controlled intervention studies are the best design to assess the relation of nutrient intake to disease progression. In a crossover study among 21 patients, those who received 180 mg of beta-carotene daily for 4 weeks experienced a small increase in the total white blood cell count, change in CD4 cell count, and percentage change in CD4/CD8 ratio compared with subjects on placebo; these parameters decreased when the subjects on the beta-carotene arm were switched to the placebo⁴⁴ (Table 2). In another study by the same investigators in which the same dose of beta-carotene was used, this effect was not observed, however.⁴⁵ Subjects in both groups of the latter study received a multivitamin supplement containing 5,000 IU of vitamin A, which may explain the lack of effect. In a placebo-controlled trial in South Africa among children born to HIV-positive women, vitamin A supplements resulted in approximately 50% reduction in diarrheal morbidity among HIV-infected children.⁴⁶ In another small placebo-controlled trial among HIV-infected pregnant women from South Africa, retinol and beta-carotene did not result in a change in viral load⁴⁷ (Table 2).

TABLE 2. Randomized, Placebo-Controlled Trials of Vitamin A in Relation to Progression of HIV Disease

Study Site and Reference	Population	Intervention	Endpoint	Measure of Effect	
				Intervention	Placebo
United States ⁴⁴	21 HIV+ subjects: 20 men, 1 woman	180 mg beta-carotene daily for 4 weeks	CD4 cell count (/mm ³)	Baseline mean (range): 339.5 (12-882) 4 weeks mean (range): 352.9 (20-846)	411.5 (22-1,379) 366.6 (12-1006)
United States ⁴⁵	72 HIV+ subjects: 63 men, 9 women	180 mg beta-carotene for 3 months; all received multivitamins	CD4 cell count (/mm ³)	Baseline mean (SD)*: 335.4 (29.5) 3 months mean (SD): 296.3 (31.9)	319.2 (28.5) 331.0 (33.3)
South Africa ⁴⁶	118 children born to HIV+ mothers	50,000 IU of retinol at 1 and 3 months, 100,000 IU at 6 and 9 months and 200,000 IU at 12 and 15 months	HIV-infected children: Diarrhea Total morbidity HIV-uninfected children: Diarrhea Total morbidity	RR (95% CI): 0.51 (0.27-0.99) RR (95% CI): 0.69 (0.36-1.31)	
South Africa ⁴⁷	24 HIV+ women at 28-32 weeks of pregnancy	30 mg beta-carotene and 5,000 IU retinol daily for 9-13 weeks	HIV viral load (log ₁₀)	Baseline mean (SD): 3.58 (0.98) 1 week postdelivery mean (SD): 3.55 (0.82)	4.04 (0.75) 4.35 (0.76)

* SD = standard deviation.

TABLE 3. Potential Mechanisms of Action of Vitamin Supplements in HIV Infection

Direction of Potential Effect*	Endpoints of Interest
↑	Disease progression
↓	Integrity of epithelia of respiratory system and gastrointestinal system
↓	Opportunistic infections
↓	Humoral immunity
↓	Cellular immunity
↓	Proinflammatory cytokines
↓	Blood HIV viral load
↓	Perinatal transmission
↓	Rate of HIV disease progression in mother
↓	Viral load in breast milk
↓	Viral load in maternal lower genital tract
↓	Integrity of mother's vaginal epithelium
↓	Placental integrity
↓	Epithelial lining in gastrointestinal system of fetus and infant
↑	Child humoral immunity
↑	Child cellular immunity

* ↓ indicates that vitamin supplements may result in a decreased occurrence of the endpoint; ↑ indicates that vitamins may result in an increased occurrence.

POTENTIAL MECHANISMS

A protective effect of vitamin A in HIV infection may be mediated through enhancing the immune system, thus reducing the severity of opportunistic infections and possibly reducing viral load among HIV-positive subjects (Table 3). Retinoid supplementation was associated with enhanced HIV replication in one *in vitro* study.⁴⁸ In another study, however, retinoic acid has been shown to inhibit HIV replication in infected cells by inducing an antiviral state in the cells.⁴⁹ Carotenoid halocynthiaxanthin may have an inhibitory effect on reverse transcriptase, an enzyme important for HIV replication.⁵⁰ Retinol supplementation of cultures with low baseline cytokine levels from HIV-seropositive individuals resulted in increased production of soluble receptors for interleukin 2, an immune system protein that has potent effects on the proliferation and maturation of several types of immune cells.⁵¹ Supplementation of retrovirally infected mice was associated with higher numbers of activated macrophages compared with infected controls and was associated with a slower death rate for retrovirally infected mice.⁵²

Even before the advent of HIV infection, vitamin A status was shown to be inversely associated with local and systemic immune function. Squamous metaplasia of the epithelial linings of the respiratory system occurs in vitamin A deficiency, leading to reduced levels of secretory immunoglobulin A, decreased mucus secretion, and weakened local barriers to infection.^{53,54} Considerable evidence shows that administration of vitamin A or beta-carotene supplements is associated with enhancement of cellular immunity, as measured by CD4 and CD8 cell counts,⁵⁵⁻⁵⁸ and humoral immunity, as evidenced by antibody response to tetanus⁵⁹ and measles antigens.⁶⁰

Vitamin A and Vertical Transmission of HIV Infection

EPIDEMIOLOGIC EVIDENCE

A growing body of data suggests that low serum levels of vitamin A among HIV-infected pregnant women is associated with a higher risk of vertical transmission of HIV. In Malawi, higher serum retinol of HIV-infected pregnant women was associated with a reduced risk of vertical transmission⁶¹; an increase of 0.45 μmol per liter in serum vitamin A levels was associated with a 44% reduction in the risk of transmission (Table 4). In the same study population, a similar increase in serum levels was associated with about 50% reduction in infant mortality.⁶² In a study from Rwanda, mothers with normal levels of vitamin A were about half as likely to have a dead infant or an HIV-positive infant as were vitamin A-deficient women.⁶³ The relation of vitamin A status and the risk of vertical transmission of HIV infection was also examined in the Women and Infants Transmission Study (WITS), a U.S. prospective study among HIV-infected pregnant women and their infants. Serum retinol was measured at two times during pregnancy. Vitamin A at any particular time was not associated with the risk of transmission. Women who had increasing serum retinol levels over time, however, were at a lower risk, whereas women who had declining serum retinol were at a higher risk of transmitting the virus.⁶⁴ Two other studies from the United States provided conflicting results: low serum vitamin A was associated with a higher risk of vertical transmission in one study,⁶⁵ but not the other.⁶⁶

Most of the limitations mentioned above for prospective studies of vitamin status and natural history also apply to studies of vertical transmission, particularly the validity of serum vitamin A as a marker of vitamin A status among infected subjects, and potential confounding by stage of HIV disease among infected women.

POTENTIAL MECHANISMS

Vitamin A supplementation may reduce the risk of vertical transmission by enhancing humoral and cellular immune function and slowing the progression of a mother's HIV disease (Table 3). Early stages of vitamin A deficiency in rats have repeatedly been shown to result in squamous metaplasia of the simple columnar epithelium of the endocervix.^{67,68} Hence, deficiency may be associated with a higher risk of abrasions of the cervix, leading to a greater risk of the baby coming in contact with infectious material during delivery. Vitamin A deficiency was associated with a higher risk of viral shedding in lower genital tract secretions in two studies from Kenya,^{69,70} possibly leading to a higher risk of intrapartum transmission of infection. Vitamin A may also reduce the risk of vertical transmission by preventing chorioamnionitis and/or enhancing placental integrity.⁷¹

Vitamin A deficiency may also be responsible, at least in part, for prematurity and low birthweight, which are associated with a higher risk of vertical transmission. Poor maternal vitamin A stores are associated with an

TABLE 4. Prospective Cohort Studies of Serum Vitamin A Levels in Relation to HIV Vertical Transmission, Infant Mortality, and Viral Isolation in Maternal Secretions

Study Site and Reference	Population	Exposure: Mother Serum Vitamin A Levels	Endpoint	Measure of Association	Variables Adjusted for
Malawi ⁶¹	474 HIV+ women	Increase of 0.45 $\mu\text{mol/liter}$	HIV infection in infant	RR (95% CI): 0.56 (0.37–0.85)	Maternal age, body mass index, CD4 cell count, birth weight, and gestational age
Malawi ⁶²	474 HIV+ women	Increase of 0.40 $\mu\text{mol/liter}$	Infant mortality	RR (95% CI): 0.47 (0.36–0.62)	Maternal age, body mass index, CD4 cell count, birthweight, and gestational age
Rwanda ⁶³	146 HIV+ women	≥ 20 vs < 20 $\mu\text{g/dl}$	Perinatal death Infant death Dead or HIV+ baby	RR (95% CI): 0.33 (0.14–0.77) 0.53 (0.27–1.00) 0.53 (0.30–0.77)	CD4 cell count, hematocrit, clinical signs
United States ⁶⁴	WITS Study (see text)	Mother serum levels	HIV infection in infant	Increase in serum retinol levels over time was associated with an apparent reduction in risk of transmission (see text)	
United States ⁶⁵	133 HIV+ women	≥ 1.05 vs < 0.70 $\mu\text{mol/liter}$	HIV infection in infant	RR (95% CI): 0.22 (0.05–0.93)	% CD4 cells, mode of delivery, gestational age, duration of membrane rupture, race
United States ⁶⁶	90 HIV+ women	Mother serum level	HIV infection in infant	Mean: Transmitters: 46.5 $\mu\text{g/dl}$ Nontransmitters: 41.1 $\mu\text{g/dl}$	None
Kenya ⁶⁹	212 HIV+ women	≥ 20 vs < 20 $\mu\text{g/dl}$	Vaginal HIV viral shedding	RR (95% CI): 0.4 (0.13–1.25)	CD4 cell count
Kenya ⁷⁰	281 HIV+ women	≥ 1.40 vs 0.70 $\mu\text{mol/liter}$	Vaginal HIV viral shedding	RR (95% CI): 0.08 (0.02–0.37)	CD4 cell count
Kenya ⁷⁴	107 HIV+ women providing 212 milk samples	≥ 40 vs < 20 $\mu\text{g/dl}$	Breast milk HIV viral shedding	RR (95% CI): < 400 CD4 cells/ mm^3 : 0.05 (0.005–0.48) ≥ 400 CD4 cells/ mm^3 : 2.5 (0.33–10.0)	Time since delivery, CD4 count

increased risk of intrauterine growth retardation, preterm birth, and decreased birthweight.^{72,73} Maternal vitamin A status after delivery may also be an important risk factor for postpartum transmission, given that vitamin A deficiency was associated with a higher risk of breast milk viral shedding among HIV-positive women in Kenya.⁷⁴ Vitamin A supplementation to mothers during pregnancy and/or lactation increases breast milk retinol levels and breastfeeding infants' vitamin A body stores,⁷⁵ which may be associated with enhanced development of the infants' immune systems and a greater ability to prevent the establishment of HIV infection.

Ongoing randomized trials are testing the hypothesis that supplementation with vitamin A (and other vitamins) can reduce vertical transmission rates. However, caution needs to be exercised in providing vitamin A supplements during pregnancy. In large doses, vitamin A has been shown to be teratogenic.⁷⁶ The World Health Organization recommends that pregnant women should not take more than 10,000 IU of vitamin A per day from supplements.⁷⁷

Vitamins B, C, D, and E and HIV Infection

EPIDEMIOLOGIC EVIDENCE

In cross-sectional studies, compared with HIV-negative subjects, those who are HIV-infected are at a greater risk of deficiency in vitamins B₁, B₂, B₆, B₁₂, C, D, and E.^{26,78-81} Most of the prospective data available on the role of various vitamins (other than vitamin A) in HIV infection are based on observational data (Table 1). It is difficult to elucidate the effects of deficiency of individual vitamins on the course of HIV disease, because levels of these nutrients are highly correlated. In a small study of 17 HIV-infected subjects, a low serum concentration of 1,25-vitamin D was associated with a higher probability of survival.⁸¹ In the San Francisco Men's Health Study,³⁶ higher intake of 11 micronutrients was associated with higher CD4 counts at baseline. Daily multivitamin use was associated with a 30% reduction in the hazard of AIDS, and a reduction of 40% in the risk of developing a CD4 cell count below 500 per mm³. In the Multicenter AIDS Cohort Study, higher intakes of niacin, vitamins B₁, B₂, B₆, were associated with slower progression to AIDS³⁷; higher intake of these vitamins and of vitamin C were also associated with a lower risk of death.³⁸ Most of the protective relations in both studies were due to intake from supplements rather than foods; however, there was limited statistical power to examine the association between deficiency states of these vitamins and the risk of progression because few subjects were consuming low levels. Also, given that these are prevalent cohorts of seropositive men at baseline, the duration of infection was not known and could not be adjusted for in the analyses. Baseline signs and symptoms and immunologic surrogates, such as CD4 levels, were taken into account, however.

In a prospective study of HIV-positive homosexual men, low serum levels of vitamin B₁₂ were associated with lower CD4 cell count over time and with a reduced

risk of progression of disease.³³ Higher serum concentrations of vitamins B₁₂ and E were also prospectively associated with longer AIDS-free time among participants in the Multicenter AIDS Cohort Study.^{34,82}

POTENTIAL MECHANISMS

Vitamins B, C, D, and E are important co-factors in many metabolic pathways. The inverse relation between levels of these vitamins and rate of progression of HIV disease is probably mediated through their protective actions on the immune system. Use of a daily multivitamin supplement reduced the risk of infection, mainly respiratory infection, among healthy, non HIV-infected elderly subjects who participated in a randomized, double-blind, placebo-controlled study.⁸³ The supplemented group also had higher numbers and better function of natural killer cells and T-cell subsets (including CD4), enhanced proliferation response to mitogen, increased interleukin 2 production, and higher antibody response to influenza vaccine.

Various animal and human studies have shown that vitamin B₆ deficiency affects both humoral and cellular immune function, including reduced lymphocyte differentiation and maturation, reduced delayed hypersensitivity responses, and possibly diminished antibody production.⁸⁴ In HIV-positive subjects, levels of vitamin B₆ were positively associated with measures of immune function, including the response of peripheral blood lymphocytes to mitogens and natural killer cell cytotoxicity, although there was no association with CD4 or CD8 cell counts, nor with serum immunoglobulin levels.³⁶ Riboflavin (B₂) deficiency impairs the ability to generate humoral antibodies in response to test antigens, but research on the effect on cell-mediated immunity is limited.⁸⁴ In clinical studies, patients with low levels of serum vitamin B₁₂ had impaired neutrophil function, whereas data from *in vitro* and animal studies indicate that B₁₂ supplements are associated with enhanced antibody function and mitogenic responses.⁸⁴

Vitamin E supplementation in studies of both laboratory and farm animals suggest that vitamin E may increase immune response to antigens, improve host resistance against challenge with microorganisms, and enhance B- and T-cell lymphocyte functions, as well as phagocyte function.⁸⁵ In HIV-positive subjects, deficiency in serum levels of vitamins C and E were correlated with lower natural killer cell counts and antibody levels.⁸⁶ By lowering the levels of free radicals which can induce the expression of HIV in human T-cell lines, vitamin E may block the replication of HIV and result in reduction in the rate of progression of HIV infection.⁸⁷ Higher vitamin E levels are also associated with increased production of interferon- γ in mice with murine AIDS, which results in increased cytotoxicity of natural killer cells, reduced production of proinflammatory cytokines including interleukin 6 and tumor necrosis factor- α , as well as increased interleukin 2, which is associated with enhanced growth and differentiation of T- and B-cells.^{87,88}

Vitamin C deficiency in animal models results in depressed cell-mediated immune response.⁸⁵ T- and B-lymphocyte proliferative responses are increased after supplementation in some human studies, and enhanced vitamin C status was associated with a lower rate of infection in an elderly population. In a cross-sectional study among patients with cystic fibrosis, vitamin C deficiency was directly related to indices of inflammation, including interleukin 6 and tumor necrosis factor- α .⁸⁹ Ascorbate resulted in reduced reverse transcriptase activity in T-lymphocytes that were chronically infected with HIV.⁹⁰ The role of antioxidant vitamins in HIV transcription and replication is supported by studies in which depletion of glutathione, the principal intracellular antioxidant, resulted in increased expression of HIV in peripheral blood mononuclear cells. Glutathione depletion is common in HIV infection, and N-acetylcysteine, an antioxidant used to replenish intracellular glutathione *in vivo* and in cells cultured *in vitro*, has been shown to inhibit cytokine-stimulated HIV transcription and replication in several studies.⁹¹

Conclusion

Protective relations between vitamin status and HIV disease progression and vertical transmission have been reported in a number of epidemiologic studies. Although the data on vertical transmission are limited to vitamin A, other vitamins may also have protective effects through their role in enhancement of maternal and/or fetal systemic immunity and reducing the risk and severity of opportunistic infections among mothers. Adequate vitamin status may also reduce vertical transmission through the intrapartum and breastfeeding routes by reducing HIV viral load in lower genital secretions and breast milk, respectively. Observational data, particularly data from cohorts of people who are already infected at baseline, may be biased if vitamin users have different durations of infection at baseline or different drug treatment, diet, or other behavior during follow-up. Randomized trials, initially including a placebo arm, are needed to accurately assess the effect of supplementation; several are under way. In developing countries, specific antiretroviral and prophylactic drugs are almost unavailable. Vitamin A, as well as other vitamins, may be among the few potential treatments that are inexpensive enough to be made available to HIV-infected persons in developing countries.

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Early reports

Randomised trial of effects of vitamin supplements on pregnancy outcomes and T cell counts in HIV-1-infected women in Tanzania

Wafaie W Fawzi, Gerard I Msamanga, Donna Spiegelman, Ernest JN Urassa, Nuala McGrath, Davis Mwakagile, Gretchen Antelman, Roger Mbise, Guillermo Herrera, Saidi Kapiga, Walter Willett, David J Hunter, for the Tanzania Vitamin and HIV Infection Trial Team*

Summary

Background In HIV-1-infected women, poor micronutrient status has been associated with faster progression of HIV-1 disease and adverse birth outcomes. We assessed the effects of vitamin A and multivitamins on birth outcomes in such women.

Methods In Tanzania, 1075 HIV-1-infected pregnant women at between 12 and 27 weeks' gestation received placebo (n=267), vitamin A (n=269), multivitamins excluding vitamin A (n=269), or multivitamins including vitamin A (n=270) in a randomised, double-blind, placebo-controlled trial with a 2x2 factorial design. We measured the effects of multivitamins and vitamin A on birth outcomes and counts of T lymphocyte subsets. We did analyses by intention to treat.

Results 30 fetal deaths occurred among women assigned multivitamins compared with 49 among those not on multivitamins (relative risk 0.61 [95% CI 0.39–0.94] $p=0.02$). Multivitamin supplementation decreased the risk of low birthweight (<2500 g) by 44% (0.56 [0.38–0.82] $p=0.003$), severe preterm birth (<34 weeks of gestation) by 39% (0.61 [0.38–0.96] $p=0.03$), and small size for gestational age at birth by 43% (0.57 [0.39–0.82] $p=0.002$). Vitamin A supplementation had no significant effect on these variables. Multivitamins, but not vitamin A, resulted in a significant increase in CD4, CD8, and CD3 counts.

Interpretation Multivitamin supplementation is a low-cost way of substantially decreasing adverse pregnancy outcomes and increasing T-cell counts in HIV-1-infected women. The clinical relevance of our findings for vertical transmission and clinical progression of HIV-1 disease is yet to be ascertained.

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Introduction

By the end of 1997, 30 million people had HIV-1 infection worldwide, of whom 90% were in the developing

world.¹ sub-Saharan Africa is home to about 65% of HIV-1-infected people. In some urban areas, more than 25% of women of reproductive age have HIV-1 infection. HIV-1 infection is also spreading rapidly in South and Southeast Asia. HIV-1-infected pregnant women have higher risks than those who are uninfected of fetal loss,^{2,3} low birthweight, preterm birth, and intrauterine growth retardation.⁴ These pregnancy outcomes are associated with higher risks of intrapartum and postpartum acquisition of HIV-1 infection,⁵ with higher infant mortality among infected⁶ and uninfected children.⁷

Micronutrient supplementation may be an important prophylactic and therapeutic measure for HIV-1-infected people and is one of the few potential treatments that is sufficiently inexpensive for low-income countries. Poor maternal micronutrient status has been associated with faster clinical and immunological progression of HIV-1 disease,^{8–10} and with adverse birth outcomes, including fetal growth retardation and death.^{11,12} These findings from observational studies are difficult to interpret because of confounding of micronutrient status by other variables, such as stage of disease or access to health care.

We did a randomised, double-blind, placebo-controlled trial to investigate the effects of supplements of vitamin A, or multivitamins, or both on progression of HIV-1 disease and on birth outcomes (fetal death, birthweight <2500 g, and preterm birth at <37 weeks as primary outcomes) and changes in T lymphocyte subsets (as secondary endpoints) among pregnant women infected with HIV-1 in Dar es Salaam, Tanzania. Only the effects on birth outcomes and T cell counts are reported here.

Methods

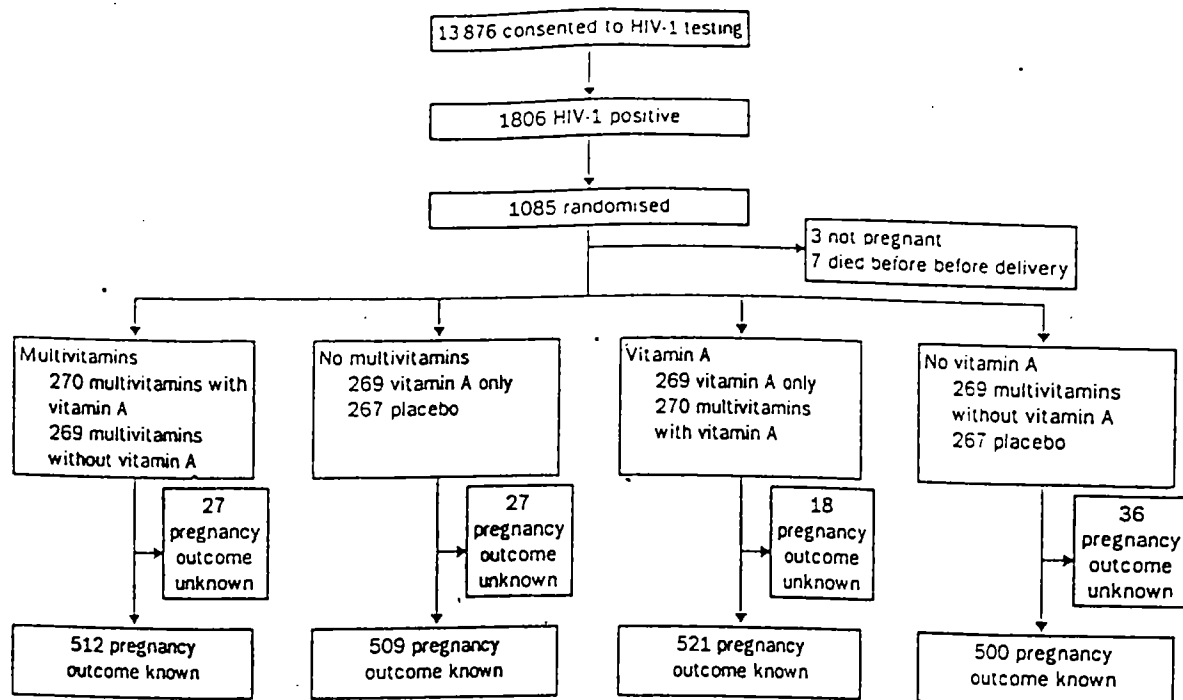
Pregnant women at between 12 and 27 weeks' gestation who were HIV-1 infected, resident in Dar es Salaam at the time of the baseline interview, and who intended to stay in the city until delivery and for 1 year thereafter were eligible. Consent was first sought for HIV-1 testing as part of prenatal screening at four antenatal clinics. At the visit after the one at which women received post-test counselling, we sought their consent for randomisation. We tested HIV-1 serostatus by ELISA (Wellcozyme, Murex Biotech Ltd, Dartford, UK) and confirmed positive results by western blot (Bio-Rad Laboratories Ltd, Hertfordshire, UK).

Women were assigned in a two-by-two factorial design. 1075 women received a daily oral dose of: vitamin A (30 mg β -carotene plus 5000 IU preformed vitamin A, n=269); multivitamins excluding vitamin A (20 mg B1, 20 mg B2, 25 mg B6, 100 mg niacin, 50 μ g B12, 500 mg C, 30 mg E, and 0.8 mg folic acid, n=269); multivitamins including vitamin A (n=270), all formulated in two tablets; or two tablets of placebo (n=267, figure). All tablets were indistinguishable and were packaged in identically coded bottles containing 90 tablets each. All women received ferrous sulphate (400 mg, equivalent to 120 mg ferrous iron) and folate (5 mg) daily, and prophylactic chloroquine phosphate (500 mg, equivalent to 300 mg of chloroquine base) weekly. At delivery, all women taking vitamin A were to receive

*Additional members listed at end of paper

Departments of Nutrition (W Fawzi *ms*, N McGrath *msc*, G Antelman *scd*, W Willett *md*), Epidemiology (D Spiegelman *scd*, W Willett, D Hunter *ms*), and Biostatistics (D Spiegelman), Harvard School of Public Health, Boston, Massachusetts, USA, and Departments of Community Health (G Msamanga *md*), Obstetrics and Gynecology (E Urassa *md*), Microbiology and Immunology (D Mwakagile *md*), and Pediatrics and Child Health (R Mbise *md*), and Epidemiology and Biostatistics (S Kapiga *md*), Muhimbili University College of Health Sciences, Dar es Salaam, Tanzania

Correspondence to: Dr Wafaie Fawzi, Department of Nutrition, Harvard School of Public Health, 665 Huntington Avenue, Boston, MA 02115, USA



Trial profile

an additional oral dose of vitamin A of 200 000 IU and the others an extra dose of a placebo. Randomisation was done in blocks of 20. At enrollment, we assigned each eligible woman the next numbered bottle of regimen. Half the stock was prepared at the beginning of the study and half was prepared with fresh product midway through the project. After 2 years of field storage all vitamins showed more than 96% potency.

At randomisation and at every monthly visit, we administered questionnaires about interim medical problems; at randomisation we tested women for sexually transmitted diseases (syphilis, gonorrhoea, and trichomoniasis), and did routine urine and stool assessments and complete blood counts. Total leucocyte counts were done with a CBC5 Coulter Counter (Coulter Corp. Miami, FL, USA), and differential white cell counts were done manually.¹⁰ Absolute T-cell subset counts of (CD4, CD8, and CD3) were done with the FACScount system (Becton-Dickinson, San Jose, CA, USA) which provides consistently similar absolute counts compared with the standard FACScan system.¹¹ Blood cell counts were done at baseline, and 6 weeks and 30 weeks postpartum.

At every visit used bottles were exchanged for new bottles, and the remaining pills counted. Overall, the mean time between randomisation and delivery was 4.0 months (SD 1.1, median 4.1). 85% of women took the single large dose of the supplement or placebo at delivery; the other 15% were not given this dose because they delivered at home or at another clinic. We assessed compliance as the percentage of prescribed tablets absent from the returned bottles. To further assess compliance, we measured plasma vitamin A concentrations at baseline and at delivery in 100 women.

The research midwife measured on a standard beam balance the weight of neonates to the nearest 10 g immediately after birth. Placentas were cleaned of blood clots and weighed on a similar scale. Midwives measured length on an infant length board, and head circumference with a non-stretchable tape, both to the nearest 0.1 cm. Gestational age was based on the date of the last menstrual period, obtained at randomisation. Miscarriage was defined as delivery before 28 weeks' gestation, stillbirth as delivery of a dead baby at or after 28 weeks' gestation, low birthweight as less than 2500 g, very low birthweight as less than 2000 g, preterm delivery as before 37 weeks, severe preterm delivery as before 34 weeks, and small size for gestational age as birthweight less than the 10th percentile for gestational age by the standards of Brenner and colleagues.¹² Women who missed their

monthly appointments were visited at home if possible. For women who left Dar es Salaam, we tried to collect information on the outcome of pregnancy from their neighbours and relatives.

We analysed the effects of multivitamins and vitamin A by intention to treat. Since analyses with and without data from twins ($n=21$ pairs) gave virtually the same results, the findings presented include data from twins. We analysed twin pregnancies as a single outcome; for continuous variables, we took the mean of the two twin values, and for categorical variables, if either infant had the outcome, we took the pregnancy to have that outcome. For treatment effects on categorical variables we calculated relative risks with 95% CIs. To test the effect of treatment we used the Mantel-Haenszel χ^2 test for categorical variables, and Wilcoxon's rank sum test for continuous variables. We analysed the effects of multivitamins and of vitamin A on categorical variables with and without adjustment for the other treatment. The results were similar, and we have presented only unadjusted results.

We investigated the effect of the vitamin supplements on absolute counts of CD4, CD8, and CD3 cells, total white blood cells, total lymphocytes, and percentage of CD4 and CD8 cells, available until Jan 15, 1998. The mean duration of follow-up in all groups by this date was 8.6 months (SD 7.2, median 6.4). Analyses of data only for participants for whom counts were done on the three specified occasions gave similar results. Analyses at the specified time points were based on the nearest value obtained within 3 months of the scheduled time. We assessed overall treatment efficacy over time with the Wei-Johnson test.¹³

For birth outcomes and T cell counts, we assessed whether the effect of one treatment (multivitamins or vitamin A) was modified by the other. We also assessed whether treatment effects on birth outcomes were modified by baseline CD4 cell count in three strata (<200 cells/ μ L, 200–499 cells/ μ L, $\geq$ 500 cells/ μ L) and within tertiles of baseline body-mass index. We used the Breslow-Day test for homogeneity to find out whether treatment effects were significantly different between strata of potential modifiers. Sample size for the trial was based on the expected effect on vertical transmission of HIV-1 infection. It would have more than 90% power to detect a 30% decrease in the risk of transmission, assuming an α error of 0.05 and a background rate of transmission of 30%, and factoring in a potential 20% loss to follow-up. All p values were two-sided and we took $p<0.05$ to be significant.

The study protocol was approved by the Research and Publications Committee of Muhimbili University College of

	Multivitamins n (%)	No multi- vitamins n (%)	Relative risk (95% CI)	p	Vitamin A n (%)	No vitamin A n (%)	Relative risk (95% CI)	p
Miscarriage	12 (2.3)	18 (3.5)	0.66 (0.32-1.36)	0.26	13 (2.5)	17 (3.4)	0.73 (0.36-1.50)	0.39
Stillbirth	18 (3.5)	31 (6.1)	0.58 (0.33-1.02)	0.05	25 (4.8)	24 (4.8)	1.00 (0.58-1.73)	1.00
Fetal death*	30 (5.9)	49 (9.6)	0.61 (0.39-0.94)	0.02	38 (7.3)	41 (8.2)	0.89 (0.58-1.36)	0.59

1021 of 1085 analysed; three not pregnant, seven died before delivery, 54 had unknown birth outcomes. *Fetal death includes both miscarriages and stillbirths.

Table 1: Fetal deaths among women who received multivitamins or vitamin A compared with those who did not receive the respective regimen

Health Sciences, the ethical committee of the National AIDS Control Program of the Tanzanian Ministry of Health, and the institutional review board of the Harvard School of Public Health. In addition to yearly review of ethics, the study was reviewed by all three committees and endorsed again after publicity about ethical conduct of AIDS trials in developing countries.

Results

Between April, 1995, and July, 1997, 13 876 pregnant women consented to HIV-1 testing. Of the 1806 who were positive, 1085 (60%) returned for their results, met the inclusion criteria, and agreed to participate in the trial. Baseline characteristics were similar in all groups for weeks of gestation, age, education, number of previous pregnancies, CD4 count in all strata, HIV-1 disease stage, body-mass index, haemoglobin concentrations, frequency of malaria, marital status, and spouse's age. The mean compliance was 91% (median 96%) between randomisation and delivery, and 88% (median 92%) at 30 weeks postpartum for all groups. Of the 1085 women who were enrolled, three of these were not pregnant (two had uterine fibroids, one was obese) and seven women died before delivery and were excluded from the trial (figure). 54 (5.9%) women were lost to follow-up by the time of delivery. Of 1021 birth outcomes, 941 were livebirths and eligible for the analyses of birthweight and preterm outcomes. We did not know date of delivery for 53 (5.6%) and could not ascertain gestational age; for 141 (15.0%) birthweight was not available because of delivery at home or at another medical facility.

There were no differences in baseline plasma vitamin concentrations between groups. At baseline, equal proportions of women (34%) who did or did not receive vitamin A had vitamin A concentrations of less than 20.0 µg/dL—vitamin A deficiency. The mean vitamin A concentrations were 26.1 µg/dL and 25.5 µg/dL, respectively ($p=0.83$). At delivery, plasma mean vitamin A concentrations were 28.8 µg/dL and 22.9 µg/dL in women who did and did not receive vitamin A, respectively ($p=0.01$). The corresponding proportions of women who had concentrations of less than 20.0 µg/dL were 22% and 50% (odds ratio 0.64 [0.47-0.88] $p=0.004$).

The mean CD4 cell count at baseline was 449 cells/µL (SD 270, median 411); most women were symptom-free.

There were 30 fetal deaths among women assigned multivitamins compared with 49 among those not given multivitamins (relative risk 0.61 [0.39-0.94] $p=0.02$; table 1). Protection against risks of miscarriage or stillbirth associated with multivitamins were of similar magnitude, but were not significant. Vitamin A did not significantly affect the risks of any fetal loss.

Mothers who received multivitamins gave birth to heavier babies than those who were not on multivitamins (mean 3048 [SD 484] vs 2948 g [522] $p=0.01$). The effect of multivitamins was due mainly to an effect on the lowest quartile of the birthweight distribution. The mean birthweights were similar among women who did and did

not receive vitamin A (3005 [507] vs 2993 g [504], $p=0.85$). Multivitamins and vitamin A had no beneficial effects on mean gestational age, length, or head circumference at birth. Women on multivitamins had heavier placentas than those not on multivitamins (526 [107] vs 507 g [104], $p=0.02$), but vitamin A had no significant effect (523 [112] vs 510 [98] g, $p=0.24$). There were no differences in haemoglobin concentrations at baseline between mothers assigned multivitamins and those assigned no multivitamins (9.3 g/dL [1.6] vs 9.5 g/dL [1.7]). At 6 weeks postpartum, women on multivitamins had gained a mean of 1.3 g/dL (2.1) compared with an increase of 0.6 g/dL (2.2) among those not on multivitamins ($p<0.0001$). Vitamin A had no effect on haemoglobin concentration.

Multivitamins significantly decreased the incidence of low birthweight by 44% (relative risk 0.56 [0.38-0.82] $p=0.003$; table 2) and the risk of very low birthweight by 58% (0.42 [0.18-1.01] $p=0.05$). Vitamin A had no effect on either outcome. The risk of low birthweight in the four groups were 17.2% on placebo, 14.5% on vitamin A alone, 9.0% on multivitamins excluding vitamin A, and 8.7% multivitamins and vitamin A. We did not find an interaction between the effect of vitamin A and multivitamins ($p=0.70$ for homogeneity).

Multivitamins decreased the risk of severe preterm delivery by 39% (0.61 [0.38-0.96]), but the effect on the risk of preterm delivery was less and not significant ($p=0.23$). Multivitamins significantly decreased the risk of being small for gestational age (table 2). Vitamin A did not alter the risk of preterm delivery or being small for gestational age.

We did not design the trial to have power to identify subgroups that may have benefited from treatment. The effect of multivitamins or vitamin A on low birthweight and preterm birth endpoints were not significantly different across strata of baseline CD4 cell counts (<200 cells/µL, 200-499 cells/µL, and ≥500 cells/µL) or tertiles of body-mass index at baseline (data not shown).

There were no differences in T cell counts at baseline between women who received multivitamins or vitamin A and those who did not. As expected during pregnancy, all T-cell subsets (CD4, CD8, and CD3) increased in all groups between baseline (mean 18 weeks' gestation), and 6 weeks postpartum. Absolute cell counts in all groups decreased at the next count at 30 weeks postpartum.

There was a significantly larger increase in CD4 cell count and percentage of CD4 cells among women assigned multivitamins than among those not on multivitamins (table 3). The mean increases between baseline and 6 weeks postpartum were 167 cells/µL and 112 cells/µL among women on multivitamins and those on no multivitamins ($p<0.001$). By 30 weeks postpartum, cell counts had decreased at the same rate, but the difference in change from baseline between the two groups was sustained ($p=0.005$).

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Endpoint	n	Multivitamins (n (%))	No multi- vitamins (n (%))	Relative risk (95% CI)	p	Vitamin A (n (%))	No vitamin A (n (%))	Relative risk (95% CI)	p
Birthweight <2500 g	800	36 (8.8)	62 (15.8)	0.56 (0.38-0.82)	0.003	47 (11.6)	51 (13.0)	0.89 (0.61-1.29)	0.54
Birthweight <2000 g	800	7 (1.7)	16 (4.1)	0.42 (0.18-1.01)	0.05	11 (2.7)	12 (3.1)	0.89 (0.40-1.98)	0.77
Preterm birth (<37 weeks)	888	96 (21.1)	106 (24.5)	0.86 (0.68-1.10)	0.23	105 (23.4)	97 (22.1)	1.06 (0.83-1.35)	0.66
Severe preterm birth (<34 weeks)	888	28 (6.2)	44 (9.1)	0.61 (0.38-0.96)	0.03	28 (8.5)	34 (7.7)	1.09 (0.70-1.70)	0.70
Low birthweight and preterm birth	768	19 (4.9)	33 (8.8)	0.55 (0.32-0.95)	0.03	28 (7.2)	24 (6.3)	1.14 (0.68-1.94)	0.62
Low birthweight and term birth	768	15 (3.8)	27 (7.2)	0.53 (0.29-0.99)	0.04	17 (4.4)	25 (6.6)	0.67 (0.37-1.21)	0.18
Small for gestational age*	768	39 (10.0)	66 (17.6)	0.57 (0.39-0.82)	0.002	48 (12.4)	57 (15.0)	0.83 (0.58-1.18)	0.29

*Small for gestational age is defined as birthweight below 10th percentile of weight for gestational age.

Table 2: Birth outcomes in women who received multivitamins or vitamin A compared with those who did not receive the respective regimen

Multivitamins also significantly increased the absolute number of CD8 cells, although not the percentage of CD8 cells (table 3). The mean increase in CD8 cells between baseline and 6 weeks postpartum was 385 cells/ μ L among women on multivitamins, compared with 289 cells/ μ L among those not on multivitamins ($p=0.001$). Between randomisation and 30 weeks postpartum, the mean difference between the two groups was 57 cells/ μ L ($p=0.06$). We also found significantly larger differences in CD3 cell counts among women on multivitamins. The mean increase between baseline and 6 weeks postpartum was 585 cells/ μ L among women on multivitamins, compared with 411 cells/ μ L among those not on multivitamins, a mean difference of 174 cells/ μ L ($p<0.001$). The increase between baseline and 30 weeks postpartum was also significantly larger among women on multivitamins ($p=0.02$).

Multivitamins had no significant effect on the T cell counts of women who received vitamin A, in any of the vitamin A strata, compared with those who were not given vitamin A.

Discussion

Multivitamin supplements decreased the risk of fetal death, low birthweight, preterm birth and small size for gestational age, whereas vitamin A supplements did not. Confounding is an unlikely explanation for these results since baseline characteristics were similar for the two groups. The protective effects of multivitamins are in accord with the findings of an observational, prospective,

non-randomised study among women in Camden, NJ, USA, most of whom were presumably HIV-1 negative. Prenatal multivitamin supplements (also containing calcium and zinc) were associated with significant decrease in the risk of low birthweight, very low birthweight, preterm birth, and severe preterm birth.¹⁸

The observed beneficial effect of multivitamins is probably mediated through improvement in fetal nutritional status, enhancement of fetal immunity, and decreased risk of infections, which together would decrease risks of intrauterine growth retardation, fetal loss, preterm birth, and low birthweight. However, how the individual vitamins produce these effects is not fully understood. Vitamins may increase placental weight, which will increase the surface area available for maternal-fetal exchange, since placentas of undernourished women are lighter and have fewer villi than those of well-nourished women.¹⁹ Women on multivitamins in our study had slightly heavier placentas than those not on multivitamins. A more compelling potential mechanism of action of the multivitamins is through improvement of mothers' haematological status: low haemoglobin concentrations are associated with higher risks of miscarriages, preterm birth, and low birthweight.²⁰ The large and significant increase in haemoglobin concentrations seen in this study supports this hypothesis.

The observed beneficial effects of multivitamins on birth outcomes in our population may have been mediated through improved maternal immune status. Women with

Endpoint	Multivitamins		No multivitamins		p	Vitamin A		No vitamin A		p
	n	Mean (SD)	n	Mean (SD)		n	Mean (SD)	n	Mean (SD)	
CD4										
Baseline	510	424 (207)	514	423 (211)	0.72	515	416 (206)	509	431 (212)	0.21
6 weeks postpartum	376	596 (312)	351	520 (339)	<0.001	375	558 (376)	352	562 (265)	0.13
30 weeks postpartum	263	522 (278)	248	482 (268)	0.05	257	496 (294)	254	509 (252)	0.23
Difference 1	373	167 (210)	349	112 (268)	<0.001	372	142 (290)	350	138 (175)	0.13
Difference 2	261	99 (208)	246	59 (167)	0.003	255	74 (210)	252	84 (167)	0.16
Overall effect	..	..	..	..	<0.001	..	..	..	..	0.29
CD8										
Baseline	510	740 (325)	514	760 (339)	0.42	515	743 (319)	509	757 (346)	0.81
6 weeks postpartum	376	1116 (528)	351	1035 (450)	0.03	375	1061 (503)	352	1094 (482)	0.32
30 weeks postpartum	263	934 (415)	248	918 (358)	0.79	257	943 (394)	254	909 (392)	0.26
Difference 1	373	385 (450)	349	289 (404)	0.001	372	316 (458)	350	363 (399)	0.04
Difference 2	261	232 (313)	246	175 (325)	0.06	255	214 (337)	252	195 (303)	0.66
Overall effect	..	..	..	..	0.05	..	..	..	..	0.13
CD3										
Baseline	510	1223 (444)	513	1243 (463)	0.81	514	1221 (438)	509	1225 (474)	0.77
6 weeks postpartum	376	1805 (748)	351	1626 (622)	0.001	375	1698 (737)	352	1741 (649)	0.20
30 weeks postpartum	263	1528 (576)	248	1480 (539)	0.57	257	1515 (563)	254	1494 (554)	0.68
Difference 1	373	585 (648)	348	411 (559)	<0.001	371	474 (660)	350	530 (557)	0.04
Difference 2	261	345 (493)	245	254 (475)	0.02	254	301 (514)	252	301 (457)	0.87
Overall effect	..	..	..	..	0.004	..	..	..	..	0.25

Difference 1 and difference 2 are changes between baseline measurements and measurements at 6 weeks and 30 weeks postpartum, respectively.

*Small size for gestational age is defined as birthweight below 10th percentile of weight for gestational age.

Table 3: T-cell measurements in women who received multivitamins or vitamin A compared with those who did not receive these regimens

advanced HIV-1 disease are at a higher risk of fetal loss, low birthweight, and preterm birth.¹⁴ Low dietary vitamin intake or serum concentrations (especially vitamins B1, B2, B6, and B12) were associated with faster progression to AIDS and decreased survival among men in observational studies.¹⁵ In our study, multivitamins had significant beneficial effects on T-cell subset counts. There were sustained increases in CD4 and CD8 cells, which are the main mediators of cellular immunity in HIV-1 infection. CD3 cell counts were also increased. The CD3 complex is expressed on CD4 and CD8 cells as well as their immature precursor cells; therefore, an increased CD3 cell count may play a part in the replenishment of the CD4 and CD8 pools that progressively diminish as patients advance in their disease. The effectiveness of multivitamins on HIV-1-disease progression remains to be determined. Improvement in T cell counts may not slow clinical progression of disease or decrease risk of mortality. In the Concorde trial, for example, individuals taking zidovudine immediately after diagnosis of HIV-1 infection had significantly higher subsequent CD4 cell counts than those who deferred treatment until they had symptoms. Ultimately, however, there was no difference in clinical progression or mortality between individuals in the two groups.¹⁶ Although CD4 cell count is an important prognostic indicator in HIV-1 disease, its role as a surrogate marker of progression is questionable.¹⁷ Because of limited follow-up time, we did not have adequate statistical power to assess the efficacy of the supplements on progression to AIDS or mortality.

The beneficial effect of multivitamin supplements on maternal immunity may decrease the risk of maternal bacterial and viral infections during pregnancy and their transmission to the fetus. We are following up babies born to women in this trial to find out whether vitamin supplements altered the risk of vertical transmission of HIV-1 infection.

We found no significant effect on birth outcomes or on T cell counts from vitamin A. Our initial hypothesis was that vitamin A may affect these endpoints through the same mechanisms as multivitamins. However, much of the previous evidence on the relations of vitamin-A deficiency and perinatal outcomes and immune function in HIV-1 infection comes from studies in which vitamin-A status was measured by serum retinol concentrations.^{18,19} Retinol is known to be decreased by the acute-phase response to infection, even in the presence of adequate liver stores of vitamin A.²⁰ Therefore, vitamin A serum concentrations may be markers of the stage of HIV-1 disease or intermittent infections rather than causally related to adverse birth outcomes. The effect of β -carotene on T cell counts has been investigated in two trials. In a small crossover study, 21 patients who received 180 mg of β -carotene daily for 4 weeks experienced small increases in the total white blood cell count, CD4 cell count, and percentage change in CD4-CD8 ratio compared with participants on placebo; these variables decreased when the participants in the carotene group were switched to placebo.²¹ In a larger study by the same investigators in which the same dose of β -carotene was used, this effect was not seen.²²

Our study design permitted simultaneous assessment of two interventions and whether the effect of one is modified by the other. Vitamin A did not, however, alter the effect of the multivitamins. Poor absorption and increased requirements of vitamin A among HIV-1-infected pregnant

women may explain the lack of effect of vitamin A. Doses higher than we used may have a beneficial effect, but the dose of β -carotene (30 mg) was high, and a dose of preformed vitamin A much higher than that used (5000 IU) has been associated with teratogenicity.²³

About a third of women had vitamin-A deficiency at baseline, which suggests an important public-health problem in this population. Although vitamin A was without a significant effect on any endpoint in this study, a slight benefit cannot be excluded, and the importance of vitamin A during pregnancy can be more fully assessed when the results from ongoing vitamin-A trials in Malawi, South Africa, and Nepal are available.

Vitamin deficiency is common among HIV-infected adults and children,²⁴ especially those in developing countries. HIV-1 infection, and adverse health outcomes associated with vitamin deficiency are also major public-health problems. The long-term clinical relevance of T-cell increase associated with multivitamin supplementation is yet to be determined. It is not clear whether protection against risks of fetal death, low birth weight, and preterm birth associated with multivitamins is applicable to HIV-1-negative women, to HIV-1-positive women in well-nourished communities, or in communities with access to antiretroviral therapy during pregnancy. Nevertheless, multivitamin supplementation provides a low-cost approach for substantially decreasing adverse pregnancy outcomes among HIV-infected women in developing countries.

Additional members of the Tanzania vitamin and HIV infection Trial Team

Iluminata Ballonzi, Jenny Coley, Max Essex, Mary Catherine Smith Fawzi, Miriam Garland, Ellen Hertzmark, Mengchi Ho, Sylvia Kaaya, Charles Kagoma, James Kiinya, Zohrah Lukmani, Jesse Mbwambo, Boris Renjifo, and Andrew Swai. Members of the Data Safety and Monitoring Board were Graham Colditz, Kenneth McIntosh, Fatma Mriho, Marcello Pagano (Chair), and Richard Platt.

Contributors

Wafae Fawzi was the principal investigator, contributed to and implemented the study design, helped with data analyses, and wrote the draft manuscript. Gerard Msamanga was the principal investigator at Muhimbili, contributed to the study design, its implementation, and the writing of the manuscript. Donna Spiegelman was the statistician and contributed to the study design and supervised the data analyses. Ernest Urassa, Davis Mwakipile, and Roger Mbiye contributed to the study design and oversaw, respectively, the obstetric, laboratory, and paediatric parts of the study implementation. Nuala McGrath and Gretchen Antelman supervised data entry and management in the field and contributed to data analyses and write-up. Saidi Kapiga and Guillermo Herrera contributed to the study design. Walter Willett contributed to the study design and advised on technical and practical issues. David Hunter contributed to study design, implementation, data analyses, and write-up. All investigators contributed to the editing of the final version of the manuscript.

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