

Original Investigation

Effect of Micronutrient Supplementation on Disease Progression in Asymptomatic, Antiretroviral-Naive, HIV-Infected Adults in Botswana

A Randomized Clinical Trial

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IMPORTANCE Micronutrient deficiencies occur early in human immunodeficiency virus (HIV) infection, and supplementation with micronutrients may be beneficial; however, its effectiveness has not been investigated early in HIV disease among adults who are antiretroviral therapy (ART) naive.

OBJECTIVE To investigate whether long-term micronutrient supplementation is effective and safe in delaying disease progression when implemented early in adults infected with HIV subtype C who are ART-naive.

DESIGN, SETTING, AND PARTICIPANTS Randomized clinical trial of supplementation with either daily multivitamins (B vitamins and vitamins C and E), selenium alone, or multivitamins with selenium vs placebo in a factorial design for 24 months. The study was conducted in 878 patients infected with HIV subtype C with a CD4 cell count greater than 350/ μ L who were not receiving ART at Princess Marina Hospital in Gaborone, Botswana, between December 2004 and July 2009.

INTERVENTIONS Daily oral supplements of B vitamins and vitamins C and E, selenium alone, or multivitamins plus selenium, compared with placebo.

MAIN OUTCOMES AND MEASURES Reaching a CD4 cell count less than 200/ μ L until May 2008; after this date, reaching a CD4 cell count of 250/ μ L or less, consistent with the standard of care in Botswana for initiation of ART at the time of the study.

RESULTS There were 878 participants enrolled and randomized into the study. All participants were ART-naive throughout the study. In intent-to-treat analysis, participants receiving the combined supplement of multivitamins plus selenium had a significantly lower risk vs placebo of reaching CD4 cell count 250/ μ L or less (adjusted hazard ratio [HR], 0.46; 95% CI, 0.25-0.85; $P = .01$; absolute event rate [AER], 4.79/100 person-years; censoring rate, 0.92; 17 events; placebo AER, 9.22/100 person-years; censoring rate, 0.85; 32 events). Multivitamins plus selenium in a single supplement, vs placebo, also reduced the risk of secondary events of combined outcomes for disease progression (CD4 cell count \leq 250/ μ L, AIDS-defining conditions, or AIDS-related death, whichever occurred earlier [adjusted HR, 0.56; 95% CI, 0.33-0.95; $P = .03$; AER, 6.48/100 person-years; censoring rate, 0.90; 23 events]). There was no effect of supplementation on HIV viral load. Multivitamins alone and selenium supplementation alone were not statistically different from placebo for any end point. Reported adverse events were adjudicated as unlikely to be related to the intervention, and there were no notable differences in incidence of HIV-related and health-related events among study groups.

CONCLUSIONS AND RELEVANCE In ART-naive HIV-infected adults, 24-month supplementation with a single supplement containing multivitamins and selenium was safe and significantly reduced the risk of immune decline and morbidity. Micronutrient supplementation may be effective when started in the early stages of HIV disease.

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Botswana, in sub-Saharan Africa, reports one of the highest rates of human immunodeficiency virus (HIV) infection in the world, with an estimated 23.4% of individuals aged 15 to 49 years having HIV infection in 2011.¹ Moreover, HIV subtype C, the subtype most prevalent in Botswana, has been associated with more prolonged early viremia and a higher set point than other HIV subtypes, with more adverse health consequences.^{2,3}

Amid discussion on when to initiate antiretroviral therapy (ART) in Africa, Botswana is one of the first resource-limited countries involved in a large-scale effort to provide ART.⁴ Persons with HIV infection who have a CD4 cell count of 350/ μ L or less have started receiving ART as of April 2012. Although most countries have provided ART to HIV-infected patients in the last decade, and the World Health Organization (WHO) has recently revised their treatment guidelines, many challenges remain in providing treatment in the early stages of the disease.⁴⁻⁶ Alternative strategies to slow progression early in HIV disease and delay an appreciable number of individuals from developing AIDS in the near future would allow additional time to prepare health care systems in resource-limited countries and allot needed resources for timely HIV interventions.⁷

Micronutrient deficiencies, known to influence immune function, are prevalent even before the development of symptoms of HIV disease and are associated with accelerated HIV disease progression.^{8,9} Micronutrient supplementation has improved markers of HIV disease progression (CD4 cell count, HIV viral load) and mortality in clinical trials; however, these studies were conducted either in the late stages of HIV disease¹⁰⁻¹² or in pregnant women.¹³ To our knowledge, there are no studies testing the effect of long-term micronutrient supplementation in early stages of HIV disease in ART-naive adults.

The B vitamins, vitamins C and E, and the trace element selenium are essential nutrients necessary for maintaining a responsive immune system.¹⁴ Selenium may also have an important role in preventing HIV replication.^{9,15,16} The objective of this randomized, double-blind, placebo-controlled clinical trial was to determine whether specific supplemental micronutrients enhance the immune system and slow HIV disease progression during the early stages of the disease in ART-naive adults.

Methods

This randomized, double-blind, placebo-controlled clinical trial consisted of a pretreatment phase (screening, and a run-in visit) followed by randomization of participants into 4 groups in a factorial design for a 24-month treatment protocol. A cohort of 878 HIV-infected ART-naive adults was recruited and followed up for 24 months, in collaboration with the Botswana-Harvard Partnership in Gaborone, Botswana, from December 2004 until July 2009. The primary objective was to determine whether the supplementation with a combination of multivitamins plus selenium is preferable to either multivitamins alone or selenium alone, in comparison with placebo, in improving immune function and slowing HIV disease progres-

sion in early stages of the disease, prior to provision of ART. Participants were eligible for the study if they had documented HIV infection, had a CD4 cell count greater than 350/ μ L and they were ART-naive; other requirements were a body mass index (BMI) greater than 18 for women and 18.5 for men (calculated as weight in kilograms divided by height in meters squared), age of 18 years or older, no current AIDS-defining conditions or history of AIDS-defining conditions, and no history of endocrine or psychiatric disorders. Women were excluded if pregnant or becoming pregnant during the study. The HIV viral load, complete blood counts, and chemistries (including parameters of renal and liver function and lipid profiles) were monitored at baseline and every 6 months, and any abnormal value resulted in patient referral and communication with the primary care physician.

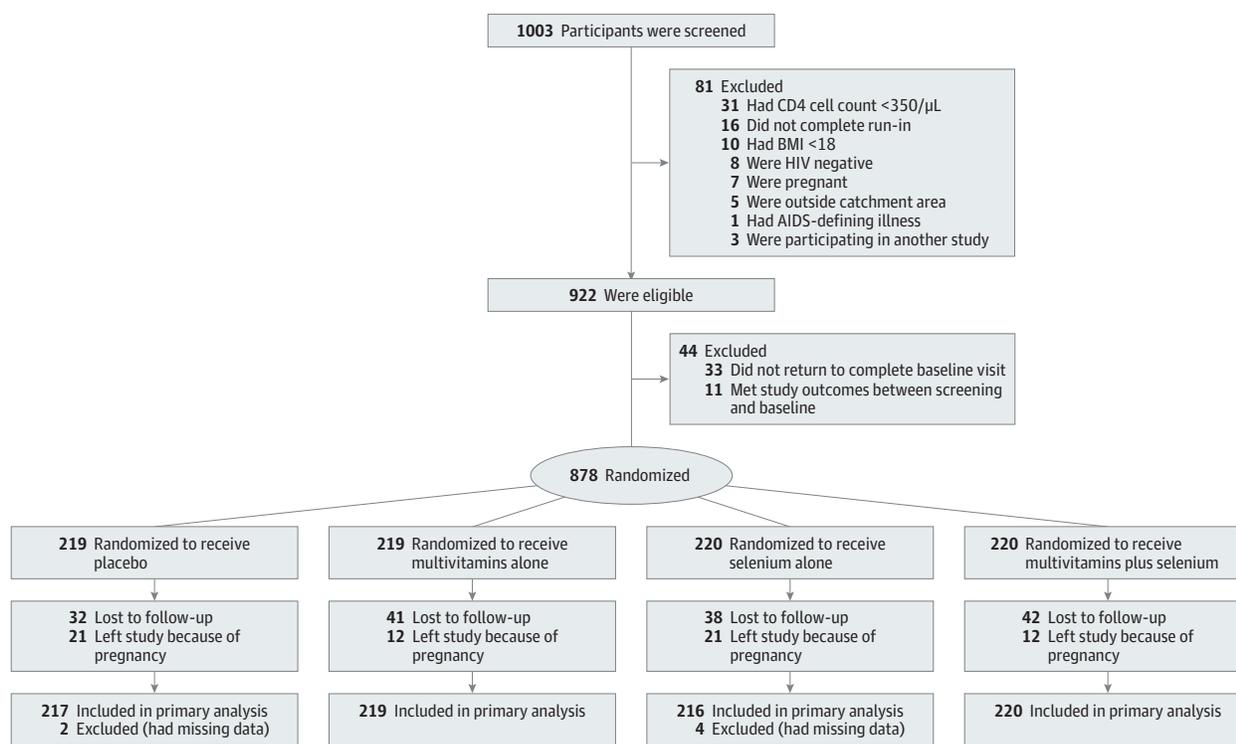
The study protocol was approved by the Florida International University institutional review board (IRB), the Harvard School of Public Health IRB, the Botswana Health Research Unit of the National Ministry of Health, and the data and safety monitoring board (DSMB). Appropriate informed consent was obtained and clinical research was conducted in accordance with guidelines for human experimentation as specified by the US Department of Health and Human Services, the authors' institutions, or both. The purpose, procedures, and potential risks and benefits of the study were explained to the prospective participants, and written informed consent was obtained.

Randomization and Intervention

The randomization flowchart is shown in the **Figure 1**. The study physicians and nurses screened individuals and conducted a run-in prerandomization phase to obtain written informed consent, confirm eligibility, and identify potentially nonadherent individuals. Demographic characteristics were collected, and the participants were counseled on supplement adherence. Participants who attended the scheduled baseline visit within a 2-week time window and returned the supplement bottles with use of more than 80% of the placebo pills dispensed in the prerandomization visit were randomized into the study using blocked randomization generated by the statistician in blocks of 20. Eligible participants were randomly assigned into one of the study groups using the next sequential number from the randomization list generated by the Botswana-Harvard Partnership Data Center.

The study groups received a daily supplement of multivitamins alone, selenium (200 μ g) alone, combination of multivitamins plus selenium, or placebo, taken as 1 pill per day. (The multivitamins included thiamin, 20 mg; riboflavin, 20 mg; niacin, 100 mg; vitamin B₆, 25 mg; vitamin B₁₂, 50 μ g; folic acid, 800 μ g; vitamin C, 500 mg; and vitamin E, 30 mg.) These supplements were chosen because improvement in immune function and delay of HIV disease progression with their use was demonstrated in our previous study.¹³ Once the participants were "on study," bottles of 35 supplements or placebo per month were dispensed. Pills were indistinguishable in shape, size, and color; they were pre-labeled for the entire study by the pharmacist with the identification number according to the assignment list, using double-blind masking. During the

Figure 1. Randomization Flowchart



Flowchart depicts the study participant screening, randomization, disposition, and loss to follow-up by group. Of the 878 participants enrolled and randomized, 872 were included in the primary analysis; data for 6 patients (0.06%) were missing. Total loss to follow-up was 17.5% (153/872), which included those who missed more than 3 monthly visits and were formally declared lost to follow-up; we were unable to contact them, and they did not return for a study visit. This loss to follow-up also included those who moved

from the catchment area and those who chose to discontinue participation. Additionally, 7.5% (66/872) had left the study because of pregnancy, for a total loss to follow-up of 25% (219/872) for the 5-year study. χ^2 analysis showed that there were no significant differences among the groups in loss to follow-up, ($df=3$, value 0.74, $P=.86$). BMI indicates body mass index; HIV, human immunodeficiency virus.

monthly visits, the remaining pills from the previous month were counted to assess adherence. Participants attended the clinic monthly for 24 months to receive the study supplement or placebo and to report adverse effects. A monthly questionnaire was administered about acceptability of the supplement, adherence, adverse effects, and intercurrent morbidity (health events occurring between the study visits). The study personnel and participants were blinded to the assignment groups. The pill doses and randomization scheme were independently validated by the Oscar E. Olson Biochemistry Laboratory, Brookings, South Dakota.

Assessments

At baseline and every 3 months, a nurse or physician performed a physical examination, obtained a medical history, and collected a blood sample for assessment of CD4 cell count. At baseline and every 6 months, HIV viral load, plasma micronutrient levels (20% subsample), and blood chemistries were determined. The medical history included intercurrent health events and prescribed medications. Medical records were reviewed to verify prescriptions and changes in health status. Adherence to the study regimen was determined with questionnaires and pill counts. Data on morbidity were collected

using questionnaires at screening and at every monthly visit and confirmed by documentation in the medical record. Cause of death was obtained through medical records and death certificates from the Botswana Ministry of Health, Department of Vital Statistics.

Laboratory Assays

Lymphocyte phenotype was determined with a 4-color immunophenotyping panel of monoclonal antibodies. Differential counts were determined using a Coulter MaxM hematology instrument and corroborated with cytocentrifuge smears. The HIV viral load was determined using an in vitro nucleic acid amplification test (Amplicor reagents and protocol; Roche-Diagnostics). For quality assurance, standard reference materials were used, and 5% of the samples underwent duplicate blind retesting.

Study Outcomes

The primary end point of this trial was HIV disease progression—specifically, time from randomization to the date of reaching CD4 cell count less than 200/ μ L, confirmed within 1 month of the first measurement. In March 2008, when the standard of care changed in Botswana to provide ART at CD4 cell

count of 250/ μ L or less,¹⁷ we requested permission to change the primary end point to the standard of treatment for the country at the time from our DSMB, the Florida International University IRB, the Harvard School of Public Health IRB, and the Botswana Health Research Unit of the National Ministry of Health. The permission was received on May 5, 2008, at which time the primary outcome for our study became a CD4 cell count of 250/ μ L or less, confirmed within 1 month of the first measurement. This end point was changed because the participants may not have reached the original end point of CD4 cell count less than 200/ μ L once they were eligible for ART at CD4 cell count of 250/ μ L or less.

Secondary end points were (1) time from the date of randomization to the date of the combined outcomes consisting of CD4 cell count of 250/ μ L or less, AIDS-defining conditions, or AIDS-related death, whichever occurred first, and (2) HIV viral load. In April 2012, after the study was completed, the ART eligibility in Botswana changed again, to a CD4 cell count of 350/ μ L or less, which was used as a third secondary end point. The composite outcome of a CD4 count of 350/ μ L or less, AIDS-defining conditions, or AIDS-related death, whichever occurred first, was the fourth secondary outcome. The sample size was increased to 878 with approval of the DSMB, all 3 IRBs, and the funding agency (National Institute on Drug Abuse). Data were censored on July 22, 2009, when the last scheduled 24-month visit was completed.

Sample Size

The sample size was calculated to detect a 25% decrease in the risk of the primary end point, the immunological decline, defined as 2 consecutive CD4 cell counts less than 200/ μ L, with the assumption that the event rate for those in the placebo group was 10 per 100 person-years. We also assumed that a minimum detectable risk was 0.75 for the primary outcome for each of the study groups compared with the placebo group, a Cox proportional hazards model would be used, the test would be 2-sided with type I error rate of .05, and the attrition rate would be 15%. The sample size estimation was within the framework of generalized linear models that is based on a non-central χ^2 approximation to the distribution score statistics.^{18,19} To examine whether taking the combination of multivitamins plus selenium, as compared with placebo, was associated with a significantly longer time to a CD4 cell count less than 200/ μ L than taking any of the other 2 regimens as compared with placebo, the sample size was adjusted based on Schoenfeld.²⁰ With a sample size of 828, the power for the primary end point was 92%. For secondary end points, such as the combined end point of CD4 cell count of 250/ μ L or less, AIDS-defining conditions, or AIDS-related death, whichever occurred first, with the sample size of 828, the power was 97%.

Statistical Analyses

Statistical analyses were performed with the use of SAS version 9.3 (SAS Institute). Data analyses were implemented using intention-to-treat principles based on randomized treatment assignment in which all available data were used and missing data were ignored. Baseline demographic and clinical characteristics were assessed and compared by treatment group with

the χ^2 test for categorical variables, with nonparametric analysis of variance (ANOVA) and Wilcoxon rank-sum test for continuous variables.

Time-to-event analyses were performed to examine whether supplementation with multivitamins or selenium delayed immunological failure compared with placebo and to examine whether taking the combination of multivitamins plus selenium was associated with a significantly longer time to immunological failure as compared with placebo, than taking multivitamins alone or selenium alone compared with placebo. Time-to-event curves were estimated with the Kaplan-Meier method and tested with a 2-sided log-rank test. Hazard ratios (HRs) and 95% confidence intervals were estimated with the use of a Cox proportional hazards model that included baseline CD4 cell count, baseline HIV viral load, age, sex, baseline BMI, and the supplementation regimens. For the survival analysis, we did not perform a multiple imputation because the rate of loss to follow-up was very close to the originally estimated rate of 15%, and the study may be affected by informative censoring on imputation.

For this trial with factorial design, we also examined the interaction effects between the multivitamin treatment and the selenium treatment. We assumed that there would be an interaction between the multivitamin and the selenium treatments. The interaction was tested using the Cox proportional hazards model. If the interaction was not significant, then the conclusions would be based on the tests of the multivitamin groups vs no-multivitamin groups and selenium groups vs no-selenium groups. If the interaction tested was significant, the conclusions would be based on various subset tests as follows. If the interactions were favorable, then multivitamins would be tested vs placebo, and selenium would be tested vs placebo. If both were significant, the combination of multivitamins and selenium in a single supplement would be concluded to be the best treatment; if just one were significant, then this one would be tested against the combination of the multivitamins and selenium in a single pill; if neither comparison were significant, the combination of multivitamins and selenium in a single supplement would be tested against placebo. The assumptions for the Cox model were assessed and met for all applications in the analyses provided herein. Schoenfeld residuals were used to assess the proportionality assumption.²¹

To examine whether supplementation influenced HIV viral load, we used a multivariable random-effects model with multiple imputation that controlled for age, sex, baseline CD4 cell count, baseline viral load (log₁₀ scale), and baseline BMI. Baseline characteristics were compared for clinically relevant differences by treatment group using nonparametric ANOVA. The differences in loss to follow-up between the groups were analyzed using χ^2 or Fisher exact test. All *P* values reported were 2-sided; statistical significance was defined as *P* < .05.

Data and Safety Monitoring

The DSMB reviewed the safety and efficacy of the supplements before the initiation of the study and annually and unblinded data at midpoint and at the end of the study. The Peto

Table 1. Baseline Characteristics of the Study Population^a

	Median (Interquartile Range)			
	Placebo (n = 217)	Multivitamins (n = 219)	Selenium (n = 216)	Multivitamins Plus Selenium (n = 220)
Age, y	33 (28-39)	31 (27-38)	32 (27-38)	32 (28-39)
CD4 cell count/ μ L	411 (327-545)	423 (333-559)	423 (347-539)	428 (336-555)
CD8 cell count/ μ L	839 (619-1193)	863 (663-1141)	835 (645-1147)	835 (617-1183)
CD4, %	25.8 (19.6-30.6)	25.8 (20.5-31.6)	25.6 (21.3-30.1)	26.2 (20.8-31.5)
CD4/CD8 ratio, %	0.52 (0.35-0.67)	0.50 (0.37-0.74)	0.51 (0.39-0.67)	0.50 (0.37-0.72)
Viral load, copies/mL	21 350 (3625-65 600)	11 800 (3890-52 300)	18 500 (3270-62 600)	11 100 (2100-53 900)
BMI ^b	22.8 (20.7-27.1)	23.1 (20.6-26.7)	23.2 (20.2-28.2)	23.6 (20.6-27.5)
Hemoglobin, g/L	12.9 (11.8-14.0)	13.3 (12.1-14.4)	12.9 (11.7-14.5)	12.9 (12.0-14.2)
Albumin, mg/dL	41.0 (38.3-43.4)	41.2 (38.9-44.2)	41.2 (38.4-43.3)	41.6 (38.6-44.0)
HDL, mg/dL	41.9 (33.1-51.5)	39.1 (31.7-50.6)	39.1 (31.2-51.0)	40.0 (31.9-48.9)
Total cholesterol, mg/dL	141 (117-166)	130 (116-155)	137 (119-161)	138 (119-157)

Abbreviations: BMI, body mass index; HDL, high-density lipoprotein cholesterol.

SI conversion factors: To convert HDL and total cholesterol to mmol/L, multiply by 0.0259.

^a A total of 872 participants were included in the primary analysis. Nonparametric Wilcoxon rank sum test showed there was no evidence to suggest that at least 1 of the 4 group medians of each parameter differed from others.

^b Calculated as weight in kilograms divided by height in meters squared.

stopping boundary was used for early stopping with nominal $P < .001$ for efficacy end points and $P = .05$ for safety end points.²² All adverse events were characterized using the Florida International University IRB form rating scale, which has a scale of 1, remote; 2, possible; 3, probable; and 4, definite.²³

Results

Micronutrients and HIV Disease Progression

A total of 878 participants were enrolled and randomized into the study. The flow of study participants throughout the clinical trial is shown in the Figure 1. Average loss to follow-up was 3.5% per study year, for a total of 17.5% (n = 153) over 5 years. Additionally, 66 participants (7.5%) left the study because of pregnancy, with no significant differences between the supplementation groups compared with the placebo group (Figure 1). The differences in loss to follow-up between the groups were analyzed using χ^2 ($df = 3$, value = 0.74, $P = .86$), and no significant differences among the groups were found. Six of 878 participants (<1%) had missing data and were not included in the analyses.

The study was conducted between December 2004 and July 2009; the median duration of study follow-up was 24 months (interquartile range [IQR], 15-24). The baseline characteristics of the study participants are shown in Table 1. The baseline median CD4 cell count was 420/ μ L (IQR, 336-550), with 33% of the participants (286/872) having a CD4 cell count of 500/ μ L or greater. The baseline median \log_{10} HIV viral load was 4.14 (IQR, 3.49-4.78). No statistical differences in any of these parameters between the 4 groups were found ($P > .05$ with nonparametric Wilcoxon test). All study participants were ART-naïve throughout the study. All participants received isoniazid prophylaxis regardless of CD4 cell count, Mantoux test status, or bacillus Calmette-Guérin vaccine status. The overall mortality in the study was low (0.34%).

Figure 2 shows the Kaplan-Meier estimates for reaching the primary end point by supplementation group. Table 2 shows

that after adjusting for age, sex, baseline BMI, baseline CD4 cell count, and baseline HIV viral load, supplementation with multivitamins as compared with placebo, and the treatment that included the combination of multivitamins plus selenium in a single supplement as compared with placebo (placebo absolute event rate [AER], 9.22/100 person-years; 32 events), significantly reduced the risk of reaching the primary end point of a CD4 cell count of 250/ μ L or less (HR, 0.54; 95% CI, 0.30-0.98; $P = .04$; AER, 4.83/100 person-years; censoring rate, 0.92; 17 events; and HR, 0.48; 95% CI, 0.26-0.88; $P = .02$; AER, 4.79/100 person-years; censoring rate, 0.92; 17 events, respectively). Selenium supplementation alone as compared with placebo did not affect the risk of reaching the primary end point (HR, 0.83; 95% CI, 0.48-1.42; $P = .50$; AER, 7.25/100 person-years; censoring rate, 0.88; 25 events).

For the secondary outcome of the composite of a CD4 cell count of 250/ μ L or less, AIDS-defining conditions, or AIDS-related death, whichever occurred first, only supplementation with the single supplement that included multivitamins plus selenium as compared with placebo (placebo AER, 10.95/100 person-years; censoring rate, 0.82; 38 events), significantly reduced risk of reaching the event (HR, 0.57; 95% CI, 0.34-0.97; $P = .04$; AER, 6.48/100 person-years; censoring rate, 0.90; 23 events). Multivitamin supplementation was not different from placebo for the risk of reaching the secondary events of the combined outcomes (HR, 0.65; 95% CI, 0.39-1.09; $P = .10$; AER, 7.10/100 person-years; censoring rate, 0.89; 25 events). Selenium supplementation alone was not different from placebo for the risk of reaching the secondary events of the composite outcome (HR, 0.76; 95% CI, 0.46-1.25; $P = .28$; AER, 8.12/100 person-years; censoring rate, 0.87; 28 events).

For the secondary end point of a CD4 cell count of 350/ μ L or less, only the supplementation with a single supplement containing multivitamins plus selenium, as compared with placebo (placebo AER, 81.12/100 person-years; censoring rate, 0.77; 51 events), significantly reduced risk of reaching the event (HR, 0.62; 95% CI, 0.40-0.97; $P = .04$; AER, 59.21/100 person-years; censoring rate, 0.85; 33 events). For the composite out-

come of a CD4 cell count of 350/ μ L or less, AIDS-defining conditions, or AIDS-related death, whichever occurred earlier, also only the supplementation with the single supplement containing multivitamins plus selenium, as compared with placebo (placebo AER, 83.44/100 person-years; censoring rate, 0.74, 57 events) significantly reduced risk of reaching the event (HR, 0.65; 95% CI, 0.43-0.99; $P = .045$; AER, 60.17/100 person-years, censoring rate, 0.83; 38 events). HIV viral load was not

Figure 2. Kaplan-Meier Estimates for Reaching the Primary End Point of CD4 Cell Count of 250/ μ L or Less by Supplementation Group

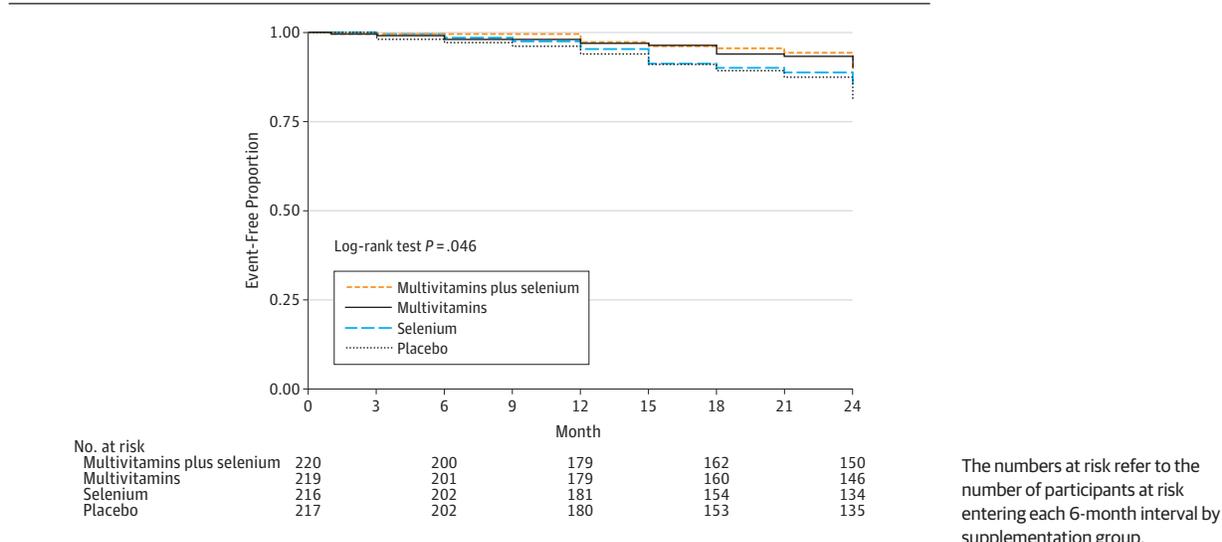


Table 2. Effect of Micronutrient Supplementation on HIV Disease Progression and Mortality Early in HIV Disease^a

Outcome	Placebo ^b		Multivitamins		Selenium Alone			Multivitamins Plus Selenium		
	% Censored and No. of Clinical Events ^c	% Censored and No. of Clinical Events ^c	HR ^d (95% CI)	P Value	% Censored and No. of Clinical Events ^c	HR ^d (95% CI)	P Value	% Censored and No. of Clinical Events ^c	HR ^d (95% CI)	P Value
CD4 cell count $\leq 250/\mu\text{L}$	85	92	0.54 (0.30-0.98)	.04	88	0.83 (0.48-1.42)	.50	92	0.48 (0.26-0.88)	.02
Clinical end points ^c	(185/217) [32]	(202/219) [17]			(191/216) [25]			(203/220) [17]		
Considering interaction			0.83 (0.48-1.42)	.50		0.76 (0.44-1.32)	.33		0.46 (0.25-0.85)	.01
CD4 cell count $\leq 250/\mu\text{L}$, AIDS-defining conditions, or AIDS-related death	82	89	0.65 (0.39-1.09)	.10	87	0.76 (0.46-1.25)	.28	90	0.57 (0.34-0.97)	.04
Clinical end points ^c	(179/217) [38]	(194/219) [25]			(188/216) [28]			(197/220) [23]		
Considering interaction			0.66 (0.39-1.10)	.11		0.73 (0.44-1.20)	.21		0.56 (0.33-0.95)	.03
CD4 cell count $\leq 350/\mu\text{L}$	77	85	0.68 (0.44-1.06)	.09	79	0.99 (0.66-1.49)	.95	85	0.62 (0.40-0.97)	.04
Clinical end points ^c	(166/217) [51]	(185/219) [34]			(171/216) [45]			(187/220) [33]		
CD4 cell count $\leq 350/\mu\text{L}$, AIDS-defining conditions, or AIDS-related death	74	81	0.75 (0.50-1.12)	.16	78	0.93 (0.63-1.37)	.40	83	0.65 (0.43-0.99)	.045
Clinical end points ^c	(160/217) [57]	(177/219) [42]			(168/216) [48]			(182/220) [38]		

Abbreviations: BMI, body mass index; HIV, human immunodeficiency virus; HR, hazard ratio.

^a Cox proportional hazards model was used to examine the effect of selenium and multivitamin supplementations, individually or jointly, on HIV disease progression. All analyses controlled for age, sex, baseline BMI, baseline CD4 cell count, and baseline HIV viral load.

^b The placebo group was the reference group for all comparisons.

^c Percent censored observations indicates the percentage of participants who did not have clinical end points during the 24 months of the study in the

numerator and the number of participants entered in the primary analyses in the denominator. The number of clinical events is indicated in brackets.

^d Hazard ratios of disease progression determined by risk of CD4 cell count $\leq 250/\mu\text{L}$; the combined end point of CD4 cell count $\leq 250/\mu\text{L}$, AIDS-defining conditions, or AIDS-related death, whichever occurred first; and the risk of CD4 cell count $\leq 350/\mu\text{L}$ alone or in combination with AIDS-defining conditions or AIDS-related death for the 3 supplementation groups compared with placebo, for the 24 months of the trial duration.

different when comparing the supplementation groups with the placebo group (see eTable 1 in the Supplement)

The interaction was examined using a multivariable Cox model, controlling for age, sex, baseline CD4 cell count, baseline HIV viral load, and baseline BMI. Because neither comparison of multivitamins vs placebo nor selenium vs placebo were significant, the treatment with a single supplement containing multivitamins plus selenium was tested against placebo and found to significantly slow HIV disease progression for both the primary end point of a CD4 cell count of 250/ μ L or less (adjusted HR, 0.46; 95% CI, 0.25-0.85; $P = .01$; AER, 4.79/100 person-years; censoring rate, 0.92; 17 events) and the secondary end point of the composite of a CD4 cell count of 250/ μ L or less, AIDS-defining conditions, or AIDS-related death, whichever occurred first (adjusted HR, 0.56; 95% CI, 0.33-0.95; $P = .03$; AER, 6.48/100 person-years, censoring rate, 0.90; 23 events).

Multivitamin supplementation alone was not statistically different from placebo for any end point determined (for the primary end point of CD4 cell count \leq 250/ μ L: adjusted HR, 0.83; 95% CI, 0.48-1.42; $P = .50$; AER, 4.83/100 person-years; censoring rate, 0.92; 17 events; for the secondary events of the combined outcomes, including CD4 cell count \leq 250/ μ L: adjusted HR, 0.66; 95% CI, 0.39-1.10; $P = .11$; AER, 7.10/100 person-years; censoring rate, 0.89; 25 events).

Selenium supplementation alone was also not statistically different from placebo for any end point determined (for primary end point: adjusted HR, 0.76; 95% CI, 0.44-1.32; $P = .33$; AER, 7.25/100 person-years; censoring rate, 0.88; 25 events; for the secondary end point of the composite with CD4 cell count \leq 250/ μ L: adjusted HR, 0.73, 95% CI, 0.44-1.20, AER, 8.12/100 person-years; censoring rate, 0.87; 28 events). The interaction was not significant when considering a CD4 cell count of 350/ μ L or less—either alone or in combination with AIDS-defining conditions or AIDS-related death.

Adherence to Intervention

Adherence with supplementation as obtained from monthly pill counts was 96% for the 5-year trial (24 months for each participant). Adherence with study visits was 89.84%.

Safety of Micronutrient Supplementation

Serious adverse events were monitored monthly using participant reports and confirmation of the reports with hospital records and primary physicians. Of the 79 serious adverse events reported by 78 participants over the duration of the trial (see eTable 2 in the Supplement), all adverse events were adjudicated as having remote relationship to the intervention by the study physicians, confirmed by the DSMB and all 3 IRBs as characterized using the Florida International University IRB form rating scale.²³ The adverse event reports caused by suicide attempts, assaults, fractures, and fatal motor vehicle crashes were not considered to be directly related to the intervention (eTable 2 in the Supplement). There were no notable differences in incidence of HIV-related and health-related adverse events among the 4 groups. Laboratory critical values for liver alanine aminotransferase, serum potassium, and cholesterol were not different between each of the study

groups and the placebo. There were 3 HIV-related deaths over the duration of the study, 1 in the placebo group and 2 in the group receiving the combined treatment of multivitamins plus selenium. Assessment of HIV viral load, metabolic profiles, and intercurrent events indicated that supplements were safe when compared with the placebo group. The study supplements were well tolerated by the participants, which contributed to the high level of adherence observed in this cohort.

Discussion

This randomized clinical trial of micronutrient supplementation was conducted for 24 months in adult men and women with HIV subtype C who were early in their HIV disease; they had a CD4 cell count greater than 350/ μ L (baseline median CD4 cell count of 420/ μ L; IQR, 336-550), were ART-naïve throughout the study, and had not yet experienced any clinical AIDS symptoms. A single supplement providing the combination of multivitamins with B vitamins, vitamins C and E, and selenium, as compared with placebo, administered early in HIV disease, reduced the risk of reaching a CD4 cell count of 250/ μ L or less in 2 years. The benefit was also evident with an earlier end point of a CD4 cell count of 350/ μ L or less, which is the current standard for providing ART in Botswana. Furthermore, the single supplement containing multivitamins plus selenium as compared with placebo reduced the risk of reaching the composite outcome of a CD4 cell count of 250/ μ L or less, the clinical manifestations of AIDS-defining conditions, or AIDS-related death. The single supplement containing multivitamins plus selenium as compared with placebo also reduced the composite outcome of a CD4 cell count of 350/ μ L or less, AIDS-defining conditions, or AIDS-related death, whichever occurred first. The effectiveness of the multivitamins without selenium as compared with placebo on the primary end point was similar in the initial analyses; however, after taking into account the interaction, the multivitamins-alone treatment was no longer significant for the primary end point or any of the secondary end points. Supplementation with selenium only also showed no effect on any of the end points measured. These results, however, demonstrate the effectiveness and safety of multivitamins and selenium when administered together in a single supplement in slowing HIV disease progression in the early stages of the disease prior to ART.

On June 30, 2013, the WHO revised their HIV treatment guidelines by recommending initiation of ART at a CD4 cell count of 500/ μ L or less; however, at the same time, the treatment progress update by WHO, UNAIDS, and UNICEF identified challenges in providing access to treatment for all eligible persons, as many of the most affected countries have limited resources to attain this standard.^{5,24} Because uncontrolled HIV replication results in immune activation and inflammation even in the early stages of HIV,²⁵ alternative interventions are needed, particularly early in HIV disease, when ART might not be available.

Micronutrient supplementation has shown benefit in slowing HIV disease progression or decreasing mortality previously; however, these studies were conducted either in late

stages of HIV disease^{10-12,26} or in pregnant women.¹³ Multivitamin and mineral supplementation compared with placebo for 3 months in our previous study in the United States¹¹ with 40 patients who had neuropathy, were late in HIV disease, and received stable ART significantly improved CD4 cell count but differed from the current study because in the previous study the patients were receiving ART and were in the late stage of HIV infection. Micronutrients provided to 481 patients with HIV subtype E without access to ART in Thailand reduced mortality, but only in patients in late stages with a CD4 cell count less than 100/ μ L.¹⁰ The difference between this Thai study and ours was that the Thai patients were in the late stage of HIV disease. Reduced mortality was also reported in 500 HIV-positive Zambians who received micronutrients as compared with placebo for 3.3 years; in contrast to our study, some of the Zambian patients had a low CD4 cell count and some started ART during the study.²⁷

Other previous clinical trials of micronutrient supplementation were conducted in patients in late stages of HIV infection who also had pulmonary tuberculosis (TB): among 213 HIV/TB co-infected patients in Tanzania, taking multivitamins and minerals compared with placebo resulted in significantly increased weight and reduced mortality.¹² Another clinical trial in 471 HIV/TB co-infected patients, also conducted in Tanzania, tested the efficacy of multivitamins compared with placebo. This study showed decreased risk of TB recurrence, increased CD4 cell count, and decreased incidence of peripheral neuropathy.²⁶ Supplementation with selenium alone compared with placebo for 9 months significantly reduced HIV viral load and indirectly improved CD4 cell count in our earlier study of drug users in the United States.¹⁵ This study, however, also differs from the current clinical trial, because a single nutrient was supplemented, all the participants were illicit drug users, and many were taking ART. These studies show a definitive benefit of micronutrient supplementation on HIV disease progression, particularly on CD4 cell count and survival in late stages of HIV infection; therefore, the uniqueness of the current clinical trial is its focus on HIV-infected patients in early stages of the disease (CD4 cell count $>350/\mu$ L at the beginning of the study) who were ART-naive throughout the study.

The largest study of micronutrient supplementation without provision of ART conducted to date enrolled 1078 pregnant women with HIV subtype C in Tanzania. Multivitamin supplementation compared with placebo significantly improved CD4 cell count and overall survival after 6 years of follow-up.¹³ However, these women were pregnant and in various stages of HIV disease with no ART available to them. In contrast, our participants in Botswana were adult men and women who were not pregnant; they initiated ART when their CD4 cell count reached 250/ μ L or less and at that point were no longer eligible to participate in our study. Thus, our clinical trial in Botswana demonstrates the benefits of micronutrient supplementation early in HIV disease, prior to ART, in reducing the risk of immune decline and morbidity and extends these findings to the early stages of the disease among the general adult population infected with HIV subtype C.

After examining the interaction effects between the multivitamin treatment and the selenium treatment in this facto-

rial trial, we found that after adjustment for age, sex, baseline CD4 cell count, baseline HIV viral load, and baseline BMI, the interaction was significant and favorable for both the primary and secondary end points. Although the treatment with multivitamins alone significantly reduced time to the primary end point in the initial analyses, when we considered the interaction, neither the multivitamins compared with placebo nor selenium compared with placebo were significant. Thus, the treatment with a single supplement containing multivitamins plus selenium was tested against placebo and found to significantly slow HIV disease progression for both the primary end point of a CD4 cell count of 250/ μ L or less and the secondary end point of the composite of a CD4 cell count of 250/ μ L or less, AIDS-defining conditions, or AIDS-related death, whichever occurred first. These results are consistent with our earlier report,¹¹ along with those of others,²⁸ that showed that selenium combined with multivitamins and other micronutrients resulted in an increase in CD4 cell count.

In addition, adding selenium to the multivitamin formula in the present study was safe, and the supplement containing multivitamins plus selenium as compared with placebo was superior to multivitamins alone or selenium alone when these study groups were compared with placebo in reducing risk of HIV disease progression. Combining the results of all supplemented groups, as compared with placebo, significantly prolonged time to HIV disease progression regarding immune outcomes and AIDS-defining conditions, showing that any micronutrient supplementation used in this trial was significantly better than placebo.²⁹ Moreover, when an earlier disease outcome (CD4 cell count $\leq 350/\mu$ L) was used, the supplement containing both multivitamins plus selenium provided benefit on delaying disease progression. This is important in regions where HIV subtype C is prevalent, because of evidence suggesting faster HIV disease progression with this subtype, as well as lack of ART availability in early stages of HIV disease.^{2-4,6,24} The evidence from our study presented herein supports provision of low-cost supplementation with multivitamins combined with selenium for HIV-infected individuals in early stages of the disease who are ART-naive to prolong adequate immune response and prevent AIDS-defining conditions.

Although the mechanisms through which the B vitamins, vitamins C and E, and selenium exert their immunomodulatory action are not fully elucidated, their antioxidant, immune-stimulatory, and viral expression properties have been documented.⁹ The B vitamins are necessary for immune function, including lymphocyte proliferation, mitogen responsiveness, NK cell cytotoxicity,^{30,31} and cell-mediated immunity.³² Vitamin C supplementation enhances proliferative responses of T and B lymphocytes in humans, affects phagocytic activity of neutrophils and macrophages,³³ and is associated with a lower rate of infections.³⁴ Vitamin E has a role as a free-radical scavenger,³⁵ regulates various cytokines, and is believed to play a role in inhibiting HIV-1 replication.³⁶ Depletion of selenium leads to impaired immune function, whereas supplementation with selenium restores or enhances immunologic activity,³⁷ and selenium deficiency increases the risk of HIV-related mortality.¹⁶ The antioxidant and antiviral prop-

erties of selenium have been documented in vitro.^{38,39} Selenium has been also found to be immunostimulatory in animal supplementation studies⁴⁰ and among elderly individuals.⁴¹

This investigation had a sound study design, adequate power, and modest drop-out rates. Adherence to the study supplements was adequate to draw conclusions from the intent-to-treat analysis. Our findings are generalizable to other HIV subtype C-infected cohorts in resource-limited settings where the provision of ART is being scaled up, rolled out, or not yet available to all in conditions similar to those in Botswana at the time of this study. The wide confidence intervals obtained indicate that the estimates of an effect were less precise and that the population value may be quite far from the sample estimate. The possible reasons are that the actual end points were less than we estimated, the sample size was relatively small, or both. Although the actual event rates were lower than originally estimated, the estimated effect size was much larger than originally projected. Thus, the study had adequate power to examine the hypotheses. The effect of supplementation on morbidity and mortality might be understated or overstated, because some of the patients who were lost to follow-up may have died or become acutely ill during the time of the study. However, there were no significant differences

in the percentage of participants lost to follow-up or in the number of deaths among the 4 study groups.

Conclusions

This clinical trial demonstrated that long-term (24-month) micronutrient supplementation of HIV-infected, ART-naive patients in the early stages of disease significantly delayed time to HIV disease progression and was safe. Multivitamins plus selenium provided as a single supplement significantly reduced the risk of reaching a CD4 cell count of 250/μL or less and a CD4 cell count of 350/μL or less. The combined outcome, which also considered AIDS-related morbidity and AIDS-related death, was also significantly reduced in the group that received multivitamins plus selenium. This evidence supports the use of specific micronutrient supplementation as an effective intervention in HIV-infected adults in early stages of HIV disease, significantly reducing the risk for disease progression in asymptomatic, ART-naive, HIV-infected adults. This reduced risk may translate into delay in the time when the HIV-infected patients experience immune dysfunction and into broader access to HIV treatment in developing countries.

ARTICLE INFORMATION

Author Contributions: Dr Baum had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

Study concept and design: Baum, Campa, Lai, Fawzi, Essex, Marlink.

Acquisition of data: Baum, Campa, Sales Martinez, Tsalaile, Burns, Farahani, van Widenfelt, Page, Bussmann, Moyo, Makhema, Thior, Essex, Marlink.

Analysis and interpretation of data: Baum, Campa, Lai, Li, Bussmann, Makhema, Essex, Marlink.

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Study supervision: Baum, Campa, Burns, Farahani, Bussman, Makhema, Thior, Essex, Marlink.

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