Multivitamin supplementation improves hematologic status in HIV-infected women and their children in Tanzania[1–3]

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ABSTRACT

Background: Anemia is a frequent complication among HIV-infected persons and is associated with faster disease progression and mortality.

Objective: We examined the effect of multivitamin supplementation on hemoglobin concentrations and the risk of anemia among HIV-infected pregnant women and their children.

Design: HIV-1–infected pregnant women ($n = 1078$) from Dar es Salaam, Tanzania, were enrolled in a double-blind trial and provided daily supplements of preformed vitamin A and β-carotene, multivitamins (vitamins B, C, and E), preformed vitamin A and β-carotene + multivitamins, or placebo. All women received iron and folate supplements only during pregnancy according to local standard of care. The median follow-up time for hemoglobin measurement for mothers was 57.3 mo [interquartile range (IQR): 28.6–66.8] and for children it was 28.0 mo (IQR: 5.3–41.7).

Results: During the whole period, hemoglobin concentrations among women who received multivitamins were 0.33 g/dL higher than among women who did not receive multivitamins ($P = 0.07$). Compared with placebo, multivitamin supplementation resulted in a hemoglobin increase of 0.59 g/dL during the first 2 y after enrollment ($P = 0.0002$). Compared with placebo, the children born to mothers who received multivitamins had a reduced risk of anemia. In this group, the risk of macrocytic anemia was 63% lower than in the placebo group (relative risk: 0.37: 95% CI: 0.18, 0.79; $P = 0.01$).

Conclusion: Multivitamin supplementation provided during pregnancy and in the postpartum period resulted in significant improvements in hematologic status among HIV-infected women and their children, which provides further support for the value of multivitamin supplementation in HIV-infected adults. *Am J Clin Nutr* 2007;85:1335–43.

KEY WORDS Anemia, HIV infection, hemoglobin, vitamins, Tanzania

INTRODUCTION

HIV infection is the chief cause of morbidity and mortality among adults and children, especially in sub-Saharan Africa. At the end of 2005, ≈40 million persons worldwide were living with HIV or AIDS (1). Anemia is a frequent complication that occurs in 20–80% of HIV-infected persons and is associated with faster disease progression and mortality (2). Therefore, interventions to prevent anemia may lead to improved health and survival potential of HIV-infected persons.

Independent of HIV infection, anemia is also prevalent in pregnancy, because of increased nutrient requirements, inadequate iron stores, and low intakes of iron and other nutrients before and during pregnancy. Similarly, among infants and children, anemia constitutes a significant problem because of the poor nutritional quality of maternal and child diets (3). Additional evidence indicates that anemia may occur as a result of chronic inflammation related to underlying infections (4). Anemia is associated with various adverse outcomes, including maternal death, low birth weight, poor mental development in children, and reduced productivity among adults. Iron supplementation, particularly during pregnancy, has been shown to raise hemoglobin concentrations, and treatment and prevention of malaria, hookworm, and other infections are other important interventions for control of anemia. Despite widespread implementation of these interventions, anemia remains a problem of important public health significance (5).

In many developing countries, diets that are deficient in iron are also lacking in other vitamins as a result of inadequate intake of fruit and foods of animal origin and the presence of absorption-inhibiting factors such as phytates. Most prior studies that examined the efficacy of micronutrient supplementation on hematologic status were conducted in Asia and Latin America (5). Prior trials included iron in the supplement; hence, it is not possible to differentiate the effect of iron from that of other nutrients (5). No study has assessed the efficacy of multivitamins alone (excluding iron) in African settings where HIV infection, malaria, and other infectious diseases are prevalent. We have completed a trial among HIV-infected Tanzanian women to examine the efficacy of maternal vitamin supplementation during pregnancy and after delivery on pregnancy outcomes and health outcomes among women and their children (6–10). All women

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received daily supplements of iron and folic acid during pregnancy, but not thereafter, according to the standard of care in Tanzania. Here, we examine the effect of the supplements on hemoglobin concentrations and the risk of anemia among the women and their children.

SUBJECTS AND METHODS

We enrolled 1078 HIV-infected pregnant women in Dar es Salaam, Tanzania, during a 2-y period starting in April 1995. Women (and their children after delivery) were followed from enrollment until the end of the study in August 2003. The detailed design of the trial was published elsewhere (6–10). In brief, eligible women were randomly assigned in blocks of 20 to a daily oral dose of 1 of 4 regimens for the total duration of follow-up: *1*) vitamin A and β-carotene alone (30 mg β-carotene + 5000 IU preformed vitamin A); *2*) multivitamins (excluding vitamin A and β-carotene) that included 20 mg thiamine (vitamin B-1), 20 mg riboflavin (vitamin B-2), 25 mg vitamin B-6, 100 mg niacin, 50 μg cobalamin (vitamin B-12), 500 mg vitamin C, 30 mg vitamin E, and 0.8 mg folic acid, henceforth referred to as multivitamins; *3*) multivitamins + vitamin A and β-carotene in the same doses as above; or *4*) placebo. To maintain pills at a reasonable size, each daily dose was prepared in 2 tablets, each containing half the dose; tablets were packaged in identical coded bottles that contained 90 tablets each. At every visit, a new bottle of regimen was given to each woman, the used bottles were taken back, and the remaining pills were counted. At delivery, women in groups 1 and 3 received an additional oral dose of vitamin A (200 000 IU), and women in groups 2 and 4 were given a placebo. All women received antenatal supplements of folic acid (5 mg) and iron (120 mg) according to the standard of prenatal care. All children received doses of vitamin A at 6-mo intervals according to standard of care in Tanzania (100 000 IU at 6 mo and 200 000 IU at 12 mo and thereafter). Active tablets and placebo were identical in size and color. All clinical and follow-up staff members were blinded to the treatment assignment. At the time of the study antiretroviral therapy was not available to most women in Tanzania, including those who participated in the study. All women consented to participate in the study. Institutional review boards at the Muhimbili University College of Health Sciences, the Tanzanian National AIDS Control Program, and the Harvard School of Public Health approved the study protocol.

Participants were followed through monthly visits to a study clinic where physicians performed a physical examination. A study nurse inquired about the health of the woman and child in the preceding period. Women were requested to provide a blood specimen at baseline and at 6-mo intervals thereafter for measurement of hemoglobin concentrations. Thin blood films with Leishman's stain were prepared and examined microscopically. Hypochromasia, microcytosis, and macrocytosis were classified into 4 levels, coded as absent, 1+, 2+, and 3+. Persons who were diagnosed with severe anemia received management of their condition according to standard of care. Investigations and treatment included stool examination for parasites, iron supplementation if indicated, and dietary counseling.

Of the 1078 women enrolled in this study, 3 were found to be not pregnant, 6 died before delivery, and 27 (2.5%) were lost to follow-up before delivery. Of the remaining 1042 women, 82 had fetal deaths, 939 had live single births, and 21 had live twin births. Among the 1078 women, 906 had a baseline assessment of hemoglobin and at least one measurement thereafter and therefore were included in these analyses (**Figure 1**). The median follow-up time, during which a mean (±SD) of 8.6 ± 4.1 measurements were performed, was 57.3 mo [interquartile range (IQR): 28.6–66.8 mo]. The number of measurements was not different when the multivitamins group was compared with the nonmultivitamins group ($P = 0.45$) and the vitamin A and β-carotene group with the no vitamin A and β-carotene group ($P = 0.38$). The corresponding P values for the comparison of the duration of follow-up by arm of treatment were 0.74 and 0.45, respectively. Blood specimens for child hemoglobin measurements and peripheral blood picture assessments were requested from the mothers at birth, every 3 mo until 18 mo of age, and every 6 mo thereafter. Of the 939 singletons, the 836 infants who had at least one measurement constitute the child cohort for these analyses (Figure 1). The mean (±SD) number of child measurements was 5.3 ± 3.3. The median time between the first and last hemoglobin measurements was 28.0 mo (IQR: 5.3–41.7 mo). The number of measurements were not different when the multivitamins group was compared with the nonmultivitamins group ($P = 0.36$) and vitamin A and β-carotene group with the no vitamin A and β-carotene group ($P = 0.33$). The corresponding P values that compared the duration of follow-up by treatment arm were 0.15 and 0.10, respectively.

The sample size of the original trial was calculated to examine the efficacy of the supplements on vertical transmission of HIV and on maternal disease progression, assuming a 30% cumulative incidence of each primary outcome (6). We have reported the primary analyses from the trial about the efficacy of each treatment arm on these outcomes (7–10).

The principal aims of this paper were to compare the effects of multivitamins and vitamin A and β-carotene on hemoglobin concentrations and risk of anemia in mothers and their children. We also investigated possible interactions between the 2 regimens and report findings on the effects of the 3 treatment arms of the trial (multivitamins alone, vitamin A and β-carotene alone, and both together) compared with placebo on the outcomes of interest, when the P value for the interaction was ≤0.10.

First, we compared baseline characteristics among the treatment arms, including sociodemographic characteristics, with the use of the Kruskal-Wallis test for continuous variables and the chi-square test for categorical variables. Then, we investigated the effects of the supplements on hemoglobin concentrations among mothers and their children by treatment arm and regimen, with the use of generalized estimating equations with the GENMOD procedure of SAS software (SAS Institute, Cary, NC) (11). An identity link function with an exchangeable working covariance structure was used. All analyses are based on the intention-to-treat principle, and P values are 2-sided. For the mothers, the treatment group means after randomization, which would have occurred if there were no baseline differences, were estimated from a model that included main effects for each treatment considered, to adjust for differences before randomization, and an interaction term for the treatment with an indicator for the visit after randomization. Treatment effects were tested for parallel structure by assessing the significance of the interaction terms in a model similar to the above, but with one treatment interaction term entered for each visit after baseline. Procedures were similar for the children, but because all measurements were after the baseline, we included the main effects of each treatment

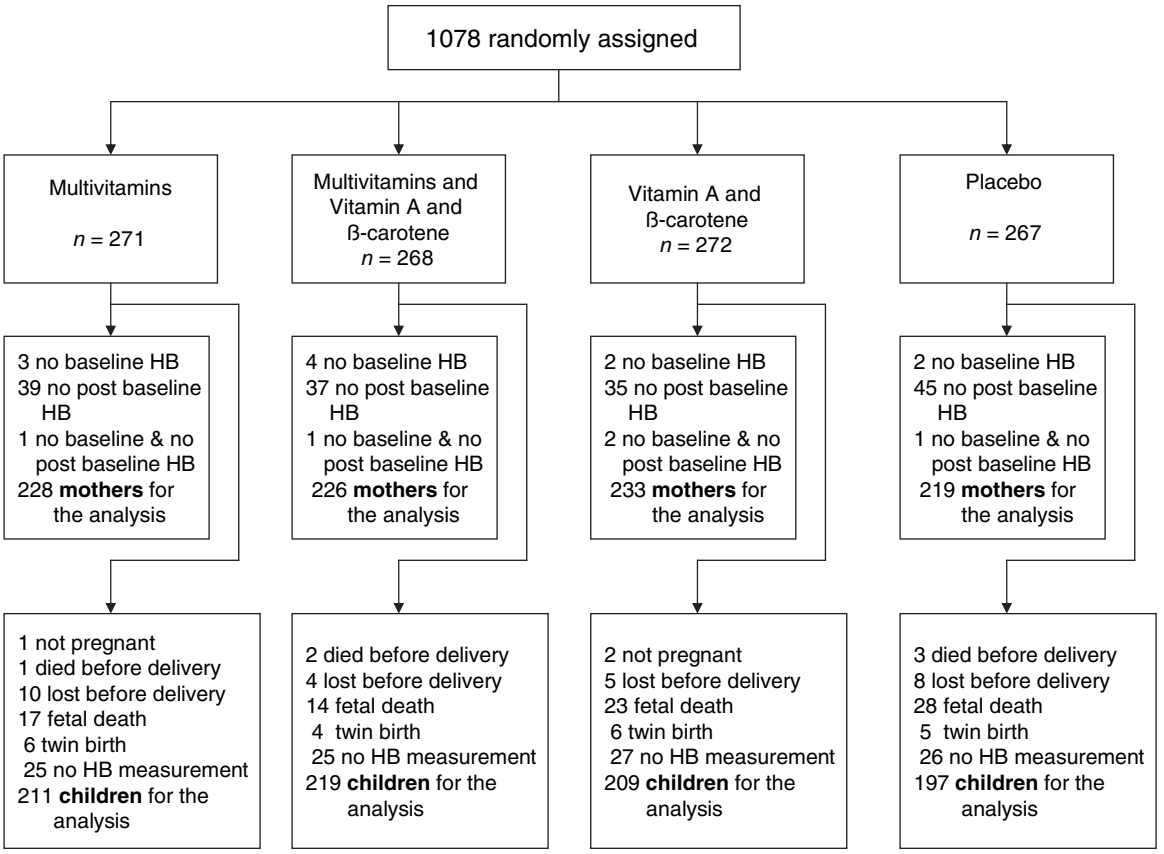

FIGURE 1. Trial profile. Hb, hemoglobin.

in the estimated means and in the hypothesis tests, in addition to the interaction terms of visit with treatment. For women, we performed analyses for the whole period, between enrollment and 70 d after delivery, the first 2 y after enrollment, and the first 4 y after enrollment. For children, we examined the effects at birth, from birth to 6 mo, the first 2 y after birth, and the first 4 y after birth. The primary analyses include data during the full duration of follow-up, and the longer times include data from the shorter times. The presentation of the different time periods is given for descriptive purposes, to aid comparison with other studies.

In mothers, to assess the statistical significance of any vitamin A-by-multivitamin interactions, cross-product terms for each visit except the baseline visit with the indicator variables for vitamin A and multivitamins were created and added to the model that included the main effects for the 2 regimens, indicator variables for each visit except the baseline visit, and cross-product terms for the 2 regimens with each visit except the baseline visit. The test for interaction assessed the statistical significance of the three-factor cross-product terms between the 2 regimens and each visit except the baseline visit. The strategy used to assess statistical interaction between the regimens in the children was similar; the only difference is the 2-factor cross-product term between the 2 regimens was also included in forming the test statistic, because all measurements in children were after baseline. When the regimen interaction was significant at $P \leq 0.05$, we displayed the P value for the contrast of the regimen compared with placebo.

We used Cox proportional hazard models to investigate the effects of the supplements on time to the first occurrence of each definition of anemia (12). For the analyses pertaining to women, those who had the endpoint of interest at baseline were excluded, but this was not necessary in the analyses of the children because all measurements were after randomization. For both mothers and their children, we assessed the effects of regimens on the risks of anemia defined as hemoglobin < 11.0 g/dL, and severe anemia was defined as hemoglobin < 8.5 g/dL. We examined the structure of peripheral blood cells as a proxy to identify effect modification by iron deficiency (presence of hypochromic and microcytic cells) and vitamin B-12 and folate deficiency (presence of macrocytic cells) (13). Hypochromic microcytic anemia was categorized as severe (hypochromasia $\geq 2+$ and microcytic cells observed), moderate and above (hypochromasia $\geq 1+$ and microcytic cells observed), and mild and above (hypochromasia $\geq 1+$ with or without microcytosis). Macrocytosis was defined as the presence of any macrocytic cells. In these analyses, the effect of each treatment under the 2×2 factorial design was assessed through the inclusion of a single term in the model for that treatment. The vitamin A-by-multivitamin interaction was assessed by a likelihood ratio test, comparing the model with the main effects of regimen only, to the analogous model augmented by the cross-product term between the 2 regimens. When the interactions were significant in the children at $P < 0.10$, we displayed the P value for the contrast of the regimen compared with placebo.

TABLE 1
Baseline characteristics of women in the 4 groups[1]

Characteristics	Vitamins B, C, and E (n = 228)	Multivitamins and vitamin A and β-carotene (n = 226)	Vitamin A and β-carotene (n = 233)	Placebo (n = 219)
Age (y)	24.8 ± 4.9[2]	24.6 ± 4.4	24.6 ± 5.1	24.6 ± 4.7
Gestational age (wk)	20.5 ± 3.1	20.3 ± 3.4	20.1 ± 3.6	20.5 ± 3.6
Education (%)				
None or adult	8.8	6.6	6.9	6.9
Primary 1–4 y	5.7	4.4	4.3	5.5
Primary 5–8 y	74.1	76.6	77.7	79.4
> 8 y	11.4	12.4	11.1	8.2
Number of prior pregnancies (%)				
0	29.3	21.4	27.4	24.4
1–3	56.9	64.3	54.9	61.0
> 3	13.8	14.3	17.7	14.6
Weight (kg)	58.2 ± 9.5	57.5 ± 8.0	57.8 ± 10.2	57.0 ± 8.6
Height (cm)	156.7 ± 5.6	156.7 ± 5.7	156.7 ± 6.2	156.8 ± 5.8
MUAC (cm)	25.8 ± 3.0	25.7 ± 2.8	25.7 ± 3.0	25.5 ± 2.9
CD4 count (/mm^3)	432 ± 199	424 ± 203	415 ± 207	424 ± 198
CD4 count <200 (%)	11.4	10.5	13.0	13.4
Hemoglobin (g/dL)	9.3 ± 1.6	9.4 ± 1.6	9.5 ± 1.7	9.6 ± 1.7
Anemia				
Hemoglobin <11.0 g/dL	85.1	84.1	82.0	79.0
Hemoglobin <8.5 g/dL	29.0	29.7	25.8	21.0
Iron deficiency (%)				
Severe	8.3	5.3	4.7	5.9
Moderate	7.9	8.0	8.6	6.4
Mild	30.3	33.2	32.2	27.4
Macrocytic	7.0	4.0	3.9	3.7
Plasma vitamin A (μg/dL)	24.7 ± 9.8	25.0 ± 8.6	24.3 ± 9.5	25.7 ± 11.9
Plasma vitamin A <20 μg/dL (%)	37.1	27.8	35.2	32.4
Plasma vitamin E (μmol/dL)	9.9 ± 3.0	10.2 ± 3.1	9.9 ± 2.9	9.9 ± 2.8
Plasma vitamin E <11.6 μmol/dL (%)	74.8	76.2	75.9	74.7
Plasma vitamin E <9.7 μmol/dL (%)	44.7	44.4	48.8	52.7

[1] MUAC, midupper arm circumference. Continuous variables were compared by using the Kruskal-Wallis test, and categorical variables were compared by using the chi-square test. None of the differences between groups were statistically significant ($P > 0.15$).
[2] $\bar{x}$ ± SD (all such values).

A data safety and monitoring board (DSMB) reviewed the study progress and interim analyses. Because of the beneficial findings on adverse pregnancy outcomes that were reported earlier from the trial (7), all women who became pregnant subsequent to May 1998 were given open-label multivitamins for the duration of their pregnancy; these women reverted to their prepregnancy-blinded regimen after delivery. No difference was noted when the multivitamins group was compared with the nonmultivitamins group ($P = 0.64$) and the vitamin A and β-carotene group with the no vitamin A and β-carotene group ($P = 0.77$). In September 2000, the DSMB recommended that vitamin A and β-carotene be eliminated from the 2 vitamin A–containing regimens because of an observed increased risk of transmission of HIV-1 to children associated with maternal vitamin A supplementation (8). Subsequently, women randomly assigned to vitamin A and β-carotene alone or to multivitamins + vitamin A and β-carotene received placebo or multivitamins that excluded vitamin A, respectively. We performed secondary analyses censoring all data at 30 September 2000, the date when the DSMB recommended dropping the vitamin A and β-carotene treatment arms. In additional secondary analyses, we censored women at the beginning of their second pregnancies, if applicable, and the results were not materially different from the ones reported here. Because results from these secondary analyses were essentially the same, only primary analyses are presented. All data analyses were performed with the use of the STATISTICAL ANALYSIS SYSTEM (SAS) version 8.0 (SAS Institute Inc, Cary, NC).

RESULTS

Women who enrolled had a mean (±SD) age of 24.7 ± 4.8 y) and gestational age of 20.3 ± 3.4 wk. The treatment groups did not differ in these or other baseline characteristics, including education, hemoglobin concentrations, $CD4^+$ and $CD8^+$ cell counts, and plasma concentrations of vitamins A and E (**Table 1**). Infant feeding practices were not significantly different across the 4 treatment arms; durations of breastfeeding were 15.3 ± 7.8 mo in the placebo group, 16.4 ± 7.1 mo in the multivitamins group, 16.2 ± 7.2 mo in the multivitamins + vitamin A and β-carotene group, and 16.2 ± 7.4 mo in the vitamin A and β-carotene group. Mean (median) compliance, evaluated as the number of tablets absent from the returned bottles at monthly visits divided by the total number of tablets the person should have taken, was high at 79% (82%) during the total follow-up period and 83% (87%) and 80% (84%) at 2 and 4 y,

TABLE 2
Effects of multivitamin supplementation of HIV-infected women ($n = 906$) on their hemoglobin concentrations (g/dL) and on their children's ($n = 836$) hemoglobin concentrations[1]

	Placebo	Vitamin A and β-carotene alone	Multivitamins alone	Multivitamins and vitamin A and β-carotene	P^2	P^3	P^4
Maternal outcomes							
Whole period	10.95 ± 0.08[5]	11.12 ± 0.16	11.38 ± 0.16	11.37 ± 0.16	0.68	0.07	0.06
≤70 d postpartum	10.19 ± 0.14	10.70 ± 0.19	11.07 ± 0.19	11.00 ± 0.19	0.20	0.0002	0.06
First 2 y	10.73 ± 0.09	11.03 ± 0.17[6]	11.32 ± 0.17[7]	11.30 ± 0.16[6]	0.49	0.001	0.03
First 4 y	11.03 ± 0.08	11.24 ± 0.16[8]	11.52 ± 0.16[7]	11.50 ± 0.16[8]	0.88	0.01	0.01
Child outcomes							
Whole period	10.02 ± 0.08	9.95 ± 0.07	10.30 ± 0.06	10.04 ± 0.07	0.18	0.0002	0.55
At birth	12.57 ± 0.15	12.90 ± 0.13	12.91 ± 0.15	12.46 ± 0.15	0.57	0.71	0.008
First 6 mo	10.48 ± 0.11	10.61 ± 0.10	10.84 ± 0.10	10.46 ± 0.10	0.17	0.14	0.02
First 2 y	9.91 ± 0.08	9.91 ± 0.08	10.22 ± 0.07	10.00 ± 0.08	0.32	0.0009	0.19
First 4 y	10.01 ± 0.08	9.94 ± 0.07	10.30 ± 0.07	10.04 ± 0.07	0.19	0.0001	0.55

[1] For the maternal outcomes, $\bar{x} \pm$ SE was obtained from generalized estimating equations, adjusted for the possible differences at baseline. For the child outcomes, $\bar{x} \pm$ SE was obtained from generalized estimating equations.
[2] P for the test for parallel structure for the vitamin A and β-carotene group compared with the no vitamin A and β-carotene group.
[3] P for the test for parallel structure for the multivitamins group compared with the nonmultivitamins group.
[4] P for the test for interaction between multivitamins and vitamin A and β-carotene.
[5] $\bar{x} \pm$ SE (all such groups).
[6] $P \leq 0.01$, [7] $P \leq 0.001$, and [8] $P \leq 0.05$ for the contrast of the regimen to placebo, reported only if the P value for the test for interaction between multivitamins and vitamin A and β-carotene was ≤0.05.

respectively. Compliance was not significantly different between the 4 treatment arms.

The factorial and regimen-specific results of hemoglobin were reported for both the mothers and their children (**Table 2**). During the whole period, women who received multivitamins had hemoglobin concentrations 0.33 g/dL higher than women who were not given multivitamins ($P = 0.07$). The difference between women in the vitamin A and β-carotene group relative to the groups that did not receive this regimen was 0.07 g/dL ($P = 0.68$; P for interaction between the 2 regimens = 0.06). The corresponding results for the period between delivery and 70 d postpartum were 0.58 g/dL ($P = 0.0002$) for multivitamins compared with no multivitamins, and 0.20 g/dL for vitamin A and β-carotene compared with no vitamin A and β-carotene ($P = 0.20$; P for interaction = 0.06). During the first 2 y after the enrollment, the difference was 0.59 g/dL ($P = 0.0002$), when the multivitamin only group was compared with the placebo group. The effects of multivitamins + vitamin A and β-carotene and vitamin A and β-carotene alone were also significantly different from placebo ($P = 0.002$ and $P = 0.01$, respectively).

Hemoglobin concentrations of children of mothers on multivitamins were 0.18 g/dL higher than did children of mother not on multivitamins ($P = 0.0002$) (Table 2). In contrast, children whose mother received vitamin A and β-carotene had 0.17 g/dL lower hemoglobin concentrations than did children of mothers who did not receive this regimen ($P = 0.18$; P for interaction = 0.55). At birth, the corresponding findings were −0.05 g/dL ($P = 0.71$) for children of mothers in the multivitamins group compared with children of mothers in the nonmultivitamins groups. The difference among children of mothers in the vitamin A and β-carotene group on average was −0.08 g/dL compared with children whose mothers did not receive vitamin A and β-carotene ($P = 0.57$; P for interaction = 0.008). We noted significant interactions between multivitamins and vitamin A for child hemoglobin at birth and at the first 6 mo and examined the effect of regimens compared with placebo. By 6 mo of age, concentrations among children of mothers in the multivitamins only group were 0.36 g/dL higher than among children of mothers given placebo ($P = 0.06$). Hemoglobin concentrations of children born to mothers who received multivitamins, including vitamin A and β-carotene, or vitamin A and β-carotene alone were not significantly different from children of mothers in the placebo group ($P = 0.61$ and $P = 0.10$, respectively).

We examined the efficacy of supplementation on the risks of anemia (hemoglobin < 11.0 g/dL) and severe anemia (hemoglobin < 8.5 g/dL). A large number of women whose hemoglobin at baseline was below the respective cutoff were excluded from these respective analyses (82.6% had hemoglobin < 11.0 g/dL, and 26.4% had hemoglobin < 8.5 g/dL), thereby reducing the statistical power to examine this question. No difference was found for the risk of anemia when multivitamins were compared with nonmultivitamins and when vitamin A and β-carotene was compared with no vitamin A and β-carotene (**Table 3**).

Compared with the children born to the mothers who were in the nonmultivitamin group, the children born to mothers who were in the multivitamin group had a reduced risk of severe microcytic hypochromic anemia [relative risk (RR): 0.60; 95% CI: 0.42, 0.85; $P = 0.004$] (**Table 4**). Significant interactions were noted between multivitamins and vitamin A and β-carotene for several child outcomes. Compared with the placebo group, the risk of microcytic hypochromic anemia was reduced ≈30% in the multivitamins alone group (RR:= 0.70; 95% CI: 0.51, 0.95; $P = 0.02$). Macrocytic anemia was significantly less likely to occur among children of women in this treatment arm (RR: 0.37; 95% CI: 0.18, 0.79; $P = 0.01$). For both endpoints, the effects of multivitamins + vitamin A and β-carotene and vitamin A and β-carotene alone were not significantly different from placebo.

TABLE 3
Effects of multivitamin supplementation of HIV-infected women ($n = 906$) on their risk of anemia[1]

Outcome	Placebo	Vitamin A and β-carotene alone	Multivitamins	Multivitamins and vitamin A and β-carotene	P^2	P^3	P^4
Hemoglobin < 8.5 g/dL							
RR (95% CI)	1.0	0.88 (0.62, 1.25)	0.81 (0.56, 1.17)	0.72 (0.49, 1.05)	0.38	0.13	0.97
n (%)	63 (36)	60 (35)	53 (33)	46 (29)			
Hemoglobin < 11.0 g/dL							
RR (95% CI)	1.0	0.88 (0.56, 1.38)	0.90 (0.56, 1.45)	0.98 (0.61, 1.56)	0.84	0.99	0.54
n (%)	39 (85)	36 (86)	31 (91)	32 (89)			
Hypochromic microcytosis[5]							
Severe							
RR (95% CI)	1.0	0.85 (0.53, 1.37)	0.74 (0.45, 1.22)	0.78 (0.47, 1.27)	0.75	0.28	0.56
n (%)	34 (17)	34 (15)	28 (13)	29 (14)			
Moderate and above							
RR (95% CI)	1.0	0.95 (0.69, 1.29)	0.75 (0.54, 1.05)	0.84 (0.60, 1.16)	0.80	0.08	0.50
n (%)	75 (39)	84 (42)	67 (35)	69 (35)			
Mild and above							
RR (95% CI)	1.0	0.97 (0.72, 1.30)	0.84 (0.62, 1.13)	0.93 (0.69, 1.25)	0.75	0.30	0.55
n (%)	90 (68)	89 (70)	83 (68)	83 (69)			
Macrocytosis							
RR (95% CI)	1.0	1.06 (0.46, 2.46)	0.62 (0.24, 1.64)	0.85 (0.35, 2.10)	0.58	0.30	0.70
n (%)	10 (5)	12 (5)	7 (3)	9 (4)			

[1] Relative risk (RR) and 95% CI were estimated from Cox regression for the contrast of the regimen to placebo. *n* is number of events.
[2] *P* value estimated from Cox regression to compare the vitamin A and β-carotene group with the no vitamin A and β-carotene group.
[3] *P* value estimated from Cox regression to compare the multivitamins group with the nonmultivitamins group.
[4] *P* value for the interaction between multivitamins and vitamin A and β-carotene.
[5] Hypochromasia and microcytosis in peripheral blood were used to define varying degrees of severity of iron deficiency. Hypochromic microcytic anemia was categorized as severe: hypochromasia ≥ 2+ and microcytic cells observed; moderate and above: hypochromasia ≥ 1+ and microcytic cells observed; and mild and above: hypochromasia ≥ 1+. Macrocytosis was defined as the presence of any macrocytic cells.

DISCUSSION

We found that supplementation with vitamins B-complex, C, and E resulted in a significant improvement in hemoglobin concentrations among women and children. This intervention also significantly reduced the risks of anemia, particularly macrocytic anemia and hypochromic microcytic anemia in children. The effects of vitamin A and β-carotene alone were mostly not significantly different from placebo. Vitamins included in the supplement may have led to better hematologic status through several mechanisms (5). Vitamin C improves intestinal absorption of iron and may also enhance mobilization of iron stores. Improved absorption of dietary iron consumed during and after pregnancy is also possible; total iron intake at baseline among women enrolled in the trial was estimated with the use of a food-frequency questionnaire to be 15 ± 4.3 mg/d; no difference was observed in intake among the 4 groups ($P = 0.81$) at enrollment to the study (Fawzi WW, unpublished data, 1995–1997). Also, supplements of iron and folate were provided to all women during the antenatal period, regardless of treatment arm. The antioxidant effects of vitamins C and E may provide protection to red blood cells from being destroyed by free radicals that are features of oxidative stress in HIV infection and other infections (14). Among the B vitamins, the prevalence of vitamin B-12 deficiency has been shown to be significantly higher in developing countries than previously anticipated (15). Vitamin B-12 is necessary for the metabolism of folate, and its deficiency is associated with megaloblastic anemia. This could explain the large beneficial effect we noted on the risk of macrocytic anemia among children. Riboflavin may also enhance intestinal absorption of iron and is also necessary for synthesis of the globin component of hemoglobin. Vitamin B-6 deficiency is associated with impaired synthesis of heme and ineffective erythropoiesis.

In contrast with our findings in pregnant women in urban Tanzania, no significant effect of multivitamin supplementation was observed on hematologic status among pregnant women in rural Nepal (16) and semirural Mexico (17). In Nepal, women were randomly assigned to 1 of 5 groups: *1*) folate, *2*) vitamin A + iron, *3*) vitamin B + zinc, *4*) vitamin C + multivitamins, and *5*) placebo. All women received daily vitamin A supplements. Supplementation with micronutrients in addition to iron and folate did not further improve hematologic status at 6 wk after delivery. In Mexico, no difference was observed at 1 mo postpartum in hemoglobin concentration or in mean ferritin and prevalence of iron deficiency. Indeed, a dramatic increase in the risk of anemia and iron deficiency was observed in both treatment arms despite high compliance with supplement use during the trial.

Several factors could explain the difference in findings among the 3 trials. Although women in Tanzania were infected with HIV, women from Nepal and Mexico were predominantly uninfected. HIV infection itself may precipitate anemia mediated by chronic inflammation (18). Mean (±SD) hemoglobin concentrations were lower among women in Tanzania (9.4 ± 1.7 g/dL) than women in Nepal (11.5 ± 1.8 g/dL) and Mexico (12.5 ± 1.4 g/dL). Differences in the doses of vitamins used in the trials may be another factor. In Tanzania, the supplement provided twice the Dietary Reference Intake (DRI) of vitamin E and multiples of the DRIs for the respective vitamins B-complex and C. Such amounts were used because of the evidence that HIV-infected persons are likely to require higher intakes to maintain

TABLE 4
Effects of multivitamin supplementation of HIV-infected women on their children's (n = 836) risk of anemia[1]

Outcome	Placebo (n = 197)	Vitamin A and β-carotene alone (n = 209)	Multivitamins (n = 211)	Multivitamins and vitamin A and β-carotene (n = 219)	P^2	P^3	P^4
Hemoglobin < 8.5 g/dL							
RR (95% CI)	1.0	0.89 (0.69, 1.14)	0.73 (0.57, 0.95)	0.87 (0.68, 1.12)	0.72	0.08	0.11
n (%)	120 (61)	122 (58)	114 (54)	125 (57)			
Hemoglobin < 11.0 g/dL							
RR (95% CI)	1.0	0.84 (0.68, 1.04)	0.83 (0.67, 1.02)	0.92 (0.75, 1.14)	0.78	0.55	0.06
n (%)	168 (85)	177 (85)	182 (86)	185 (84)			
Hypochromic microcytosis[5]							
Severe							
RR (95% CI)	1.0	0.87 (0.56, 1.37)	0.51 (0.31, 0.84)	0.61 (0.38, 1.00)	0.88	0.004	0.38
n (%)	38 (19)	38 (18)	26 (12)	28 (13)			
Moderate and above							
RR (95% CI)	1.0	0.90 (0.67, 1.22)	0.70 (0.51, 0.95)[6]	0.92 (0.68, 1.23)	0.38	0.12	0.08
n (%)	84 (43)	88 (42)	78 (37)	94 (43)			
Mild and above							
RR (95% CI)	1.0	0.89 (0.71, 1.13)	0.73 (0.58, 0.92)[7]	0.96 (0.77, 1.21)	0.23	0.14	0.02
n (%)	140 (71)	146 (70)	143 (68)	160 (73)			
Macrocytosis							
RR (95% CI)	1.0	0.55 (0.27, 1.09)	0.37 (0.18, 0.79)[7]	0.87 (0.47, 1.59)	0.73	0.37	0.004
n (%)	21 (11)	13 (6)	10 (5)	21 (10)			

[1] Relative risk (RR) and 95% CI were estimated from Cox regression for the contrast of the regimen to placebo. n is number of events.
[2] P value estimated from Cox regression to compare the vitamin A and β-carotene group with the no vitamin A and β-carotene group.
[3] P value estimated from Cox regression to compare the multivitamins group with the nonmultivitamins group.
[4] P value for the interaction between multivitamins and vitamin A and β-carotene.
[5] Hypochromasia and microcytosis in peripheral blood were used to define varying degrees of severity of iron deficiency. Hypochromic microcytic anemia was categorized as severe: hypochromasia $\geq$ 2+ and microcytic cells observed; moderate and above: hypochromasia $\geq$ 1+ and microcytic cells observed; and mild and above: hypochromasia $\geq$ 1+. Macrocytosis was defined as the presence of any macrocytic cells.
[6] $P \leq 0.05$ and [7] $P \leq 0.01$ for the contrast of the regimen to placebo, reported only if the P value for the test for interaction between multivitamins and vitamin A and β-carotene was ≤ 0.10.

an adequate status (19). In Nepal, a single DRI of most nutrients was used, whereas 100–150% of the DRI was included in the supplements in the Mexican trial. The DRI is the amount recommended for healthy women in North America, and it is conceivable that even in the absence of HIV infection this amount is inadequate for persons in many developing countries, given the relatively higher burden of undernutrition and parasitic infections (20). In a recently completed trial from Guinea-Bissau, pregnant women who received prenatal micronutrient supplements at twice the DRI had infants of higher birth weight than did the women who received only a single DRI or those who were given placebo (21).

In another trial from Tanzania, a micronutrient-fortified beverage was associated with reduced risk of anemia in pregnancy and improved hemoglobin concentrations among women in a largely arid part of the country (22). The beverage contained iron in addition to 10 other micronutrients. Although the iron component may have contributed to the hematologic improvements, it is not possible to quantify its effect independent of the other nutrients.

Our findings of the beneficial effect of multivitamins were sustained beyond the end of pregnancy through the first 2 y and even 4 y of the trial. We previously reported that women who received multivitamins had significantly slower disease progression, higher CD4 cell counts, and lower viral load. Anemia has been associated with higher risk of mortality, faster disease progression, and wasting in the same cohort (23, 24) and for other studies (2). Thus, the improvement in hematologic status may provide an additional mechanism for the improved clinical outcomes. It is also possible that the enhanced clinical outcomes resulted in further reduction in the risk of anemia through better absorption of essential nutrients and reduced oxidative stress that contributes to the cause of anemia. Thus, supplementation would help interrupt a vicious cycle that leads to increased severity of anemia, worse clinical outcomes, and ultimately death.

Maternal multivitamin supplementation continued through the postpartum period resulted in improvements in hemoglobin concentrations among the children. This benefit may be attributed to improved vitamin concentrations in breast milk (25) and enhanced infant micronutrient status (26), and it is consistent with reduced risks of mortality, morbidity, and undernutrition among children in the same trial (8, 27, 28). Two placebo-controlled trials were conducted in Peru (29) and India (30) to isolate the effect of multivitamin supplements from iron. In both trials, multivitamins provided for 10 and 12 wk, respectively, resulted in higher hemoglobin concentrations than did placebo.

At baseline, hypochromasia and microcytosis were prevalent in this population, suggesting that iron deficiency is an important contributor to the cause of anemia. Iron supplementation was provided to all women in this trial during pregnancy only according to local standard of care; unfortunately, we have no data on the compliance of women with these supplements. There are, however, some concerns about the provision of iron in HIV infection because it may contribute to oxidative stress and lead to faster disease progression (31). Data from randomized trials are

needed to examine the safety and efficacy of iron supplementation in the context of HIV infection. Iron deficiency is prevalent in children, particularly among children aged 6–24 mo, when they are increasingly dependent on an iron-deficient diet, and the quantity in breast milk may be suboptimal (32). Although this suggests that iron supplementation may be an important intervention during that period, a meta-analysis of 28 controlled clinical trials of iron supplementation concluded that diarrhea was more common in children who received iron supplements (33). Higher rates of mortality in malaria-endemic areas were found in a large iron trial from the Pemba Island in Zanzibar, Tanzania (34). These findings suggest that further data are needed before such supplementation is implemented. Multivitamin supplementation may therefore be a safe approach to addressing anemia, possibly even among persons affected by iron deficiency anemia through mobilization of iron stores and increased absorption of dietary iron.

Vitamin A supplementation has previously been shown in some studies to improve hematologic status and is thought to increase mobilization of iron stores (35). This finding was not supported by our study or by the trials from Nepal (16) and Mexico (17) in which the respective supplements contained vitamin A. In our trial all children, regardless of the regimen assigned to their mothers, were given periodic large doses of vitamin A, starting at 6 mo of age according to the national standard of care. This supplementation may have reduced the chances of finding a protective effect of maternal supplements, if such an effect existed. The lack of effect of vitamin A and β-carotene on anemia is consistent with the null effect of this supplement on various maternal and infant outcomes in the same cohort (7, 27).

Although the prevalence of anemia is lower among HIV-negative women and children than women and children who are HIV-positive, this condition constitutes an important public health problem even in the absence of HIV infection. For example, among 9-mo-old uninfected children in Uganda, 77% were reported to be anemic compared with 91% among HIV-infected infants of the same age (36). Similarly, among pregnant women, anemia was highly prevalent (>50%) in a cohort of women in Tanzania regardless of their HIV status (37). The effects of multivitamins in this study may not be generalizable to HIV-negative women, and several trials are under way to examine this question. Among HIV-infected women, multivitamin supplementation provided during pregnancy and in the postpartum period resulted in significant improvements in hematologic status among women and their children, providing further support for the value of multivitamin supplementation to HIV-infected adults (38, 39).

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The authors' responsibilities were as follows—WWF (Harvard principal investigator) and DH: study design, study implementation, data analyses, and writing of manuscript; GIM: study design and daily management of the field study; RK, EV, and FM: review of data analyses plan; DS and RW (study statisticians): study design and supervision of data analyses; and all authors: contributed to the editing of the final version of the manuscript. None of the authors had a conflict of interest.

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