

Antenatal Vitamin A Supplementation Increases Birth Weight and Decreases Anemia among Infants Born to Human Immunodeficiency Virus–Infected Women in Malawi

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Vitamin A is essential for immunity and growth. A controlled clinical that involved 697 human immunodeficiency virus (HIV)–infected pregnant women was conducted to determine whether vitamin A prevents anemia, low birth weight, growth failure, HIV transmission, and mortality. Women received daily doses of iron and folate, either alone or combined with vitamin A (3 mg retinol equivalent), from 18–28 weeks' gestation until delivery. In the vitamin A and control groups, respectively, the mean (\pm SE) birth weights were 2895 ± 31 g and 2805 ± 32 g ($P = .05$), the proportions of low-birth-weight infants were 14.0% and 21.1% ($P = .03$), the proportions of anemic infants at 6 weeks postpartum were 23.4% and 40.6% ($P < .001$), and the respective cumulative proportions of infants who were HIV infected at 6 weeks and 24 months of age were 26.6% and 27.8% ($P = .76$) and 27.7% and 32.8% ($P = .21$). Receipt of vitamin A improved birth weight and neonatal growth and reduced anemia, but it did not affect perinatal HIV transmission.

Vitamin A is essential for normal immune function, growth, maintenance of mucosal surfaces, and hematopoiesis [1]. In many developing countries, vitamin A deficiency is common and is a major cause of morbidity and mortality among infants, children, and pregnant women [2]. Vitamin A deficiency among pregnant women with HIV infection has been associated with low birth weight [3, 4], infant growth failure [5], faster pro-

gression of HIV disease among infants [6], increased infant mortality [3], and higher rates of mother-to-child transmission of HIV [7, 8]. Low maternal plasma vitamin A concentrations were independently associated with higher rates of mother-to-child transmission of HIV after adjusting for maternal CD4⁺ lymphocyte count [7, 8]. Improvement of vitamin A status through supplementation or fortification has been shown to reduce child morbidity and mortality due to infectious diseases, particularly diarrheal diseases, measles, and malaria, but not nonmeasles respiratory disease [9–11]. A recent clinical trial in Nepal showed that both antenatal vitamin A and β -carotene supplementation reduced mortality related to pregnancy [12] and symptoms of illness in women during late pregnancy [13]. To date, the >100 clinical trials involving vitamin A have shown that vitamin A supplementation enhances immunity and reduces the morbidity and mortality of some infectious diseases [14].

The rates of adverse infant outcomes, such as low

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birth weight, growth failure, and infant mortality, are relatively high among infants born to HIV-infected women [3–5]. In developing countries, appropriate low-cost interventions are needed to improve infant health, and antenatal vitamin A supplementation is a potentially promising strategy. We hypothesized that providing antenatal vitamin A supplementation to HIV-infected pregnant women would improve birth outcomes, such as low birth weight, infant growth failure, infant mortality, and mother-to-child transmission of HIV. To address this hypothesis, we conducted a controlled clinical trial of vitamin A supplementation for HIV-infected pregnant women in Blantyre, Malawi.

METHODS

The study population consisted of pregnant women of 18–28 weeks' gestation who were seen at the antenatal clinic of the Queen Elizabeth Central Hospital (Blantyre, Malawi) from November 1995 through December 1996. The Queen Elizabeth Central Hospital is the main hospital for Blantyre, a city of ~300,000 inhabitants. Pregnant women received instructions in prenatal care, AIDS education, HIV testing, pre- and posttest HIV counseling, physical examination, and treatment for sexually transmitted diseases and malaria. Height and weight were recorded, and gestational age was estimated from the most recent reported menstrual period.

Written informed consent was obtained from all participants in the study. The study protocol was approved by 4 different independent ethical review committees: the Johns Hopkins School of Medicine (Baltimore, MD); the Malawi Health Sciences Research Committee, Government of Malawi (Lilongwe); the National Cancer Institute, National Institutes of Health (Bethesda, MD); and the Ministry of Health and Population, Government of Malawi (Lilongwe). Final approval was given by the Office for Protection from Research Risk, National Institutes of Health (Bethesda, Maryland).

All women received orally administered daily doses of iron (30 mg of elemental iron) and folate (400 μ g) from the time of study enrollment until delivery. One-half of the women were randomized to receive daily doses of orally administered vitamin A (3 mg retinol equivalent [10,000 IU]; the Vitamin A group) from the time of study enrollment until delivery. Treatment assignment was determined by use of a computer's random-number generator, and treatment assignment was concealed by prepacking study supplements in sequentially numbered series assigned to study identification numbers. Mothers were assigned an original study identification number at enrollment and were given the next sequentially numbered opaque bottle with supplements. Supplements containing vitamin A, iron, and folate were identical in appearance to the supplements containing iron and folate. Tablet counts

were conducted every 4 weeks to assess adherence to the supplementation regimen. Birth weight was measured at delivery using a digital scale. Infant weight and length were measured at 6, 10, and 14 weeks of age and every 3 months from 6 to 24 months of age using a digital scale and a Shorr child-measuring board (Shorr Productions) by use of standardized techniques [15]. All women received oral vitamin A (30 mg retinol equivalent) at 6 weeks postpartum, as per policy of the Malawi Ministry of Health (Lilongwe).

At the time of screening, serum samples obtained from the mothers were assessed for the presence of HIV-1 antibody by EIA (Wellcozyme [Wellcome Diagnostics] and Genetic Systems EIA [Genetic Systems]). Both EIAs were required to yield positive results for a woman to be considered HIV positive. Immunoblotting (Bio-Rad Laboratories) was used to confirm HIV status for women with equivocal HIV EIA results. At enrollment, a second blood sample was obtained by venipuncture, and plasma was separated immediately and frozen at -70°C . Plasma HIV load was measured by quantitative RT-PCR (Roche Amplicor HIV-1 Monitor, version 1.5; Roche Diagnostics), and these assays were run and validated in the AIDS Clinical Trials Group reference laboratory at Johns Hopkins Hospital (by J.B.J.). A complete blood cell count was done by use of an automated cell counter (Coulter). Percentages of CD4⁺ and CD8⁺ lymphocytes were determined using standard flow cytometry methods [16].

Six weeks and 12 months after delivery, blood samples were obtained from the infants, and qualitative DNA PCR was used to detect HIV-1 DNA on a blood spot on filter paper [17]. At 6 weeks after delivery, infant hemoglobin concentrations were measured by use of a HemoCue instrument (HemoCue). At 12 months after delivery, infant hemoglobin concentrations were measured by use of an automated analyzer (Coulter). PCR for HIV-1 DNA and validation studies were conducted at the virology laboratories of the National Cancer Institute (Fort Detrick, Maryland). At 24 months after delivery, serum samples obtained from the infants were assessed for the presence of HIV-1 antibody by EIA, as described above. Both EIAs were required to yield positive results for an infant to be considered HIV positive at 24 months of age.

At 6 weeks postpartum, a sample of milk was obtained from either breast by manual expression, and breast milk was immediately aliquoted and stored in a sample archive at -70°C until analysis for milk vitamin A concentrations was conducted [18]. Maternal plasma vitamin A concentrations were measured at enrollment and at 38 weeks of gestation using high-pressure liquid chromatography [7].

The sample size of ~350 women per treatment group was based on 80% power to detect a 30% reduction in mother-to-child transmission of HIV, with 1:1 allocation, no matching, a 2-sided test, and $\alpha = .05$. Comparisons between groups were

made using Student's *t* test for continuous variables with a normal distribution. Plasma HIV load (in copies per milliliter) was transformed by \log_{10} to achieve a normal distribution. The Wilcoxon rank-sum test was used for nonparametric comparison between groups of variables that could not be transformed into a normal distribution, such as HIV load in breast milk. χ^2 Test and Fisher's exact test were used to compare categorical variables between groups. Multivariate logistic regression models were used to examine the relationship between maternal risk factors and low birth weight.

Weight-for-height, weight-for-age, and height-for-age indices were calculated using EpiInfo software, version 6.04 (Centers for Disease Control and Prevention). Body mass index was calculated with the Quetelet index (weight/height²). Growth standards from the National Center for Health Statistics were used as a reference standard [19]. Anemia in infants was defined as a hemoglobin level of <110 g/L. A plasma vitamin A concentration of <0.70 $\mu\text{mol/L}$ was considered to be consistent with vitamin A deficiency in mothers. Differences in the 12-month mortality were calculated by logistic regression. Analyses were conducted with SAS (SAS Institute) and S-PLUS (Mathsoft) software.

RESULTS

Six hundred ninety-seven women were enrolled in the study at 18–28 weeks' gestation. There were 340 women in the vitamin A group and 357 women in the control group. At enrollment, the mothers in the 2 groups were not significantly different with regard to age, body mass index, gestational age, CD4⁺ or CD8⁺ lymphocyte count, plasma HIV load, plasma vitamin A concentration, and plasma folate concentration (table 1). At 38 weeks' gestation, maternal plasma vitamin A concentrations were significantly higher in the vitamin A group

than in the control group (table 2). Mean plasma folate concentrations were similar in both groups. More than 95% of women in both groups took >90% of study supplements, as ascertained by tablet counts. Infant birth outcomes and cumulative HIV infection statuses are shown in table 2. In the vitamin A group, birth outcomes were ascertained for 306 women (297 live births, including 12 twins, plus 8 stillbirths and 7 abortions), and 34 women were lost to follow-up before delivery (32 moved out of area and 2 could not be located). In the control group, birth outcomes were ascertained for 317 women (325 live births, including 16 twins, plus 6 stillbirths and 5 abortions), and 29 women were lost to follow-up before delivery (25 moved out of area and 4 could not be located). Five hundred eighty-six women (84.1%) returned for delivery at Queen Elizabeth Central Hospital. Among infants born to these women, the mean birth weight was significantly higher ($P = .05$) and the proportion with low birth weight was significantly lower ($P = .03$) among those born to mothers given vitamin A compared with the control group.

There were 14 pairs of twins (6 in the vitamin A group and 8 in the control group), and, because twins are known to have lower birth weight and higher mortality rates, these twin pairs were excluded from the birth weight and mortality analyses. Of the 6 twin pairs in the vitamin A group, both twins in 5 pairs were not infected with HIV at 6 weeks of age, and the HIV infection status was not determined for 1 pair. Of 8 twin pairs in the placebo group, both twins in 2 pairs were HIV infected at 6 weeks of age, both twins in 4 pairs were not infected with HIV at 6 weeks of age, and the HIV infection status was not determined for 2 twin pairs. Because the HIV infection status was the same among both twins in every twin pair tested and remained concordant in follow-up, for each mother with twins, the infant's HIV infection status result at

Table 1. Baseline characteristics of HIV-infected mothers who received vitamin A supplementation (vitamin A group) and of those in a control group.

Characteristic	Vitamin A group (<i>n</i> = 340)	Control group (<i>n</i> = 357)	<i>P</i>
Age, years	23.9 ± 0.2	23.7 ± 0.2	.54
Gestational age, weeks	23.3 ± 0.1	23.2 ± 0.1	.79
Body mass index, weight/height ²	22.6 ± 2.3	22.7 ± 2.5	.31
CD4 ⁺ lymphocyte count, cells/ μL	429 ± 14	436 ± 13	.87
CD8 ⁺ lymphocyte count, cells/ μL	837 ± 26	866 ± 26	.44
CD4:CD8 ratio	0.56 ± 0.01	0.56 ± 0.01	.74
Plasma \log_{10} HIV load, copies/mL	4.43 ± 0.05	4.29 ± 0.05	.45
Plasma vitamin A level, $\mu\text{mol/L}$	0.74 ± 0.01	0.71 ± 0.01	.24
Plasma folate level, ng/mL	6.3 ± 0.1	6.7 ± 0.1	.08

NOTE. Data are mean ± SE.

Table 2. Maternal and infant health outcomes for HIV-infected mothers who received vitamin A supplementation (vitamin A group) and for those in a control group.

Characteristic	Vitamin A group	Control group	<i>P</i>
Maternal vitamin A level, $\mu\text{mol/L}^{\text{a}}$	0.80 \pm 0.02	0.67 \pm 0.02	.0001
Percentage of mothers with a vitamin A level of <0.70 $\mu\text{mol/L}$	50.8	51.0	.86
Maternal folate level, ng/mL ^a	14.2 \pm 0.5	14.5 \pm 0.8	.73
Vitamin A level in breast milk, $\mu\text{mol/L}^{\text{b}}$	1.57 \pm 0.07	1.12 \pm 0.03	.0001
Percentage of infants <2500 g at birth ^c	14.0	21.1	.03
Birth weight, g ^c	2895 \pm 31	2805 \pm 32	.05
Weight in g, by age			
6 weeks	4627 \pm 55	4458 \pm 55	.03
14 weeks	5925 \pm 68	5885 \pm 72	.68
6 months	7360 \pm 81	7405 \pm 83	.69
Length in cm, by age			
6 weeks	53.7 \pm 0.2	53.0 \pm 0.2	.03
14 weeks	58.6 \pm 0.2	58.3 \pm 0.2	.33
6 months	64.1 \pm 0.2	64.2 \pm 0.2	.69
Infant hemoglobin level in g/L, by age			
6 weeks	116 \pm 1	112 \pm 1	.04
12 months	106 \pm 1	107 \pm 1	.69
Percentage of infants with anemia, by age			
6 weeks	23.4	40.6	.001
12 months	56.9	55.3	.76
No. of HIV-infected infants/total no. (%), by age			
6 weeks	62/233 (26.6)	66/237 (27.8)	.76
12 months	65/238 (27.3)	80/250 (32.0)	.25
24 months	67/242 (27.7)	82/250 (32.8)	.21
No. of infants who were HIV negative at 6 weeks but HIV positive by 24 months/total no. (%)	5/176 (2.8)	14/182 (7.7)	.04
No. of infants who died/total no. (%), by age			
<6 weeks	12/285 (4.2)	20/309 (6.5)	.22
12 months	58/285 (20.4)	58/309 (18.8)	.64
24 months	69/285 (24.2)	68/309 (22.0)	.53

NOTE. Data are mean \pm SE, unless otherwise indicated.

^a At 38 weeks of gestation.

^b At 6 weeks postpartum.

^c Excluding twin births.

6 weeks of age was considered to be a single outcome in the statistical analysis.

The cumulative HIV infection status of infants is shown in table 2. In the vitamin A and control groups, 12 and 20 infants, respectively, died before HIV infection status could be determined at 6 weeks of age. There were no significant differences in the proportion of HIV-infected infants at 6 weeks or 12 or 24 months between the 2 treatment groups. The proportion of infants who were not infected with HIV at the age of 6 weeks but who were HIV infected by 24 months—that is, infants who were likely infected via breast-feeding—was higher in the control group than it was in the vitamin A group ($P = .04$). The

overall cumulative proportions of infants who were HIV infected at the ages of 6 weeks and 12 and 24 months were 27.2%, 29.7%, and 30.3%, respectively. The denominators are not consistent for each visit, because some infants had missed earlier visits but showed up for later visits. Infant mortality rates in the vitamin A and control groups were not significantly different.

At 6 weeks of age, the mean hemoglobin concentration was significantly higher and the proportion of infants with anemia was significantly lower among infants born to women who received vitamin A than it was among infants born to women in the control group. The findings of univariate and multivar-

iate models for low birth weight are shown in table 3. In both univariate and multivariate analyses, vitamin A supplementation, maternal age, CD4⁺ lymphocyte count, and body mass index at enrollment were significantly associated with low birth weight. Lack of vitamin A supplementation was independently associated with low birth weight in multivariate analyses.

DISCUSSION

This study shows that antenatal vitamin A supplementation at the dosage of 3 mg retinol equivalent (10,000 IU) per day during the second and third trimester of pregnancy improves birth weight and growth among infants born to HIV-infected women. To our knowledge, this is the first study to show that provision of vitamin A supplementation during pregnancy improves birth weight and neonatal growth. Previous observational studies have shown that low plasma vitamin A concentrations during pregnancy were associated with low birth weight [3, 4] and infant growth failure [5]. A longitudinal study in Uganda also showed that linear and ponderal growth were also lower among HIV-infected infants with low plasma vitamin A concentrations [20]. In the present trial, the differences in weight and length were no longer significant after 6 weeks postpartum, but women in both arms of the trial received vitamin A (60 mg retinol equivalent given orally), presumably improving the content of vitamin A in breast milk and, thus, infant vitamin A status in both treatment groups after 6 weeks. Thus, this indirect vitamin A supplementation in both arms of the study would be expected to reduce any growth differences between treatment groups after 6 weeks.

Vitamin A is known to play a role in infant and child growth [2], and all-*trans* retinoic acid, the active metabolite of vitamin A, is known to regulate the expression of growth hormone [21]. A recent trial in Tanzania showed that provision of antenatal vitamin A supplementation for HIV-infected women did not affect birth weight [22]; however, there was relatively less vitamin A deficiency among the study subjects in Tanzania. In Tanzania and Malawi, ~34% and 51% of women, respec-

tively, had plasma vitamin A concentrations of <0.70 μmol/L during the second trimester of pregnancy. The intervention with vitamin A in the study in Tanzania consisted of a smaller dosage of vitamin A (1.5 mg retinol equivalent [5000 IU] per day) plus high-dose β-carotene (30 mg per day). In contrast, multimicronutrient supplementation, without vitamin A, was shown to reduce low birth weight, preterm birth, and fetal deaths [22]. There may be additional beneficial effects of maternal vitamin A supplementation for their infants. A study in Durban, South Africa, showed that antenatal vitamin A or β-carotene supplementation helped maintain the integrity of the gut among HIV-infected infants, a factor that may reduce diarrheal morbidity [23]. The maintenance of gut integrity by vitamin A may possibly have contributed to the apparent reduction in later perinatal transmission that occurred among infants who were not infected with HIV at 6 weeks in the vitamin A group.

Although observational studies show that low concentrations of plasma vitamin A in mothers are associated with higher rates of mother-to-child transmission of HIV [7, 8] and increased genital shedding of HIV [24], clinical trials in Tanzania and South Africa have not shown that either vitamin A or multimicronutrient supplementation has an impact on mother-to-child transmission of HIV [25, 26]. Our trial in Malawi also corroborates the finding that vitamin A supplementation has no overall impact on mother-to-child transmission of HIV. This study suggests that antenatal vitamin A supplementation may give some protection against mother-to-child transmission of HIV among infants who were not infected with HIV by 6 weeks of age, but the mechanism by which vitamin A supplementation could protect against HIV transmission between 6 weeks and 24 months of age is unclear. The mother's plasma HIV level during pregnancy is the strongest predictor of mother-to-child transmission of HIV [27]. No correlation has been observed between plasma vitamin A concentration and plasma HIV level, or between vitamin A concentration in breast milk and HIV load in breast milk (authors' unpublished data). A limitation

Table 3. Findings from univariate and multivariate models for low birth weight among infants born to HIV-infected mothers who received or did not receive vitamin A supplementation.

Variable	Univariate OR (95% CI)	<i>P</i>	Multivariate OR (95% CI)	<i>P</i>
Vitamin A supplementation	0.61 (0.39–0.96)	<.03	0.58 (0.36–0.93)	<.03
Mother's age, years	0.90 (0.86–0.96)	<.0004	0.91 (0.86–0.96)	<.0009
Mother's CD4 lymphocyte count, 50 cells/μL ^a	0.93 (0.89–0.98)	<.009	0.93 (0.88–0.98)	<.006
Mother's body mass index, weight/height ²	0.87 (0.79–0.96)	<.009	0.88 (0.80–0.98)	<.03

^a Per increase of 50 cells/μL.

of the present study is that CD4⁺ lymphocyte counts and HIV loads were not determined for infants.

To our knowledge, this is the first study to demonstrate that provision of vitamin A supplements to mothers during pregnancy reduces anemia among their infants. Previous trials have suggested that vitamin A supplementation can reduce the prevalence of anemia. For example, daily supplementation with vitamin A plus iron reduced anemia more than did iron alone among pregnant women in Indonesia [28] and children in Guatemala [29]. In the present study, the vitamin A concentration in breast milk at postpartum week 6 was significantly higher in the vitamin A group than it was in the control group, and it is likely that these breast-feeding infants born to vitamin A-supplemented mothers had better vitamin A status. Vitamin A appears to reduce anemia through diverse biological mechanisms, including the enhancement of growth and differentiation of erythrocyte progenitor cells, potentiation of immunity to infection and reduction of the anemia of infection, and mobilization of iron stores from tissues [30], but it does not appear to influence the production of erythropoietin by the kidneys in response to anemia [31]. It is unclear whether vitamin A supplementation influences other factors in breast milk that might affect infant erythropoiesis, such as concentrations of iron or erythropoietin in breast milk [32].

An estimated 500,000 infants are infected with HIV every year, and most of the new cases are found in sub-Saharan Africa [33]. In 1999, nevirapine, an inexpensive antiretroviral medication, was shown to reduce mother-to-child transmission of HIV by approximately one-half [34], and short-course zidovudine treatment also was shown to have an effect on mother-to-child transmission of HIV [35, 36]. Although vitamin A supplementation did not affect mother-to-child transmission of HIV, it did appear to improve infant health. Given its low cost and potential benefit for improving birth outcomes and morbidity of infants, antenatal micronutrient supplementation should also be considered part of a low-cost package of care for HIV-infected women in developing countries.

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