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HIV-1 Shedding in Women: Trial of Vitamin A

Jared Murray Baeten

A dissertation submitted in partial fulfillment of the requirements for the degree of

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Program Authorized to Offer Degree: Epidemiology
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Chair of the Supervisory Committee:

Joan K. Kreiss

Reading Committee:

Shirley A. A. Beresford

King K. Holmes

Joan K. Kreiss

Thomas D. Koepsell

Date: July 31, 2001
Vitamin A deficiency has been associated with increased vaginal shedding of HIV-1 infected cells among pregnant and non-pregnant HIV-1 infected women, as well as with increased mother-to-child HIV-1 transmission, suggesting that deficiency may increase infectivity of women with HIV-1. We conducted a randomized, double-blind, placebo-controlled trial of 6 weeks of daily oral vitamin A (10,000 IU retinyl palmitate) among 400 HIV-1 infected non-pregnant women in Mombasa, Kenya. The primary aim was to assess the effect of supplementation on vaginal shedding of HIV-1 and HIV-1 infected cells. At follow-up, we found no statistically significant difference in the prevalence of HIV-1 DNA (18% vs. 21%, p=0.4) or the quantity of HIV-1 RNA (3.12 vs. 3.00 log_{10} copies/swab, p=1.0) in vaginal secretions of women receiving vitamin A compared to women receiving placebo. No effect of supplementation on plasma HIV-1 viral load or CD4 or CD8 counts was observed, and no effect was seen among women who were vitamin A deficient at baseline.

To better understand the disparity between the earlier observational studies and the results of our randomized trial, we conducted a cross-sectional study using data from the 400 HIV-1 infected trial participants as well as 200 HIV-1 uninfected women who did
not participate in the randomized trial because of their HIV-1 status. The objective was to examine the relationships between vitamin A deficiency and HIV-1 status, HIV-1 disease stage, and the acute phase response. Vitamin A deficiency was independently associated with HIV-1 infection (OR 2.7, 95% CI 1.9-4.0) and the acute phase response (OR 2.8, 95% CI 1.9-4.1). Among HIV-1 infected women, vitamin A deficiency and the acute phase response were both independently associated with higher HIV-1 plasma viral load and lower CD4 count. After supplementation as part of the randomized trial, HIV-1 infected women having an acute phase response had no increase in serum vitamin A levels. Thus, among HIV-1 infected individuals, low serum vitamin A concentrations are associated with more active infection and the acute phase response, and may not reflect true deficiency.

Our results suggest that vitamin A supplementation may be ineffective for treating or preventing HIV-1 infection.
TABLE OF CONTENTS

List of Figures .................................................................................................................. ii

List of Tables .................................................................................................................. iii

Introduction ....................................................................................................................... 1

Chapter I ......................................................................................................................... 13

Vitamin A Supplementation and HIV-1 Shedding in Women: Results of a Randomized Clinical Trial

Chapter II ....................................................................................................................... 42

Vitamin A Deficiency and the Acute Phase Response among HIV-1 Infected and Uninfected Women in Kenya

Conclusion ....................................................................................................................... 69

Bibliography .................................................................................................................... 77
LIST OF FIGURES

Figure 1.1 Flow Diagram of Trial Participants .................................................. 36
LIST OF TABLES

Table 1.1 Baseline Characteristics .......................................................... 37
Table 1.2 Effect of vitamin A supplementation ....................................... 39
Table 1.3 Effect of vitamin A supplementation, by enrollment vitamin A status .... 41
Table 2.1 Demographic and medical characteristics ...................................... 62
Table 2.2 Severity of HIV-1 disease, vitamin A deficiency, and the acute phase response among seropositive women .......................................................... 64
Table 2.3 Odds of vitamin A deficiency, by HIV-1 and acute phase response status .. 66
Table 2.4 Effect of vitamin A supplementation among HIV-1 seropositive women, stratified by enrollment vitamin A and acute phase response status ............ 68
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DEDICATION

For my parents and all my teachers
INTRODUCTION

The World Health Organization estimates that 5.3 million people worldwide became newly infected with HIV-1 during the year 2000, reflecting nearly 58 million cumulative infections over the course of the epidemic (1). More than 95% of new infections will occur in developing countries, where few options exist for treatment of HIV-1 and where prevention efforts are often limited. Simple, inexpensive intervention strategies to prevent and treat HIV-1 infection are urgently needed.

HIV-1 infected women may shed the virus in vaginal and cervical secretions, and epidemiologic studies have demonstrated that genital tract shedding of HIV-1 is a risk factor for mother-to-child HIV-1 transmission (2-5). Women who shed HIV-1 in the genital tract are likely to be more infectious to sexual partners as well (6-8), as there is strong agreement between epidemiologic correlates of female-to-male HIV-1 transmission and correlates of female genital shedding of HIV-1 (6, 7).

The first studies of female genital HIV-1 shedding used culture techniques to demonstrate the presence of the virus (9, 10). Viral culture was often complicated by bacterial and yeast contamination (11), however, so subsequent studies have used nucleic acid amplification. HIV-1 DNA PCR has been used to detect HIV-1 infected cells and HIV-1 RNA PCR has been used to detect cell-associated and cell-free virions. Culture-based studies found infectious virus both in the cell pellet of centrifuged secretions as well as in filtered supernatant, implying that transmissible HIV-1 may exist as both cell-associated virus and free virions (10). The roles of HIV-1 and HIV-1 infected cells in
transmission have not been conclusively determined, but animal studies have implicated both as potentially the infectious agent at mucosal sites (12-17). Epidemiologic studies have found that both HIV-1 DNA and HIV-1 RNA are associated with greater likelihood of mother-to-child HIV-1 transmission (3-5).

Studies of HIV-1 shedding in female genital secretions have detected the virus in only a fraction of the HIV-infected women investigated. A number of studies have sought to elucidate host characteristics that correlate with the presence of detectable HIV-1 in cervical and vaginal secretions, as identification of correlates of shedding of HIV-1 in genital tract secretions may suggest intervention strategies to prevent heterosexual and perinatal transmission. The largest study to separately examine cervical and vaginal secretions for HIV-1 DNA included 318 HIV-1 infected women in Mombasa, Kenya (18). In this study, serum vitamin A concentration was strongly predictive of vaginal shedding. In an analysis which adjusted for CD4 count, prevalence of detection odds ratios were 12.9 [95% confidence interval (CI) 2.7-62.3] for severe vitamin A deficiency (<20 μg/dl), 8.0 (95% CI 1.7-38.0) for moderate vitamin A deficiency (20-29.9 μg/dl), and 4.9 (95% CI 1.0-23.5) for low normal serum vitamin A (30-39.9 μg/dl), when compared to concentrations within the high normal range (≥40 μg/dl).

In two studies among HIV-1 infected women in Nairobi, low serum concentrations of vitamin A were associated with detection of HIV-1 DNA in vaginal secretions during pregnancy (19) and in breastmilk (20). Among members of a large observational cohort of HIV-1 infected pregnant women in Malawi, maternal serum vitamin A concentration and maternal CD4% were independently predictive of vertical
transmission (21). Compared to women with serum vitamin A levels ≥40 μg/dl, the relative risks of transmission of infection among women with serum vitamin A levels of 20-29.9 μg/dl and <20 μg/dl were 3.6 (95% CI 1.3-9.6) and 4.4 (95% CI 1.6-11.9), respectively. Together, the results of these studies strongly suggested that depletion of vitamin A could increase infectivity of women with HIV-1.

Studies from the United States have shown that low serum micronutrient concentrations are unusually common in adults with HIV-1 infection, including deficiencies in vitamins A, B6, B12, E, copper, selenium, and zinc (22-24). Among these, vitamin A has long been known to play a key role in maintaining normal immune function (25). An association between vitamin A deprivation and infectious diseases was well known from animal studies as early as the 1920's, when vitamin A was called the "anti-infective vitamin" (26). Observational studies in humans have shown that there is a strong reciprocal relationship between vitamin A deficiency and infectious diseases—each seems to increase the risk of the other (27). Deficiency leads to depressed humoral and cellular immunity, increased susceptibility to infectious diseases, and heightened infectious morbidity and mortality (28).

Vitamin A is essential for maintaining epithelial integrity of the respiratory, gastrointestinal, and genitourinary systems, and deficiency is accompanied by changes in the formation of epithelial structures (29). Keratinization and stratification of the vaginal epithelium is one of the earliest and most consistent manifestations of vitamin A deficiency in animal models, and the return of vaginal integrity was found to be an extremely specific and rapid response to replenishment of vitamin A stores (30-33).
Pathologic evidence of mucosal compromise has been described among vitamin A deficient children, even in the absence of the most clinically observable feature of deficiency disease, xerophthalmia (34, 35).

In persons without HIV-1 infection, vitamin A deficiency has generally been a disease of children and of pregnant and lactating women in developing countries (28, 36). Numerous supplementation trials with vitamin A have been conducted in an effort to improve immune status and reduce infectious morbidity and mortality among populations at risk for deficiency. Among children with measles, a meta-analysis concluded that supplementation reduced mortality by more than 60% (37). Furthermore, this study found that regular, community-based supplementation reduced general childhood mortality by 30%. A smaller trial found that supplementation increased the absolute number and proportion of CD4 T-cells in deficient children (38). Regular vitamin A distribution demonstrated a 30% reduction in episodes of malaria in children participating in a trial in Papua New Guinea (39). Another large field trial in Nepal of regular low-dose supplementation in adult women showed a 40% reduction in pregnancy-related mortality (40). On the basis of these studies and others, the World Health Organization recommends regular, high-dose, universal vitamin A supplementation for infants, children, and lactating women in countries where deficiency is common (41).

In persons with HIV-1 infection, vitamin A deficiency has been associated with enhanced immune dysfunction. Deficiency was associated with lower CD4 counts in a cross-sectional study from Baltimore (42) and with faster decline in CD4 count over time in a longitudinal cohort study from Miami (43). A case-control study from Rwanda
found deficiency to be more common among women with rapid progression to AIDS compared to those with slow progression (44). Longitudinal analysis of a cohort of HIV-1 infected injection drug users in Baltimore showed that vitamin A deficiency was associated with increased mortality (relative risk 6.3, 95% CI 2.1-18.6) (45), and a larger nested case-control analysis revealed this association to be independent of wasting (odds ratio 4.6, 95% CI 1.8-11.3) (46).

Overall, the observational data associating low serum vitamin A concentrations with genital tract shedding of HIV-1 and mother-to-child HIV-1 transmission as well as with HIV-1 disease progression were strong and consistent. Thus, in 1998, we initiated a randomized, double-blind, placebo-controlled trial of vitamin A supplementation among HIV-1 infected women in Mombasa, Kenya. The primary goal of the study was to assess the effect of vitamin A supplementation on vaginal shedding of HIV-1 in a population with a high prevalence of vitamin A deficiency. Secondary goals included assessing the effect of supplementation on HIV-1 plasma viral load and CD4 and CD8 counts as well as comparing the prevalence of vitamin A deficiency in HIV-1 infected and uninfected women in this population.
Notes to Introduction


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CHAPTER I
VITAMIN A SUPPLEMENTATION AND HIV-1 SHEDDING IN WOMEN:
RESULTS OF A RANDOMIZED CLINICAL TRIAL

Summary
Observational studies have associated vitamin A deficiency with vaginal shedding of human immunodeficiency virus type 1 (HIV-1) infected cells and mother-to-child HIV-1 transmission. To assess the effect of vitamin A supplementation on vaginal shedding of HIV-1, a randomized, double-blind, placebo-controlled trial of 6 weeks of daily oral vitamin A (10,000 IU retinyl palmitate) was conducted among 400 HIV-1 infected women in Mombasa, Kenya. At follow-up, there was no significant difference in the prevalence of HIV-1 DNA (18% vs. 21%, p=0.4) or the quantity of HIV-1 RNA (3.12 vs. 3.00 log_{10} copies/swab, p=1.0) in vaginal secretions of women receiving vitamin A compared to women receiving placebo. No effect of supplementation on plasma HIV-1 viral load or CD4 or CD8 counts was observed, and no effect was seen among women who were vitamin A deficient at baseline. Vitamin A supplementation is unlikely to decrease the infectivity of women infected with HIV-1.

Introduction
More than 95% of new human immunodeficiency virus type 1 (HIV-1) infections occur in developing countries, where few options exist for treatment of HIV-1 or HIV-1-related opportunistic infections [1]. Heterosexual transmission of HIV-1 is the principal
mode of infection for adults and represents the vast majority of total HIV-1 infections. Effective strategies to control the HIV-1 pandemic would ideally combine interventions that reduce infectivity of HIV-1 infected individuals with those that reduce susceptibility of exposed individuals. HIV-1 infected women can shed HIV-1 and HIV-1 infected cells in cervical and vaginal secretions, and there is epidemiologic evidence that those who shed more virus in the genital tract are more infectious [2-4].

Several studies have sought to elucidate characteristics that are associated with detection of HIV-1 in genital secretions of infected women, as identification of modifiable correlates may suggest intervention strategies to prevent sexual and vertical transmission. In 1997, we reported the results of a large cross-sectional study of correlates of shedding of HIV-1 infected cells in cervical and vaginal secretions of HIV-1 infected women [5]. Serum vitamin A concentration was strongly related to vaginal shedding. In analysis adjusting for CD4 count, prevalence of detection odds ratios were 12.9 for severe vitamin A deficiency (<20 μg/dl), 8.0 for moderate vitamin A deficiency (20-29.9 μg/dl), and 4.9 for low normal serum vitamin A (30-39.9 μg/dl), when compared to concentrations within the high normal range (≥40 μg/dl). These findings reinforced those of earlier observational studies associating low serum vitamin A levels with increased vaginal shedding of HIV-1 infected cells during pregnancy [6], increased shedding of HIV-1 infected cells in breastmilk [7], and increased mother-to-child HIV-1 transmission [8]. In addition, low serum vitamin A concentrations have been associated with lower CD4 counts [9], faster disease progression [10], and increased mortality [11] among HIV-1 infected individuals.
Vitamin A has long been known to play a key role in normal immune function [12]. Deficiency leads to depressed humoral and cellular immunity as well as heightened morbidity and mortality, especially from infectious diseases [13]. The most striking manifestations of vitamin A deficiency involve changes in the formation of epithelial structures, as vitamin A is essential for maintaining epithelial integrity of the ocular, respiratory, gastrointestinal, and genitourinary systems [14]. Large randomized trials conducted among HIV-1 uninfected individuals in developing countries have shown vitamin A supplementation can increase CD4 counts among deficient children [15], reduce childhood mortality [16], and decrease pregnancy-related mortality among adult women [17].

The consistency of the observational data relating vitamin A deficiency and HIV-1 disease and the positive results of supplementation trials in HIV-1 uninfected populations suggest that vitamin A supplementation might be an effective and simple intervention for persons with HIV-1 infection. Thus, in 1998 we initiated a randomized, double-blind, placebo-controlled trial of vitamin A supplementation among HIV-1 infected women in Mombasa, Kenya. The primary goal of this study was to assess the effect of supplementation on shedding of HIV-1 and HIV-1 infected cells in vaginal secretions. Secondary outcome measures included the effect of supplementation on CD4 and CD8 counts and plasma HIV-1 viral load. We hypothesized that supplementation would decrease shedding and plasma viral load and would increase CD4 and CD8 counts, and that these effects might be greatest among women having low serum vitamin A concentrations at baseline.
Materials and Methods

Study participants and procedures. Between September 1998 and June 2000, women attending the female outpatient clinic at Coast Provincial General Hospital in Mombasa, Kenya were offered serologic testing for HIV-1. Seropositive women meeting the study inclusion criteria were offered participation in the trial. Exclusion criteria included: age under 18 or over 45 years, use of vitamin supplements within the prior 3 months, or pregnancy within the prior 3 months. Women using oral contraceptive pills within the prior 3 months were also excluded because serum retinol levels are raised in these individuals, rendering evaluation of true vitamin A status difficult [18].

At baseline, women were interviewed with a standard questionnaire, covering demographic characteristics, medical history, and sexual and contraceptive practices. Serum and EDTA-anticoagulated blood were obtained, and general physical and pelvic examinations were performed. Urine testing for human chorionic gonadotropin was performed to exclude current pregnancy (QuickVue, Quidel, San Diego, CA, USA). Vaginal and cervical secretions were collected for PCR detection of HIV-1. All pelvic examinations were conducted by one of two investigators (J.M.B. and R.S.M.). Women who were menstruating were not examined and were asked to return for examination once menstruation had ended. Women with signs or symptoms of STDs (i.e., abnormal cervical or vaginal discharge or cervical or vaginal ulcers) were asked to return for examination one week after completing treatment.

Genital samples for HIV-1 PCR assays were collected with plastic-handled
Dacron swabs. Vaginal secretions were sampled by rolling a swab 3 full turns against the lateral vaginal wall, avoiding pooled secretions. Swabs for HIV-1 DNA PCR were placed in dry cryovials, and swabs for HIV-1 RNA PCR were placed in 1 ml freezing medium (70% RPMI, 20% fetal calf serum, 10% DMSO, with added penicillin, streptomycin, and amphotericin B). Cryovials were stored on ice until transfer to a −70°C freezer. Vials containing freezing medium were transferred to liquid nitrogen after overnight slow freezing in an isopropanol-insulated container.

Women were randomized to a six-week daily supply of gelatin capsules containing either 10,000 IU vitamin A as retinyl palmitate or placebo. This dosage is recommended by the World Health Organization for correction of symptomatic vitamin A deficiency in women of childbearing age [19]. It is appropriate for daily administration to women with or without demonstrated vitamin A deficiency and is safe for use during pregnancy and lactation. Capsules were distributed in prescription bottles containing an alarm set to remind patients of their daily dosage (RemindRx, IBV Technologies, Seattle, WA, USA). Use of these bottles has been associated with significantly improved compliance with daily medication in this population [20]. After the first 50 vitamin A and 50 placebo patients were enrolled, a third arm was added to the study to evaluate the effect of multivitamin supplementation. Thus, women were randomized to one of three potential regimens: vitamin A, multivitamin, or placebo. The current analysis is restricted to the vitamin A/placebo comparison.

Women were asked to return after six weeks for follow-up. A follow-up questionnaire was administered, blood was collected, and a pelvic examination including
specimen collection was performed as at enrollment. A pill count was conducted at follow-up to assess compliance. All women were provided with 4 weeks of daily dosage of 10,000 IU vitamin A at the follow-up visit, to ensure adequate treatment of vitamin A deficiency in the placebo group, since levels of serum retinol were not available in the field.

Computer-generated block randomization was used to assign treatment group. Blocks were of random size. Pill bottles were coded with random numbers, and the regimens were indistinguishable. Field researchers were blinded to treatment assignments, and the bottle code was held by the study statistician (B.A.R.) during the trial. Capsules were prepared and packaged by Tishcon, Corp. (Westbury, NY, USA). Vitamin A capsules tested after 12 months of field storage retained 100% activity.

Laboratory methods. HIV-1 serology was by ELISA (Detect HIV-1/2, BioChem ImmunoSystems, Montreal, Canada). Positive tests were confirmed with a second ELISA (Recombigen, Cambridge Biotech, Worchester, MA, USA). Absolute CD4 and CD8 counts were determined using a semi-automated system (Zymmune CD4/CD8 Cell Monitoring Kit, Bartels Inc., Issaquah, WA, USA). The lower limit of quantification for this system is 25 cells/μl. Light-protected sera were tested for vitamin A by high-pressure liquid chromatography [21]. Using standard definitions, deficiency was defined by concentrations below 30 μg/dl and severe deficiency by concentrations below 20 μg/dl [12].

Cryovials containing vaginal swabs and plasma for HIV-1 PCR were shipped in liquid nitrogen or on dry ice to the University of Washington. Swab samples were tested
for HIV-1 DNA by nested PCR amplification of the \textit{gag} gene [22]. This assay is able to detect a single copy of HIV-1 DNA. The Gen-Probe HIV-1 viral load assay (Gen-Probe, San Diego, CA, USA), which is a transcription-mediated amplification, was used for quantitative detection of HIV-1 RNA in plasma and vaginal samples [22, 23]. The lower limit of quantification is 25 copies per test. For plasma, where up to 500 \( \mu l \) was tested, the limit was thus 50 copies/ml. For vaginal swabs, where 1/5 of the media in which the swab was eluted was tested (200 \( \mu l \)), the lower limit of quantification was 125 copies/swab.

\textit{Data analysis.} The study was designed with a sample size of 200 participants in each arm for 80\% power to detect a 3-fold difference in the prevalence of vaginal shedding of HIV-1 DNA using a 2-sided test with \( \alpha=0.05 \) and 15\% loss to follow-up, based on a baseline prevalence of vaginal HIV-1 DNA shedding of 14\% as seen in the earlier observational study which served as the basis for this trial [5]. We postulated a priori that supplementation might have the greatest effect among women with enrollment serum vitamin A levels indicative of deficiency or severe deficiency, and this study sample size allowed for detection of a 2-fold and 3-fold difference in vaginal HIV-1 DNA shedding, respectively, among these two subgroups, assuming the prevalence of deficiency and its relationship with shedding were the same as in the earlier observational study. When this trial was initiated, there was limited information on HIV-1 plasma or genital RNA load in this population.

All analyses were intent-to-treat. Vaginal and plasma HIV-1 RNA viral loads and CD4 counts below the limits of quantification were imputed to be half those limits.
Comparisons of categorical variables were performed using $\chi^2$ tests and comparisons of continuous variables using Mann-Whitney U-tests. The effect of supplementation on our primary and secondary outcome measures at follow-up was evaluated using multivariate linear or logistic regression models. To approximate normality, vaginal and plasma viral loads were log transformed and CD4 and CD8 counts were square root transformed. Multivariate regression models were adjusted for the baseline value of the outcome measure as well as for the square root of enrollment CD4 count, since enrollment CD4 count differed between the randomization groups. Further adjustment for other baseline measures did not substantially affect the results. All statistical analyses were conducted using SPSS 10.0 (SPSS, Chicago, IL, USA).

Results

Study population. Of 2021 women tested for antibodies to HIV-1, 1026 (51%) were seropositive (Figure 1). Eight hundred fifty-seven (84%) HIV-1 infected women returned to receive their results, and 650 (76%) were enrolled in the study. Two hundred women received active vitamin A and 200 received placebo. The remaining 250 women participated only in the multivitamin arm of the trial and were not included in this analysis. Two hundred seven women received their results but did not enroll in the study, of whom 131 (63%) declined study participation. The remaining 76 women were excluded from the study for the following reasons: age $\geq$45 years (12 women), pregnancy (14 women), use of oral contraceptive pills (10 women), too sick to participate (19 women), other reasons (21 women).
The median age of enrolled women was 28 years (range 18-45 years). Study participants were of low socioeconomic status, with 71% living in a one-room home, 93% sharing a toilet with other households, 5% having running water within their home, and 16% owning a television. Fifty-two percent were married, and 10% were widowed. Only 9% reported a history of prostitution. Eighty-six percent had been pregnant at least once, and 13% were breastfeeding. Progesterone-based hormonal contraception (Depo-Provera or Norplant) was used by 20%. Oral contraceptive pill use within the previous 3 months was a study exclusion criterion, and only 2% had used this method 3-6 months before enrollment.

HIV-1 related immunosuppression was common in this population, with 173 women (43%) having CD4 counts <200. The median CD4 count was 226. Three-quarters of participants had symptoms and/or signs of HIV-1 disease. The median HIV-1 plasma viral load was 5.44 (range 2.33-7.66) log_{10} copies/ml [275,630 (range 214-46,000,000) copies/ml].

Vitamin A deficiency was extremely common in this population. At baseline, 234 women (59%) had serum concentrations <30 µg/dl (vitamin A deficiency) and 104 (26%) had concentrations <20 µg/dl (severe vitamin A deficiency).

HIV-1 DNA was detected in 22% (86/400) of vaginal swabs at enrollment. Two hundred ninety-four vaginal swabs (74%) had HIV-1 RNA above the lower limit of quantification of 125 copies/swab. The median concentration of HIV-1 RNA in vaginal swabs was 3.32 (range <2.10-6.15) log_{10} copies/swab.

Demographic and medical characteristics at baseline were generally comparable
between the two randomization groups (Table 1.1). However, slight differences, some of which were statistically significant, were present for some of the outcome measures at enrollment. The median CD4 count among women randomized to vitamin A was slightly higher than that among women randomized to placebo (240 vs. 203 cells/μl, p=0.06). They also had a higher median CD8 count (630 vs. 560, p=0.03). Women receiving vitamin A had a 0.20 log_{10} lower median plasma HIV-1 viral load (p=0.07) and a 0.34 log_{10} lower median vaginal HIV-1 viral load (p=0.08) than women receiving placebo.

There were no statistically significant differences in the prevalence of HIV-1 DNA in vaginal samples (p=0.8) or of vaginal HIV-1 RNA ≥125 copies/swab (p=0.2), in median serum vitamin A concentration (p=0.4), or in the prevalence of vitamin A deficiency or severe deficiency (p=0.5 and p=0.4, respectively).

**Follow-up.** Three hundred and fifty-four women (89% of those enrolled) returned for follow-up. This included 176 (88%) randomized to the vitamin A arm and 178 (89%) randomized to the placebo arm (p=0.8). The median time to follow-up was 42 days (range 32-445 days), and 326 women (92% of those with follow-up) had their follow-up examination within 8 weeks of enrollment. Median time to follow-up did not differ by randomization group (p=0.9).

The 46 women lost to follow-up were more immunosuppressed than the women who completed the study (median CD4 count 98 vs. 239, p<0.001). They also had a higher median plasma viral load (5.99 vs. 5.36 log_{10} copies/ml, p<0.001), though there was no statistically significant difference at enrollment in the prevalence of HIV-1 DNA in their vaginal swabs (22% vs 21%, p=1.0) or in their median vaginal HIV-1 RNA
concentration (3.56 vs. 3.29 log_{10} copies/swab, p=0.14). They had a lower median
enrollment serum vitamin A concentration (22.2 vs. 28.2 μg/dl, p<0.001) and were more
likely to be deficient (78% vs. 56%, p=0.004) or severely deficient (43% vs. 24%,
p=0.004) in vitamin A.

Of the 354 participants who returned for follow-up, 318 (90%) returned their
medication bottle. The median number of pills remaining was 0 (range 0-15), and 307
(97%) had 2 or fewer pills remaining. There was no difference between the two
randomization groups in the median number of pills not consumed (p=0.4). Among the
36 women who did not return their medication bottle, 34 (94%) reported 2 or fewer pills
were remaining. Thus, overall, 341 women (96% of those with follow-up) took at least
95% of their prescribed pills.

*Effect of vitamin A supplementation.* At follow-up, there was no statistically
significant difference in the prevalence of HIV-1 DNA detected in vaginal swabs from
women randomized to vitamin A when compared with women randomized to placebo
(18% vs. 21%, p=0.4) (Table 1.2). In a multivariate logistic regression model, which
controlled for baseline vaginal HIV-1 DNA and baseline CD4 count, no effect of vitamin
A supplementation on follow-up vaginal HIV-1 DNA detection was seen (adjusted odds
ratio 0.7, 95% confidence interval [CI] 0.4-1.4). The median vaginal HIV-1 RNA
centration at follow-up did not differ between the two randomization groups (3.12 vs.
3.00 log_{10} copies/swab, p=1.0), and no statistically significant effect of vitamin A was
demonstrated after controlling for baseline CD4 count and vaginal HIV-1 RNA (adjusted
regression coefficient 0.15, 95% CI –0.05-0.35). The prevalence of vaginal HIV-1 RNA
≥125 copies/swab was also not significantly different at follow-up in univariate and multivariate analyses.

Among the secondary outcome measures, plasma viral load was similar at follow-up for women receiving vitamin A or placebo, and multivariate analysis did not alter this finding. There was a difference in median CD4 count between the two randomization groups at follow-up, but in a multivariate model which adjusted for CD4 count at baseline, no significant effect of supplementation was retained. Similarly, the median follow-up CD8 counts for the two randomization groups were slightly different, but no effect of supplementation was found in multivariate analysis.

*Subgroup analyses.* We had hypothesized a priori that vitamin A supplementation might have a greater effect among the subgroup of women who had serum vitamin A concentrations indicative of deficiency (<30 μg/dl) or severe deficiency (<20 μg/dl) at baseline. Among those who were deficient, there was no significant difference in the prevalence of HIV-1 DNA or the quantity of HIV-1 RNA in vaginal swabs at follow-up, nor was there any difference in median plasma HIV-1 viral load, CD4 count, or CD8 count for women who received active vitamin compared with those who received placebo (Table 1.3). Adjustment for baseline differences between the two groups did not affect these findings (data not shown). Similarly, among women with serum vitamin A levels indicative of severe vitamin A deficiency at enrollment, those receiving active vitamin A had a similar prevalence of vaginal HIV-1 DNA, median vaginal HIV-1 RNA quantity, median plasma HIV-1 viral load, median CD4 count, and median CD8 count as those receiving placebo at the follow-up visit (Table 1.3). Adjustment for baseline factors did
not change these results (data not shown).

Because the population of women enrolled in the trial was relatively immunosuppressed, we considered a posteriori whether immunosuppression limited response to vitamin A supplementation. Thus, we repeated the analyses in Table 1.2, including an interaction term to assess whether the effect of the vitamin was different among the 223 women who had CD4 counts >200 at baseline (56% of the total study sample). For each of the outcome measures, there was no evidence of a statistical interaction for this subgroup (p-value for interaction term >0.05 in all models), suggesting that the effect of vitamin A did not differ by baseline CD4 count.

**Effect of supplementation on serum vitamin A concentration.** At follow-up, women who had received vitamin A had higher serum vitamin A concentrations than those who had received placebo (median 29.4 vs. 26.8 μg/dl, p=0.03). However, the prevalence of vitamin A deficiency was similar between the two groups [94/176 (53%) vs. 106/178 (60%), p=0.2]. There was a trend for severe vitamin A deficiency to be less common among women receiving vitamin A [34/176 (19%)] compared to women receiving placebo [48/178 (27%)] (p=0.09). Among women who were vitamin A deficient at baseline, supplementation was associated with a higher median serum vitamin A concentration at follow-up compared to placebo (25.1 vs. 21.2 μg/dl, p=0.03), but this was not true among women who were severely vitamin A deficient at baseline (19.0 vs. 17.9 μg/dl, p=0.5).
Discussion

In this randomized trial, we found no statistically significant effect of vitamin A on shedding of HIV-1 or HIV-1 infected cells in vaginal secretions, HIV-1 plasma viral load, or CD4 or CD8 counts. Among women with serum vitamin A concentrations indicative of deficiency or severe deficiency at enrollment, no significant effect of vitamin A supplementation on any of the outcome measures was observed. Also, we found no evidence for any difference in effect among those women who had CD4 counts >200 at baseline.

This trial was motivated by the results of a cross-sectional study done among women in Mombasa that found low serum vitamin A concentrations to be strongly predictive of vaginal shedding of HIV-1 infected cells [5]. Other correlates of female genital tract shedding of HIV-1 have been identified in cross-sectional studies, including sexually transmitted diseases and high plasma HIV-1 viral load [5, 24]. Prospective studies have demonstrated that shedding can be reduced by strategies targeted at these correlates, such as STD treatment [22, 25] and antiretroviral therapy [26], suggesting that genital tract shedding is amenable to intervention. We were disappointed that vitamin A supplementation did not reduce shedding in a similar fashion, and we considered possible explanations for our results.

One possible explanation for our findings could be a lack of statistical power. However, we had high study power to detect an effect of vitamin A supplementation for each of our outcome measures. The prevalence of vaginal HIV-1 DNA at enrollment
(22%) was higher and the rate of loss to follow-up lower (11%) than had been expected when the study was designed. Consequently, we had >95% power to detect a 3-fold difference in vaginal HIV-1 DNA prevalence between the vitamin A and placebo groups at follow-up and 74% power to detect a 2-fold difference. We had 80% power to detect a 10% difference in vaginal HIV-1 quantity, a 5% difference in plasma HIV-1 viral load, and an 18% difference in CD8 count. Thus, it is unlikely that an inadequate sample size is responsible for our findings.

Second, the dosage of vitamin A used may have been inadequate. However, this dosage was already >10-fold higher than the U.S. Recommended Dietary Allowance [27] and is the recommended treatment for adult women with corneal lesions as a result of very severe vitamin A deficiency [19]. No women in this study had such lesions. The dosage of vitamin A administered in this trial was similar to that used in three trials of vitamin A for preventing mother-to-child HIV-1 transmission [28-30].

A third possible reason we may have failed to show any effect of supplementation is that our intervention may have been of insufficient length. However, vitamin A treatment of symptomatic deficiency requires only 4 weeks of daily supplements [19], and we used 6 weeks of supplementation in this trial. Reductions in vaginal HIV-1 shedding have been demonstrated within 3 weeks of initiating STD treatment [22]. This suggests that our 6 weeks of follow-up was sufficient to demonstrate any change in vaginal shedding, though it would be interesting to know whether any changes would become apparent with longer follow-up. Overall, it seems unlikely that insufficient vitamin A dose or insufficient time to follow-up are responsible for our results.
A fourth reason for the lack of effect of vitamin A in our study may be that the participants were too advanced in HIV-1 disease stage to respond adequately to supplementation. We found no evidence for a difference in effect of vitamin A between the subgroups of women with CD4 counts >200 and ≤200 at baseline. Trials among women at earlier stages of HIV-1 infection would be needed to more definitively demonstrate whether vitamin A supplementation has any benefit for women before the development of significant immunosuppression.

A fifth explanation for failing to demonstrate any significant effect of vitamin A in this study is that serum vitamin A levels are not indicative of true vitamin A deficiency in this population. The observational studies relating vitamin A deficiency to disease progression, mother-to-child transmission, and vaginal shedding relied upon serum vitamin A levels to classify deficiency, and, though they adjusted for CD4 count, incomplete control for severity of HIV-1 disease may have led to biased findings. Some have suggested that the acute phase response, which is known to lower serum vitamin A levels during some infections even in the presence of adequate liver vitamin A stores, operates in persons infected with HIV-1 [30]. In this trial, women randomized to receive vitamin A had higher serum vitamin A concentrations at follow-up than those randomized to placebo, indicating that the supplement was able to raise vitamin A levels. However, there was no difference in follow-up serum vitamin A concentrations between the two arms among those women who were severely vitamin A deficient at enrollment, though this group would seem to be the most likely to respond to therapy. If low vitamin A levels in part reflect more advanced or active HIV-1 infection, rather than true
deficiency, then this may explain the failure to respond to supplementation. More
detailed analyses of the relationships between serum vitamin A levels, HIV-1 disease
stage, and the acute phase response may help to explain the disappointing findings of this
and other randomized trials of vitamin A for HIV-1 infection.

Our results are consistent with those of other clinical trials of vitamin A for
prevention of HIV-1 transmission [31]. Among HIV-1 infected individuals in the United
States, daily beta-carotene (a pro-vitamin A carotenoid) supplementation did not increase
CD4 count [32], and one-time, high-dose supplementation demonstrated no effect on
HIV-1 plasma viral load [33, 34]. Randomized trials in Malawi, South Africa, and
Tanzania found daily low-dose vitamin A failed to decrease mother-to-child HIV-1
transmission [28-30]. Our study demonstrated no reduction in vaginal HIV-1 shedding
after 6 weeks of low-dose vitamin A supplementation among HIV-1 infected women with
a high prevalence of vitamin A deficiency. The short duration of follow-up precludes
conclusions about the effect of vitamin A supplementation on disease progression and
HIV-1 related mortality, though we found no effect on HIV-1 plasma viral load or CD4
count. The disparity between the results of the randomized trials and those of the earlier
observational studies that found an association between low serum vitamin A levels and
faster disease progression and increased infectivity among HIV-1 infected individuals
emphasizes the importance of randomized clinical trial designs in evaluating intervention
strategies to prevent and treat HIV-1 infection. On the basis of our results and those of
other randomized trials, it appears unlikely that there is a role for vitamin A
supplementation in reducing infectivity or preventing transmission of HIV-1.
Notes to Chapter 1


Figure 1.1 Flow Diagram of Trial Participants
### Table 1.1 Baseline Characteristics

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Vitamin A Arm N=200</th>
<th>Placebo Arm N=200</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Demographics</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age, years</td>
<td>28 (18-45)</td>
<td>28 (18-45)</td>
</tr>
<tr>
<td>Education, years</td>
<td>7 (0-12)</td>
<td>7 (0-12)</td>
</tr>
<tr>
<td>Currently married</td>
<td>103 (52%)</td>
<td>103 (52%)</td>
</tr>
<tr>
<td>Number of rooms in home</td>
<td>1 (1-6)</td>
<td>1 (1-5)</td>
</tr>
<tr>
<td>Toilet in home</td>
<td>18 (9%)</td>
<td>9 (5%)</td>
</tr>
<tr>
<td>Running water in home</td>
<td>13 (7%)</td>
<td>6 (3%)</td>
</tr>
<tr>
<td>Own a television</td>
<td>34 (17%)</td>
<td>31 (16%)</td>
</tr>
<tr>
<td>Smoking</td>
<td>9 (5%)</td>
<td>1 (1%)</td>
</tr>
<tr>
<td>Alcohol use</td>
<td>19 (10%)</td>
<td>18 (9%)</td>
</tr>
<tr>
<td>History of prostitution</td>
<td>20 (10%)</td>
<td>14 (7%)</td>
</tr>
<tr>
<td><strong>Obstetric and contraceptive history</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gravidity</td>
<td>2 (0-10)</td>
<td>2 (0-10)</td>
</tr>
<tr>
<td>Months since last pregnancy</td>
<td>59 (3-312)</td>
<td>59 (3-312)</td>
</tr>
<tr>
<td>Currently breastfeeding</td>
<td>24 (12%)</td>
<td>29 (15%)</td>
</tr>
<tr>
<td>Depo-Provera or Norplant for contraception</td>
<td>34 (17%)</td>
<td>47 (24%)</td>
</tr>
<tr>
<td>Practice vaginal douching</td>
<td>95 (48%)</td>
<td>104 (52%)</td>
</tr>
<tr>
<td><strong>HIV-1 disease history</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Any HIV-1 related symptom$^1$</td>
<td>143 (72%)</td>
<td>137 (69%)</td>
</tr>
<tr>
<td>Any HIV-1 related sign$^2$</td>
<td>60 (30%)</td>
<td>55 (28%)</td>
</tr>
<tr>
<td>Body-mass index</td>
<td>21.5 (14.6-45.1)</td>
<td>20.8 (14.9-31.9)</td>
</tr>
<tr>
<td><strong>Vitamin A status</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vitamin A (μg/dl)</td>
<td>27.8 (3.5-81.0)</td>
<td>27.2 (2.8-57.2)</td>
</tr>
<tr>
<td>&lt;30 μg/dl (deficiency)</td>
<td>114 (57%)</td>
<td>120 (60%)</td>
</tr>
<tr>
<td>&lt;20 μg/dl (severe deficiency)</td>
<td>48 (24%)</td>
<td>56 (28%)</td>
</tr>
<tr>
<td><strong>Outcome measures</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vaginal HIV-1 DNA</td>
<td>44 (22%)</td>
<td>42 (21%)</td>
</tr>
<tr>
<td>Vaginal HIV-1 RNA (log$_{10}$ copies/swab)</td>
<td>3.16 (&lt;2.10-6.07)</td>
<td>3.50 (&lt;2.10-6.15)</td>
</tr>
<tr>
<td>Vaginal HIV-1 RNA ≥125 copies/swab</td>
<td>141 (71%)</td>
<td>153 (77%)</td>
</tr>
<tr>
<td>Plasma HIV-1 RNA (log$_{10}$ copies/ml)</td>
<td>5.34 (2.33-7.08)</td>
<td>5.54 (2.43-7.66)</td>
</tr>
<tr>
<td>CD4 count (cells/μl)</td>
<td>240 (&lt;25-1010)</td>
<td>203 (&lt;25-1117)</td>
</tr>
<tr>
<td>CD8 count</td>
<td>630 (111-2000)</td>
<td>560 (&lt;25-3992)</td>
</tr>
</tbody>
</table>
Footnotes to Table 1.1

1 Includes a history of fever >1 month, diarrhea >1 month, cough >1 month, weight loss >5 kg, or itching skin rash during past year.

2 Includes the presence of oral thrush, oral hairy leukoplakia, oral ulcers, maculopapular rash, or Kaposi’s sarcoma on physical examination.
Table 1.2  Effect of vitamin A supplementation

<table>
<thead>
<tr>
<th>Outcome measure</th>
<th>Median (range) or number (%)</th>
<th>p-value</th>
<th>Adjusted OR (95% CI)</th>
<th>Adjusted regression coefficient (95% CI)</th>
<th>Adjusted p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vaginal HIV-1 DNA</td>
<td>31 (18%)</td>
<td>0.4</td>
<td>0.7 (0.4-1.4)</td>
<td></td>
<td>0.3</td>
</tr>
<tr>
<td>Vaginal HIV-1 RNA (log_{10} copies/swab)</td>
<td>3.12 (&lt;2.10-5.63)</td>
<td>1.0</td>
<td>0.15 (-0.05-0.35)</td>
<td></td>
<td>0.2</td>
</tr>
<tr>
<td>Vaginal HIV-1 RNA ≥125 copies/swab</td>
<td>121 (69%)</td>
<td>0.6</td>
<td>1.5 (0.9-2.5)</td>
<td></td>
<td>0.1</td>
</tr>
<tr>
<td>Plasma HIV-1 RNA (log_{10} copies/ml)</td>
<td>5.33 (2.63-7.20)</td>
<td>0.1</td>
<td>0.06 (-0.02-0.15)</td>
<td></td>
<td>0.2</td>
</tr>
<tr>
<td>CD4 count (cells/μl)</td>
<td>272 (&lt;25-997)</td>
<td>0.04</td>
<td>0.34 (-0.22-0.90)</td>
<td></td>
<td>0.2</td>
</tr>
<tr>
<td>CD8 count (cells/μl)</td>
<td>719 (97-2001)</td>
<td>0.08</td>
<td>0.06 (-0.91-1.04)</td>
<td></td>
<td>0.9</td>
</tr>
</tbody>
</table>
Footnotes to Table 1.2

1 One woman in the placebo arm did not have vaginal specimens collected at follow-up.

2 For categorical variables (vaginal HIV-1 DNA, vaginal HIV-1 RNA ≥125 copies/swab), adjusted odds ratio and p-value from multivariate logistic regression model. For continuous variables (vaginal HIV-1 RNA, plasma HIV-1 RNA, CD4 count, CD8 count), regression coefficient and p-value from multivariate linear regression model. For each outcome measure, results are adjusted for the square root of baseline CD4 count and for the baseline value of that outcome measure (e.g., vaginal HIV-1 DNA is adjusted for the square root of baseline CD4 count and for HIV-1 DNA detection at the baseline visit).

3 The adjusted regression coefficients for CD4 and CD8 counts are from multivariate models using the square root of CD4 or CD8 count at follow-up as the dependent variable.
Table 1.3  Effect of vitamin A supplementation, by enrollment vitamin A status

<table>
<thead>
<tr>
<th>Outcome measure</th>
<th>Vitamin A Arm N=94</th>
<th>Placebo Arm N=104</th>
<th>p-value</th>
<th>Severe vitamin A deficient (&lt;20 µg/dl) at enrollment</th>
<th>Vitamin A Arm N=35</th>
<th>Placebo Arm N=49</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vaginal HIV-1 DNA</td>
<td>21 (22%)</td>
<td>21 (20%)</td>
<td>0.7</td>
<td></td>
<td>9 (26%)</td>
<td>11 (22%)</td>
<td>0.7</td>
</tr>
<tr>
<td>Vaginal HIV-1 RNA (log_{10} copies/swab)</td>
<td>3.54 (&lt;2.10-5.63)</td>
<td>3.30 (&lt;2.10-5.85)</td>
<td>0.7</td>
<td>3.78 (&lt;2.10-5.63)</td>
<td>3.43 (&lt;2.10-5.85)</td>
<td></td>
<td>0.3</td>
</tr>
<tr>
<td>Vaginal HIV-1 RNA ≥125 copies/swab</td>
<td>70 (74%)</td>
<td>73 (70%)</td>
<td>0.5</td>
<td>29 (83%)</td>
<td>38 (78%)</td>
<td></td>
<td>0.6</td>
</tr>
<tr>
<td>Plasma HIV-1 RNA (log_{10} copies/ml)</td>
<td>5.61 (3.09-7.20)</td>
<td>5.56 (2.40-6.70)</td>
<td>0.8</td>
<td>5.83 (4.52-7.20)</td>
<td>5.69 (4.05-6.70)</td>
<td></td>
<td>1.0</td>
</tr>
<tr>
<td>CD4 count (cells/µl)</td>
<td>228 (&lt;25-817)</td>
<td>206 (&lt;25-840)</td>
<td>0.2</td>
<td>218 (&lt;25-550)</td>
<td>155 (&lt;25-770)</td>
<td></td>
<td>0.2</td>
</tr>
<tr>
<td>CD8 count (cells/µl)</td>
<td>655 (170-1595)</td>
<td>614 (187-3242)</td>
<td>1.0</td>
<td>763 (243-1528)</td>
<td>689 (223-1430)</td>
<td></td>
<td>0.5</td>
</tr>
</tbody>
</table>
CHAPTER II

VITAMIN A DEFICIENCY AND THE ACUTE PHASE RESPONSE AMONG HIV-1 INFECTED AND UNINFECTED WOMEN IN KENYA

Summary

Among HIV-1 infected individuals, vitamin A deficiency has been associated with faster disease progression and greater infectivity in observational studies, but randomized clinical trials have shown no effect of vitamin A supplementation. To examine the relationships between vitamin A deficiency and HIV-1 status, HIV-1 disease stage, and the acute phase response, a cross-sectional study of 400 HIV-1 infected and 200 HIV-1 uninfected women in Mombasa, Kenya was conducted. Among the HIV-1 infected women, the effect of vitamin A supplementation was examined in a randomized trial. Vitamin A deficiency was independently associated with HIV-1 infection [odds ratio (OR) 2.7, 95% confidence interval (CI) 1.9-4.0] and the acute phase response (OR 2.8, 95% CI 1.9-4.1). Among HIV-1 infected women, vitamin A deficiency and the acute phase response were both independently associated with higher HIV-1 plasma viral load and lower CD4 count. HIV-1 infected women having an acute phase response had no increase in serum vitamin A levels after supplementation. Among HIV-1 infected individuals, low serum vitamin A concentrations are associated with more active infection and the acute phase response. These results provide possible explanations for the disparity between observational studies and randomized trials of vitamin A for HIV-1 infection.
Introduction

Vitamin A has long been known to play a key role in normal immune function (1). Deficiency is associated with heightened susceptibility to infectious diseases as well as increased infectious disease morbidity and mortality (2). Randomized clinical trials among women and children in developing countries have demonstrated that vitamin A supplementation reduces infectious disease morbidity and mortality among populations with high prevalences of deficiency (3, 4).

In the United States, nutritional deficiencies, including vitamin A deficiency, have been found to be unusually common among HIV-1 infected individuals (5, 6). Because vitamin A supplementation is an inexpensive and simple intervention, a role for vitamin A in HIV-1 infection has been investigated in a number of studies (7). Longitudinal cohort studies from the United States have found vitamin A deficiency (when defined as serum retinol <30µg/dl) to be associated with faster decline in CD4 count over time (8) and increased HIV-1 related mortality (9). A case-control study from Rwanda found deficiency to be more common among women with rapid progression to AIDS compared to those with slow progression (10). Studies from Kenya and Malawi showed deficiency to be strongly associated with increased detection of HIV-1 infected cells in vaginal secretions and breastmilk of infected women (11-13) and with increased risk of mother-to-child HIV-1 transmission (14).

Despite the consistency of these findings from observational studies, randomized clinical trials have failed to demonstrate benefit of supplementation in HIV-1 disease (15). Beta-carotene (a pro-vitamin A carotenoid) supplementation failed to increase CD4
count (16) and high dose vitamin A supplementation failed to decrease HIV-1 plasma viral load (17, 18) in randomized trials among HIV-1 infected individuals in the United States. Three randomized trials in Africa showed no effect of daily low-dose vitamin A supplementation on mother-to-child HIV-1 transmission (19-21), and we demonstrated no effect of low-dose supplements on HIV-1 plasma viral load, CD4 count, and genital tract shedding of HIV-1 among non-pregnant Kenyan women (22).

One possible explanation for the lack of beneficial effect in these trials is that serum vitamin A concentration does not accurately reflect vitamin A tissue stores in HIV-1 infected individuals and instead is simply a marker of more active or more advanced disease. While the observational studies controlled for CD4 count, incomplete control for severity of HIV-1 disease (e.g., confounding by HIV-1 plasma viral load) may have contributed to biased findings in these studies. Some have suggested that the acute phase response, which is known to artificially lower serum vitamin A levels in individuals with some acute illnesses, is responsible for lower vitamin A levels in HIV-1 infected compared with uninfected persons and, among those who are infected, for lower levels among those with more advanced disease (21). The acute phase response describes the series of physiologic events that accompany acute and chronic inflammatory states, including trauma, infection, and autoimmune diseases (23). Elevated serum concentrations of C-reactive protein (CRP) and α₁-acid glycoprotein (AGP) are indicative of an acute phase response and have been used to identify individuals potentially misclassified as vitamin A deficient based on depressed serum retinol concentration in the context of an acute phase reaction (24).
To address these issues, we conducted a cross-sectional study of HIV-1 infected and uninfected women in Mombasa, Kenya, to examine the relationships between serum vitamin A concentration and HIV-1 status, HIV-1 disease stage, and markers of the acute phase response (i.e., CRP and AGP). Among the HIV-1 infected women, we further assessed the effect of acute phase markers on response to low-dose vitamin A supplementation in a randomized, placebo-controlled trial.

Materials and Methods

Study participants and procedures. Between September 1998 and June 2000, we conducted a randomized clinical trial of vitamin A supplementation among HIV-1 infected women attending Coast Provincial General Hospital in Mombasa, Kenya (22). The goal of the trial was to assess the effect of supplementation on genital tract shedding of HIV-1, plasma HIV-1 viral load, and CD4 count. Individual, confidential counseling and serologic testing for HIV-1 were offered to women in the female outpatient clinic, with results made available after one week. HIV-1 seropositive women who consented to testing and who returned to receive their results were offered enrollment in the trial. Four hundred HIV-1 seropositive women were enrolled, and data collection for the cross-sectional study of vitamin A status and HIV-1 infection was completed during the enrollment visit. HIV-1 seronegative women were not eligible for the randomized trial, but those who returned for their results were eligible for participation in the cross-sectional study. Two hundred HIV-1 seronegative women were randomly selected by enrolling the first consenting HIV-1 seronegative woman after every 2 HIV-1
seropositive women enrolled. The study protocol was approved by the institutional review boards of the University of Washington and the University of Nairobi. All participants provided written informed consent.

Exclusion criteria for the randomized trial were as follows: age under 18 or over 45 years, use of vitamin supplements within the prior 3 months, pregnancy within the prior 3 months, or oral contraceptive pill use within the prior 3 months, the last because use may alter serum retinol levels (25). These criteria also applied to the HIV-1 seronegative women who participated in the cross-sectional study. No women had ocular signs of vitamin A deficiency (xerophthalmia or Bitot’s spots). No HIV-1 seropositive women used antiretroviral therapy.

All women were interviewed with a standard questionnaire, covering demographic characteristics, medical history, and sexual and contraceptive practices. Serum and EDTA-anticoagulated blood were obtained. Serum was light-protected after collection.

For the supplementation trial, HIV-1 seropositive women were randomized to a six-week daily supply of gelatin capsules containing either 10,000 IU vitamin A as retinyl palmitate or placebo. The regimens were indistinguishable, and field researchers were blinded to treatment assignments. Women were asked to return after six weeks for follow-up, at which time blood was again collected as at enrollment. A pill count was done to assess compliance.

HIV-1 seronegative women were provided with 4 weeks of daily dosage of 10,000 IU vitamin A after completion of the cross-sectional study, and HIV-1 positive
women were given the same dose of vitamin A after completion of the randomized trial, to ensure adequate treatment of vitamin A deficiency since levels of serum retinol were not available in the field.

*Laboratory methods.* HIV-1 serology was by ELISA (Detect HIV-1/2, BioChem ImmunoSystems, Montreal, Canada), and positive serologies were confirmed with a second ELISA (Recombigen, Cambridge Biotech, Worchester, MA, USA). Absolute CD4 and CD8 counts were determined for HIV-1 seropositive women using a semi-automated system (*Zymmune CD4/CD8 Cell Monitoring Kit*, Bartels Inc., Issaquah, WA, USA), with a lower limit of quantification of 25 cells/μl.

Serum and plasma were separated within 4 hours of collection and stored in cryovials at −70°C. For laboratory analyses, cryovials were shipped on dry ice to the University of Washington. For HIV-1 seropositive participants, HIV-1 plasma RNA viral loads were quantified previously (22) using the Gen-Probe quantitative HIV-1 RNA assay (Gen-Probe, San Diego, CA, USA) (26), with a lower limit of quantification of 50 copies/ml of plasma.

Light-protected sera were tested for vitamin A by high-pressure liquid chromatography (27). According to standard definitions, we used serum retinol concentrations <30 μg/dl to reflect vitamin A deficiency (28).

To identify patients with an acute phase response, we measured serum concentrations of C-reactive protein (CRP) and α1-acid glycoprotein (AGP) by nephelometry (Dade Behring, Marburg, Germany) (24). These are known as positive
acute phase proteins because their serum concentrations increase during the acute phase response. Serum concentrations of CRP $\geq$10 mg/l and/or AGP $\geq$1.2 g/l were used to define an acute phase response, as per manufacturers' instructions and standard usage (29, 30), although we recognize that a variety of demographic factors influence serum acute phase protein concentrations (31).

Data analysis. All statistical analyses were conducted using SPSS 10.0 (SPSS, Chicago, IL, USA). Comparisons of categorical variables were conducted using $\chi^2$ tests and comparisons of continuous variables using Mann-Whitney U-tests and Spearman's correlation coefficient. Logistic regression was used for multivariate analyses. Evidence of confounding was said to be present if addition of a potential confounder produced a $>10\%$ change in the odds ratio (OR) for the association under investigation.

Results

Study population. Two thousand twenty-one women were screened for antibodies to HIV-1, of whom 1026 (51%) were seropositive and 977 (48%) were seronegative. Eighteen (1%) had discordant ELISA results. One thousand six hundred ninety-nine women (84% of those screened) returned for their results, including 857 (84%) HIV-1 seropositive and 828 (85%) HIV-1 seronegative women ($p=0.5$). As described previously (22), 400 HIV-1 seropositive women were enrolled in the vitamin A randomized trial. They also participated in the cross-sectional study of vitamin A status and the acute phase response presented here. In addition, two hundred HIV-1 seronegative women were enrolled in the cross-sectional study.
The HIV-1 seropositive women were slightly older, less educated, and less likely to be currently married than the HIV-1 seronegative women (Table 2.1). They also were less likely to have characteristics associated with higher socioeconomic status, such as owning a television or having running water in the home. They had had more pregnancies and were more likely to drink alcohol or report a history of prostitution. The median body-mass index was lower for seropositive women.

*Vitamin A deficiency and HIV-1 infection.* HIV-1 seropositive women had lower serum vitamin A concentrations than HIV-1 seronegative women (Table 2.1) and were significantly more likely to have vitamin A concentrations reflecting deficiency [OR 3.5, 95% confidence interval (CI) 2.4-5.2]. None of the demographic factors listed in Table 2.1 confounded the relationship between HIV-1 serostatus and vitamin A status.

The population of HIV-1 seropositive women recruited for this study was relatively immunosuppressed, with a median CD4 count of 226 (range <25-1117) and a median HIV-1 plasma viral load of 275,630 (range 214-46,000,000) copies/ml (Table 2.1). Among these women, vitamin A deficiency was associated with higher HIV-1 plasma viral load and lower CD4 count (Table 2.2). Serum vitamin A levels were significantly correlated with both of these markers of HIV-1 disease severity ($r=-0.29$, $p<0.001$ for HIV-1 plasma viral load and $r=0.24$, $p<0.001$ for CD4 count). By multivariate logistic regression modeling, each 1 log$_{10}$ increase in plasma viral load was associated with a 1.6 (95% CI 1.2-2.2) fold increased odds of vitamin A deficiency while each 100 cell/µl increase in CD4 count was associated with a 0.9 (95% CI 0.8-1.0) fold decrease. Symptoms and signs of HIV-1 disease were more common among women who
were vitamin A deficient (Table 2.2).

**Acute phase response and HIV-1 infection.** Serum concentrations of CRP and AGP were significantly higher among HIV-1 seropositive than among HIV-1 seronegative women (Table 2.1). When women were defined as having an acute phase response based on CRP $\geq 10$ mg/l and/or AGP $\geq 12$ g/l, the prevalence of an acute phase response was 44% (174/400) among HIV-1 seropositive women and 14% (28/200) among HIV-1 seronegative women (OR 4.7, 95% CI 3.0-7.5). This relationship was not confounded by any of the demographic factors listed in Table 2.1.

Among HIV-1 seropositive women, symptoms and signs of HIV-1 disease were more common among those having CRP and/or AGP levels reflecting an acute phase response (Table 2.2). Women with an acute phase response had a higher median HIV-1 plasma viral load and a lower median CD4 count. CRP levels were correlated with plasma viral load ($r=0.29$, $p<0.001$) and with CD4 count ($r=-0.23$, $p<0.001$), as were AGP levels ($r=0.43$, $p<0.001$ for plasma viral load and $r=-0.34$, $p<0.001$ for CD4 count). In a logistic regression model containing viral load and CD4 count, each 1 log$_{10}$ increase in HIV-1 plasma viral load was associated with a 2.0 (95% CI 1.5-2.8) fold increased odds of being acute phase response positive and each 100 cell/µl increase in CD4 count was associated with a 0.8 (95% CI 0.7-0.9) fold decrease.

**Vitamin A deficiency and acute phase response.** Among HIV-1 seropositive women, there was a moderately strong and statistically significant negative correlation between serum vitamin A concentration and both the concentrations of CRP ($r=-0.45$, $p<0.001$) and AGP ($r=-0.43$, $p<0.001$). However, among HIV-1 seronegative women,
there was no correlation between serum retinol concentration and CRP levels ($r=0.06$, $p=0.4$) and only a weak correlation with AGP levels ($r=0.15$, $p=0.03$).

In multivariate logistic regression analysis, HIV-1 infection and the acute phase response (elevated CRP and/or AGP) were independently associated with vitamin A deficiency ($<30\ \mu g/dl$) (OR 2.7, 95% CI 1.9-4.0 and OR 2.8, 95% CI 1.9-4.1, respectively). Women who were HIV-1 infected and who had an acute phase response were at greatest risk of vitamin A deficiency (Table 2.3). An interaction term between HIV-1 status and acute phase response status added to the logistic regression model had a trend for statistical significance ($p=0.1$). Among HIV-1 infected women, 55% (129/234) of those with vitamin A deficiency also had an acute phase response.

*Vitamin A deficiency, acute phase response, and effect of supplementation.* After completing the cross-sectional study, the 400 HIV-1 seropositive women were randomized to receive 6 weeks daily supplementation with low-dose vitamin A or placebo (200 in each arm). As described elsewhere (22), demographic and medical characteristics and follow-up were comparable between the two arms. Three hundred fifty-four women (89%) returned for follow-up, at a median of 42 days. Compliance with the intervention was good, with ~95% of women taking ≥95% of their assigned pills.

Overall, women receiving vitamin A had higher serum vitamin A concentrations at follow-up than women receiving placebo (Table 2.4). When stratified by enrollment acute phase response status, only those women with no acute phase response at enrollment responded. Since low vitamin A concentrations were strongly associated with having an acute phase response, we further examined the subgroup of women who were
vitamin A deficient at enrollment, stratified by acute phase response status. Within these subgroups, supplementation had no effect on serum vitamin A levels among women having an acute phase response.

Discussion

The results of this large cross-sectional study and randomized trial offer new information on the interrelationships between vitamin A status, HIV-1 infection, HIV-1 disease stage, and the acute phase response. We found both low serum vitamin A concentrations and the acute phase response to be more common among HIV-1 infected than uninfected women. Among HIV-1 infected women, more active HIV-1 disease was associated with lower vitamin A and higher positive acute phase protein concentrations, and these were strongly related to each other as well. Finally, we found that women having an acute phase response had no increase in serum vitamin A levels after supplementation, even when they had low serum vitamin A concentrations at baseline.

Our finding that vitamin A deficiency was more common among HIV-1 infected than uninfected women agrees with several studies from the United States which found an association between HIV-1 infection and multiple micronutrient deficiencies (5, 6). To our knowledge, ours is the first study to assess this question among women in a developing country, traditionally a group with a high prevalence of vitamin A deficiency. HIV-1 infection and the acute phase response were independently associated with an increased odds of deficiency in this study, and when both were present, the likelihood increased substantially, suggesting that they may act synergistically to lower serum
vitamin A concentrations. The population of HIV-1 infected women in this study were relatively immunosuppressed, and it is possible that the relationships we observed would be different in healthier women.

Others have found vitamin A deficiency to be associated with lower CD4 counts among HIV-1 infected individuals (8). We found deficiency to be independently related to higher HIV-1 plasma viral load as well. Previous observational studies which found an association between vitamin A deficiency and HIV-1 disease progression (9), genital shedding (12, 13), and mother-to-child transmission (14) adjusted for CD4 count in their analyses, but not for viral load. This suggests that uncontrolled confounding may have contributed to their findings, as HIV-1 plasma viral load is a strong predictor of these outcomes (32-34).

Among HIV-1 infected women in this study, higher HIV-1 viral load, lower CD4 count, and symptoms and signs of HIV-1 disease were related to the presence of an acute phase reaction, as was vitamin A deficiency. Synthesis of positive acute phase proteins like CRP and AGP increases in response to infections (35). Others have shown that elevated concentrations of acute phase proteins are associated with lower serum vitamin A levels (36), even during subclinical infection (37). Acute phase reactions are thought to depress serum vitamin A concentrations by decreasing release of the vitamin A-retinol binding protein complex from the liver (24). It is unknown whether the transient decrease in serum vitamin A associated with the acute phase response has any protective role, though similar decreases in serum iron are thought to sequester iron from infectious organisms and increase macrophage activity (29, 35).
The physiologic significance of low serum vitamin A concentrations in the context of an acute phase response is unclear (24). The relationship between the two was first described in Thai adults with malaria, who had low serum vitamin A concentrations but high intakes of pro-vitamin A carotenoids and no evidence of clinical vitamin A deficiency disease, suggesting that they were not truly deficient (38). Evidence of an acute phase response has been used to identify individuals as potentially misclassified as vitamin A deficient in large surveys (29). Among HIV-1 infected women in this study, the majority with serum vitamin A concentrations reflecting deficiency also had an acute phase response. Those having an acute phase reaction showed no increase in serum vitamin A levels after vitamin A supplementation, while those without an acute phase reaction did respond to the supplement. In addition to the possibility of uncontrolled confounding by HIV-1 plasma viral load noted above, misclassification of vitamin A deficiency as a result of the acute phase response may also explain the disparity between observational studies and randomized trials of vitamin A in HIV-1 infection. We did not study the effect of vitamin A supplementation among the HIV-1 uninfected women, though it would be interesting to know if an acute phase reaction limits the response to supplementation among these women as it did among HIV-1 infected women.

The major limitation of our study is that a cross-sectional analysis does not allow for determination of the causal direction of the relationships between vitamin A deficiency, HIV-1 status and disease stage, and the acute phase response. However, based on previous studies, it seems biologically plausible that the acute phase response depresses serum vitamin A levels and that more severe HIV-1 disease could stimulate an
acute phase reaction. Our randomized trial data showed that having an acute phase reaction prevents response to vitamin A supplementation, suggesting that the low serum vitamin A levels associated with an acute phase reaction may not reflect vitamin A deficiency. Three studies (17, 18, 22) have demonstrated no effect of vitamin A supplementation on HIV-1 plasma viral load, which suggests that higher viral load depresses serum vitamin A levels, rather than vitamin A deficiency causing higher viral load. Thus, we hypothesize that low serum vitamin A levels are a consequence, rather than a cause, of more active viral replication among HIV-1 infected individuals.

Overall, this study confirms an association between the acute phase response and low serum vitamin A levels, and it suggests that more active HIV-1 infection may act through this mechanism to depress serum vitamin A concentrations. The findings of earlier observational studies that found an association between low serum vitamin A levels and HIV-1 progression and infectivity should be reevaluated, since low vitamin A levels may simply be a marker of higher HIV-1 plasma viral load and a related acute phase response. Randomized trials have shown that vitamin A supplementation fails to increase CD4 count (16, 22), decrease viral load (17, 18, 22), or decrease infectivity (19-22) among HIV-1 infected individuals. Our results provide possible explanations for the failure of randomized trials of vitamin A supplementation and suggest that a role for supplementation in treating or preventing HIV-1 infection should be reconsidered.
Notes to Chapter 2


Table 2.1  Demographic and medical characteristics

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>HIV-1 seropositive N=400</th>
<th>HIV-1 seronegative N=200</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Demographics</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age, years</td>
<td>28 (18-45)</td>
<td>26 (18-42)</td>
<td>0.006</td>
</tr>
<tr>
<td>Education, years</td>
<td>7 (0-12)</td>
<td>8 (0-16)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Currently married</td>
<td>206 (52%)</td>
<td>133 (67%)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Number of rooms in home</td>
<td>1 (1-6)</td>
<td>1 (1-10)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Toilet in home</td>
<td>27 (7%)</td>
<td>32 (16%)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Running water in home</td>
<td>19 (5%)</td>
<td>27 (14%)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Own a television</td>
<td>65 (16%)</td>
<td>46 (23%)</td>
<td>0.05</td>
</tr>
<tr>
<td>Smoking</td>
<td>10 (3%)</td>
<td>1 (1%)</td>
<td>0.09</td>
</tr>
<tr>
<td>Alcohol use</td>
<td>37 (9%)</td>
<td>5 (3%)</td>
<td>0.002</td>
</tr>
<tr>
<td>History of prostitution</td>
<td>34 (9%)</td>
<td>0 (0%)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Gravidity</td>
<td>2 (0-10)</td>
<td>1 (0-11)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Currently breastfeeding</td>
<td>53 (13%)</td>
<td>20 (10%)</td>
<td>0.3</td>
</tr>
<tr>
<td>Body-mass index</td>
<td>21.2 (14.6-45.1)</td>
<td>22.9 (13.3-48.2)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Plasma HIV-1 RNA</td>
<td>275,630 (214-)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>(copies/ml)</td>
<td>46,000,000</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>CD4 count (cells/μl)</td>
<td>226 (&lt;25-1117)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Vitamin A status</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vitamin A (μg/dl)</td>
<td>27.4 (2.8-81.0)</td>
<td>36.5 (15.1-78.5)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>&lt;30 μg/dl (deficiency)</td>
<td>234 (59%)</td>
<td>57 (29%)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Acute phase proteins</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CRP (mg/l)</td>
<td>3.2 (&lt;0.2-207.0)</td>
<td>1.0 (&lt;0.2-89.8)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>≥10 mg/l</td>
<td>103 (26%)</td>
<td>19 (10%)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>AGP (g/l)</td>
<td>1.0 (0.4-4.3)</td>
<td>0.7 (0.3-3.1)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>≥1.2 g/l</td>
<td>162 (41%)</td>
<td>18 (9%)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Acute phase response'</td>
<td>174 (44%)</td>
<td>28 (14%)</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>
Footnotes to Table 2.1

1 Defined as having either CRP $\geq 10$ mg/l and/or AGP $\geq 1.2$ g/l.
Table 2.2  Severity of HIV-1 disease, vitamin A deficiency, and the acute phase response among seropositive women

<table>
<thead>
<tr>
<th></th>
<th>Vitamin A Deficiency</th>
<th></th>
<th>Acute Phase Response</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Median (range) or number (%)</td>
<td></td>
<td>Median (range) or number (%)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Deficient / number (N=234)</td>
<td>Not deficient / number (N=166)</td>
<td>p-value</td>
<td>Present / number (N=174)</td>
</tr>
<tr>
<td>Plasma HIV-1 RNA (copies/ml)</td>
<td>457,110 (270-46,000,000)</td>
<td>165,970 (214-16,000,000)</td>
<td>&lt;0.001</td>
<td>719,370 (2,700-46,000,000)</td>
</tr>
<tr>
<td>CD4 count (cells/μl)</td>
<td>191 (&lt;25-1117)</td>
<td>288 (&lt;25-1010)</td>
<td>&lt;0.001</td>
<td>156 (&lt;25-832)</td>
</tr>
<tr>
<td>Any HIV-1 related symptom</td>
<td>171 (73%)</td>
<td>109 (66%)</td>
<td>0.1</td>
<td>140 (81%)</td>
</tr>
<tr>
<td>Any HIV-1 related signs</td>
<td>83 (36%)</td>
<td>32 (19%)</td>
<td>&lt;0.001</td>
<td>74 (43%)</td>
</tr>
<tr>
<td>Any symptom or sign</td>
<td>183 (78%)</td>
<td>111 (67%)</td>
<td>0.01</td>
<td>149 (86%)</td>
</tr>
</tbody>
</table>
Footnotes to Table 2.2

1 Defined as serum retinol concentration $<30 \, \mu g/dl$.

2 Defined as having either CRP $\geq 10 \, mg/l$ and/or AGP $\geq 1.2 \, g/l$.

3 Includes a history of fever $>1$ month, diarrhea $>1$ month, cough $>1$ month, weight loss $>5$ kg, or itching skin rash during past year.

4 Includes the presence of oral thrush, oral hairy leukoplakia, oral ulcers, maculopapular rash, or Kaposi’s sarcoma on physical examination.
Table 2.3  Odds of vitamin A deficiency, by HIV-1 and acute phase response status

<table>
<thead>
<tr>
<th>HIV-1</th>
<th>Acute phase response'</th>
<th>Vitamin A deficiency (&lt;30 μg/dl)</th>
<th>n/total (%)</th>
<th>OR (95% CI)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative</td>
<td>Negative</td>
<td></td>
<td>47/172 (27%)</td>
<td>1.0</td>
<td></td>
</tr>
<tr>
<td>Negative</td>
<td>Positive</td>
<td></td>
<td>10/28 (36%)</td>
<td>1.5 (0.6-3.7)</td>
<td>0.4</td>
</tr>
<tr>
<td>Positive</td>
<td>Negative</td>
<td></td>
<td>105/226 (47%)</td>
<td>2.3 (1.5-3.6)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Positive</td>
<td>Positive</td>
<td></td>
<td>129/174 (74%)</td>
<td>7.6 (4.6-12.7)</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>
Footnotes to Table 2.3

1 Defined as having either CRP $\geq 10$ mg/l and/or AGP $\geq 1.2$ g/l.
<table>
<thead>
<tr>
<th>Status at enrollment visit</th>
<th>Vitamin A</th>
<th>Placebo</th>
<th>p-value</th>
<th>Vitamin A</th>
<th>Placebo</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>All women</td>
<td>27.8</td>
<td>27.2</td>
<td>0.4</td>
<td>29.4</td>
<td>26.8</td>
<td>0.03</td>
</tr>
<tr>
<td>Vitamin A &lt;30 μg/dl</td>
<td>22.0</td>
<td>20.3</td>
<td>0.8</td>
<td>25.1</td>
<td>21.2</td>
<td>0.03</td>
</tr>
<tr>
<td>Acute phase response</td>
<td>21.2</td>
<td>22.3</td>
<td>0.6</td>
<td>24.9</td>
<td>24.3</td>
<td>0.7</td>
</tr>
<tr>
<td>No acute phase response</td>
<td>31.0</td>
<td>30.1</td>
<td>0.6</td>
<td>31.4</td>
<td>28.3</td>
<td>0.02</td>
</tr>
<tr>
<td>Vitamin A &lt;30 μg/dl and acute phase response</td>
<td>17.4</td>
<td>17.6</td>
<td>0.9</td>
<td>19.9</td>
<td>19.3</td>
<td>0.9</td>
</tr>
<tr>
<td>Vitamin A &lt;30 μg/dl and no acute phase response</td>
<td>24.5</td>
<td>25.3</td>
<td>0.9</td>
<td>27.3</td>
<td>23.3</td>
<td>0.002</td>
</tr>
</tbody>
</table>
CONCLUSION

Summary

The two original studies presented here offer new information on the role of vitamin A in HIV-1 infection. In a randomized clinical trial among 400 HIV-1 infected women in Mombasa, Kenya, who had a prevalence of vitamin A deficiency of nearly 60%, we found no effect of daily low-dose vitamin A supplementation on vaginal shedding of HIV-1 or HIV-1 infected cells. Additionally, no effect was seen on HIV-1 plasma viral load, CD4 count, or CD8 count. Our results were consistent with other randomized clinical trials among HIV-1 infected individuals that have shown no effect of vitamin A supplementation on CD4 count, plasma viral load, or HIV-1 transmission from mother to child. Earlier observational studies had associated low serum vitamin A concentrations with faster disease progression and greater infectivity among HIV-1 infected individuals, however, and the reasons for the disparity between the observational reports and the results of the randomized trials are unclear. Thus, we conducted a cross-sectional analysis of data collected from our 400 trial participants as well as from 200 HIV-1 seronegative women who had been screened for the study but not enrolled. We found HIV-1 seropositive women were significantly more likely to be vitamin A deficient and to have an acute phase response than seronegative women. Among the seropositive women, deficiency and the acute phase response were strongly associated with higher HIV-1 plasma viral load and lower CD4 count. HIV-1 seropositive women having an acute phase reaction demonstrated no increase in serum vitamin A levels as a result of
vitamin A supplementation. These results suggest that low serum vitamin A levels in HIV-1 infected individuals may in part reflect more active or advanced disease rather than true deficiency, offering a possible explanation for the disappointing results of vitamin A supplementation trials. Overall, these two studies suggest there is no clear role yet for vitamin A in preventing or treating HIV-1 infection.

Future Research

Nutritional interventions for HIV-1 infection have been considered promising because they are inexpensive and could be distributed widely, even without regard to HIV-1 serostatus. These considerations would be particularly advantageous in the developing world, where resources are scarce, HIV-1 testing is uncommon, and malnutrition and nutritional deficiency are highly prevalent. To date, however, there is only limited evidence that micronutrient supplementation offers significant and sustained benefits to HIV-1 infected individuals.

Despite the disappointing findings of randomized vitamin A trials for prevention of HIV-1 transmission or treatment of HIV-1 infection, there is some evidence that directed vitamin A supplementation may be beneficial for HIV-1 infected children in areas of high vitamin A deficiency. Two studies in HIV-1 infected children from South Africa and Tanzania showed significantly reduced morbidity from diarrheal diseases as a result of supplementation (1, 2). Another showed that high-dose supplements given to children with HIV-1 led to statistically significant increases in CD4 count and in total lymphocytes at four weeks compared with placebo (3). More recently, high-dose
supplementation given to HIV-1 infected children hospitalized with pneumonia in Tanzania was associated with a 63% (95% CI 5-86, p=0.04) reduction in mortality during 2 years of follow-up (4). Thus, vitamin A supplementation, which has repeatedly been associated with decreased childhood morbidity and mortality in areas where deficiency is highly prevalent, appears to show similar benefits among HIV-1 infected children in these areas. The effect of vitamin A supplementation on infectious morbidity and mortality among HIV-1 infected adults has not been thoroughly investigated.

In addition to vitamin A, other micronutrients have been investigated for a relationship with HIV-1 disease. Antioxidant micronutrient deficiencies may influence HIV-1 pathogenesis, as suggested by evidence that oxidative stress enhances HIV-1 replication in vitro and is more common in infected individuals than in uninfected controls (5, 6). Deficiencies in specific antioxidants (e.g., selenium and vitamin E) have been associated with faster progression to AIDS (7), increased HIV-1 related mortality (8, 9), and increased female genital shedding of HIV-1 infected cells (10). Clinical studies suggest that there may be a role for antioxidant supplementation in HIV-1 disease. One small trial found a significant increase in CD4/CD8 ratio after 12 weeks of selenium supplementation (11). Another showed a trend for a decrease in HIV-1 plasma viral load during 3 months of high-dose supplementation with vitamins E and C (12). Higher intake of some antioxidant micronutrients (vitamins E and C) has been associated with slower progression to AIDS in two observational studies (13, 14). Finally, one small trial of high-dose mixed carotenoid supplements showed a trend (p=0.06) for decreased mortality among patients with AIDS (15). In general, however, the potential for
antioxidant supplementation to decrease HIV-1 disease progression or transmission requires further study. A randomized trial of selenium supplementation to decrease HIV-1 related mortality among injection drug users is currently on-going in Miami.

Since HIV-1 infection has been associated with multiple micronutrients deficiencies and since there is significant physiologic interaction between micronutrients, it has been suggested that combinations of micronutrients may offer greater benefits than single nutrient supplementation alone. Few studies have been conducted. In a randomized placebo-controlled trial in Tanzania of a multivitamin supplement to prevent mother-to-child HIV-1 transmission, randomization to the multivitamin was associated with higher CD4 counts among the mothers after delivery and higher birthweights among their infants (16), but the intervention had no effect on HIV-1 transmission (17). Short-term supplementation of HIV-1 infected individuals with diarrhea and wasting in Zambia showed no effect on morbidity or mortality (18). Finally, in our trial, 250 women who were not presented here participated in an assessment of supplementation with a multivitamin. These results are not yet available.

In conclusion, while nutritional deficiencies have been associated with adverse outcomes and greater infectivity among HIV-1 infected individuals, there is currently little evidence to support widespread use of micronutrient supplementation as an intervention to treat HIV-1 or prevent its transmission. Controlled clinical trials such as this one offer the best approach to discerning whether simple, inexpensive interventions like micronutrient supplementation offer any benefits to persons infected with HIV-1.
Notes to Conclusion


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VITA

Jared Murray Baeten

Office Address:

International AIDS Research and Training Program
University of Washington
Box 359909
325 Ninth Avenue
Seattle, Washington 98104
(206) 731-2822
jbaeten@u.washington.edu

Personal Data:

Birthdate: June 20, 1973
Birthplace: Green Bay, Wisconsin
Citizenship: USA

Education:

1991-1995 BA Chemistry and Religious Studies, Summa cum laude
Washington University, St. Louis, MO

1995-present MD/PhD student
School of Medicine and Department of Epidemiology
University of Washington, Seattle, WA

Training Positions:

1991-1995 Research Assistant, Departments of Chemical Engineering, Plastic
and Reconstructive Surgery, and Chemistry, Washington
University
1993-1995 Tutor, Washington University
1994-1995 Teaching Assistant, Department of Chemistry, Washington
University
1996-2001 Tutor, School of Medicine, University of Washington
1997  Teaching Assistant, Department of Epidemiology, University of Washington
1997-1998 Research Assistant, Department of Epidemiology, University of Washington
1998-2000 Visiting Research Associate, Department of Medical Microbiology, University of Nairobi, Kenya

Awards:

1993, 1994 Research Fellowships, Department of Chemistry, Washington University
1994 Sigma Xi
1994 Barry M. Goldwater Scholar
1995 Phi Beta Kappa
1995 Sowden Prize in Chemistry (for best graduating senior in Chemistry)
1997 Student Subspecialty Award, Neurosciences, Western Student Medical Research Forum
1997 Lange Award (for best second year medical student)
1998-2000 Traineeship Award, International AIDS Research and Training Program, University of Washington
2000-2001 Traineeship Award, Center for AIDS and STD, University of Washington
2001 Outstanding Student Award, Department of Epidemiology

Publications:


Lavreys L, Baeten JM, Overbaugh J, Panteleeff DD, Chohan BH, Richardson BA, Mandaliya K, Ndinya-Achola JO, Kreiss JK. Acute HIV-1 infection illness is associated with higher viral load among women in Mombasa, Kenya. In preparation.

Abstracts:


Baeten J, Richardson B, Martin H, Nyange P, Lavreys L, Ngugi E, Mandaliya K, Bwayo J, Kreiss J. Rapid decline in risk of HIV-1 acquisition after enrollment in a vaccine preparedness cohort of Kenyan prostitutes: implications for the design of HIV-1 vaccine