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Cholecalciferol Supplementation Does Not Affect the Risk of HIV Progression, Viral Suppression, Comorbidities, Weight Loss, and Depression among Tanzanian Adults Initiating Antiretroviral Therapy: Secondary Outcomes of a Randomized Trial

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ABSTRACT

Background: Observational studies suggest that blood concentrations of 25-hydroxyvitamin D [25(OH)D] are associated with morbidity, viral suppression, and mortality among adults living with HIV.

Objectives: We evaluated the effect of cholecalciferol (vitamin D_3) supplementation on the risk of HIV disease progression, HIV-1 viral suppression, comorbidities, weight change, and depression among HIV-infected individuals that were initiating antiretroviral therapy (ART) in Dar es Salaam, Tanzania.

Methods: We conducted a randomized, double-blind, placebo-controlled trial of vitamin D_3 supplementation among 4000 HIV-infected adult men and nonpregnant women initiating ART with insufficient serum 25(OH)D concentrations (<30 ng/mL). Participants were randomly assigned to receive either weekly 50,000-IU doses for 4 wk followed by daily 2000 IU vitamin D_3 until 1 y or a matching placebo regimen given in weekly followed by daily doses until 1 y. Participants were followed up at weekly visits for the first month followed by monthly visits thereafter. We conducted intent-to-treat analyses to assess the effect of vitamin D_3 supplementation on the secondary trial outcomes of HIV progression or death, viral suppression, comorbidities, change in BMI, >10% weight loss, incident wasting, and depression.

Results: During follow-up, 345 participants (17.2%) in the vitamin D_3 group and 371 participants (18.6%) in the placebo group experienced HIV disease progression or death and there was no difference in risk between groups (RR: 0.91; 95% CI: 0.79, 1.06). Vitamin D_3 supplementation did not affect the risk of an unsuppressed HIV-1 viral load (>1000 copies/mL) after 6 mo (RR: 1.10; 95% CI: 0.87, 1.41) and there was also no effect on change in BMI, risk of >10% weight loss, wasting, comorbidities, and depression (P values >0.05).

Conclusions: Vitamin D supplementation did not affect the risk of HIV progression, viral suppression, common morbidities, weight-related indicators, or depression among adults initiating ART in Tanzania. This trial was registered at clinicaltrials.gov as NCT01798680. *J Nutr* 2022;152:1983–1990.

Keywords: vitamin D, dietary supplements, micronutrients, HIV, antiretroviral therapy, body weight, depression, clinical trial, sub-Saharan Africa

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Introduction

Vitamin D deficiency is estimated to affect ~1 billion people globally with an additional 50% of the global population being vitamin D insufficient (1). In sub-Saharan Africa, it is estimated that 34.2% of the population is vitamin D deficient as defined by serum or plasma 25-hydroxyvitamin D [25(OH)D] concentrations < 20 ng/mL; however, there is a high degree of heterogeneity in the prevalence of deficiency among countries and populations within the region (2). Although vitamin D deficiency is common in the general population, people living with HIV may be at higher risk than the general population owing to factors related to HIV infection, including inflammation and comorbidities, as well as exposure to antiretroviral medications (3, 4). In a prior observational study in Tanzania, we found that 9.2% of adults initiating antiretroviral therapy (ART) had vitamin D deficiency [serum 25(OH)D < 20 ng/mL] and 43.6% were vitamin D insufficient [serum 25(OH)D = 20–30 ng/mL] (5).

Vitamin D is important for calcium homeostasis and bone health; however, more recent evidence suggests it may play a role in multiple extraskeletal conditions, including infectious and noncommunicable diseases (6). Observational studies have found that low vitamin D status is associated with an increased risk of acute respiratory infections (ARIs) and randomized trials have identified beneficial effects of vitamin D supplementation on the incidence of ARIs (7–9). In the context of HIV, observational studies have found vitamin D deficiency to be associated with an increased risk of HIV disease progression, increased risk of comorbidities, and mortality (6, 10, 11). A previous observational study conducted in HIV-infected individuals in Tanzania found that vitamin D deficiency at ART initiation was associated with an increased risk of mortality, pulmonary tuberculosis (PTB), and oral thrush along with wasting and weight loss (5, 12). Observational data have also shown associations between low vitamin D and increased risk of depression in the general population (13, 14) and depression is a common comorbidity among people living with HIV in sub-Saharan Africa (15, 16). To the best of our knowledge, no randomized trials to date have evaluated the effect of vitamin D supplementation on HIV disease progression, viral suppression, common comorbidities related to HIV or ART, weight change, and depression in sub-Saharan Africa.

We conducted a randomized, double-blind, placebo-controlled trial of cholecalciferol (vitamin D_3) supplementation in HIV-infected adults initiating ART in Tanzania. We previously reported no effect of vitamin D_3 supplementation on the coprimary trial outcomes of mortality and incidence of PTB (17). We also found that the vitamin D_3 supplementation regimen increased serum 25(OH)D concentrations as expected (17). In this study, we present the effect of vitamin D supplementation on secondary trial outcomes including HIV progression or death, viral suppression, morbidity, weight change, and depression.

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Abbreviations used: ARI, acute respiratory infection; ART, antiretroviral therapy; HSCL-25, Hopkins Symptom Checklist-25; PTB, pulmonary tuberculosis; ToV4, Trial of Vitamins—4; vitamin D_3, cholecalciferol; 25(OH)D, 25-hydroxyvitamin D.

Methods

The Trial of Vitamins—4 (ToV4) was a randomized, double-blind, placebo-controlled trial (NCT01798680) of vitamin D_3 supplementation conducted among 4000 HIV-infected adults that were initiating ART in Dar es Salaam, Tanzania. The trial protocol and the efficacy results for the primary trial outcomes of all-cause mortality and incidence of PTB have been reported elsewhere (17, 18). The trial was approved by the Harvard TH Chan School of Public Health Institutional Review Board (IRB13-0231), the Tanzanian National Health Research Ethics Sub-Committee (National Institute for Medical Research/HQ/R.8a/Vol. IX/1658), and the Tanzania Food and Drug Authority (Tanzania Medicines and Medical Devices Authority 13/CTR/0005/3). Written informed consent was obtained from all participants.

Briefly, the trial was conducted from February 2014 to March 2018 at 4 large HIV care and treatment centers. Participants were eligible for enrollment if they met the following inclusion criteria: *1*) adult men or women aged ≥18 y, *2*) documented HIV diagnosis, *3*) initiating ART at the time of randomization, *4*) insufficient serum 25(OH)D concentration <30 ng/mL at the screening visit, *5*) intended to stay in Dar es Salaam for ≥1 y after enrollment, and *6*) provided written informed consent. Exclusion criteria were *1*) pregnant women and *2*) enrolled in any other clinical trial. All study participants were provided with HIV care and treatment that adhered to Tanzanian national guidelines (18). Efavirenz–lamivudine–tenofovir was the first-line ART regimen during the trial. Participants received co-trimoxazole prophylaxis if their CD4 T-cell count was <200 cells/μL. Participants diagnosed with tuberculosis received directly observed therapy with a 6-mo short-course regimen comprising a 2-mo intensive phase of daily rifampicin–isoniazid–pyrazinamide–ethambutol followed by a 4-mo continuation phase of daily rifampicin and isoniazid. All participants also received psychological counseling and general nutritional counseling on diet for people living with HIV as the standard of care at each clinic visit. No additional food or nutritional supplements were provided as standard of care.

Screening and randomization

The screening visit for ToV4 was integrated into the HIV care and treatment program ART eligibility visit. CD4 T-cell count was measured at the ART eligibility visit (FACS-Calibur System, Becton Dickinson). Serum 25(OH)D concentrations were measured with a commercial enzyme immunoassay (Immunodiagnostics) and test procedures have been presented in the trial protocol (18). Participants who were eligible for randomization and consented to enrollment were randomly assigned in a 1:1 ratio to the vitamin D_3 supplementation or placebo group. The randomization list was computer-generated and stratified by study clinic. Participant allocation was completely concealed through the use of prelabeled, sequential participant identification numbers. The trial participants, study staff, and investigators were blinded to the randomly assigned groups.

The vitamin D_3 supplementation group received oral supplements containing 50,000 IU vitamin D_3 to be taken under direct observation at the randomization visit and once a week for the next 3 wk at study clinic visits (total of 4 doses, each 50,000 IU), followed by daily oral supplements containing 2000 IU vitamin D_3 to be taken at home starting at the fourth week until trial discharge at 1 y postrandomization. The trial protocol presents complete details on the rationale for the selection of the vitamin D_3 supplementation regimen (18). Briefly, the regimen was intended to increase 25(OH)D concentrations quickly and safely with the weekly 50,000 IU supplements during the first month of ART followed by maintenance of 25(OH)D concentrations thereafter with the daily 2000-IU supplements until trial discharge at 1 y. The placebo group received matching oral placebo supplements taken at identical times under direct observation for the first month and then daily from 1 mo until trial discharge. Both the vitamin D_3 and the placebo supplements were manufactured by Tishcon Corporation and were identical in appearance, taste, smell, and weight.

At the randomization visit, study participants received a full clinical examination and tuberculosis screening from study physicians and were assessed for WHO HIV disease stage. Participants suspected of having

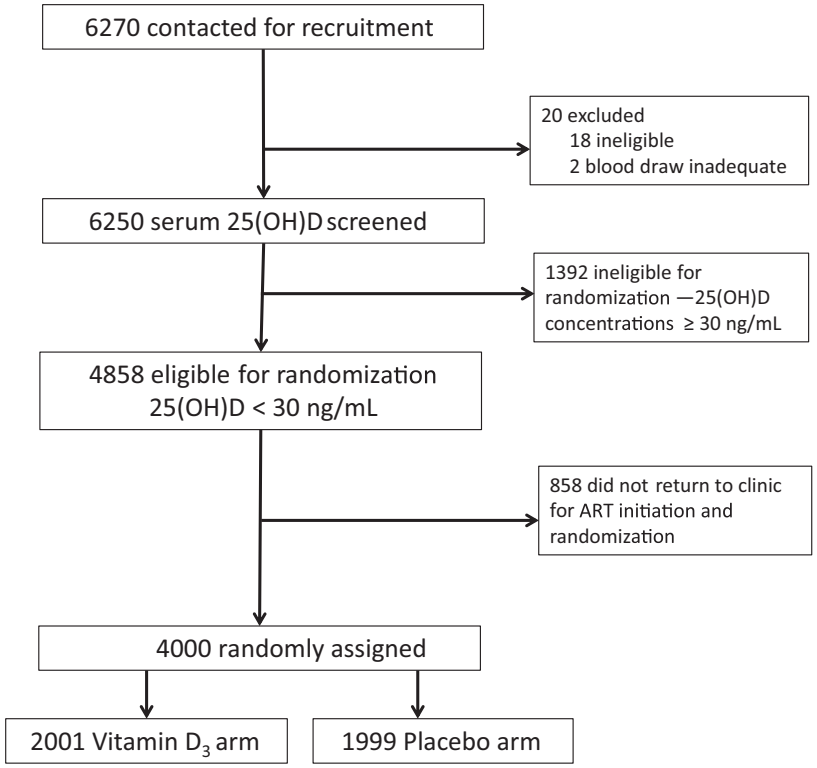

FIGURE 1 Trial flow diagram for recruitment of HIV-infected adult men and nonpregnant women with insufficient serum 25(OH)D concentrations (<30 ng/mL) at ART initiation. ART, antiretroviral therapy; vitamin D_3, cholecalciferol; 25(OH)D, 25-hydroxyvitamin D.

PTB had a chest X-ray and provided a sputum sample on the day PTB was suspected, were given a container for an early-morning sputum sample collected the next day, and had a third sputum sample collected at the clinic the following day. Sputum smears were stained using the Ziehl–Nielsen technique and examined for acid-fast bacilli. PTB was diagnosed when ≥1 sputum smear had acid-fast bacilli detected or if there were radiological features suggestive of PTB when sputum smears were negative. Study nurses assessed participant height and weight, then collected information on sociodemographic characteristics and self-reported symptoms during the last 28 d, and administered the Hopkins Symptom Checklist (HSCL-25) to assess depressive symptoms (19).

Participant follow-up procedures

All participants were followed at weekly clinic visits for 3 wk (weeks 1, 2, and 3) followed by monthly clinic visits starting at 1 mo until discharge at 12 mo postrandomization. At all clinic visits, physicians performed a full clinical examination, screened for PTB, and assessed WHO HIV disease stage. In addition, at all visits research nurses administered a questionnaire that included symptoms reported within the last 28 d, and measured participant weight. Nurses administered the HSCL-25 to assess depressive symptoms at the 6- and 12-mo visits. Nurses directly observed participants taking the trial regimen during the 3 weekly clinic visits and conducted pill counts at the monthly clinic visits when participants took the regimen at home. Adherence was quantified separately for weekly and monthly supplement doses. The adherence percentage for the directly observed weekly doses was calculated as the number of weekly doses taken by the participant divided by the number of weekly doses expected. The adherence percentage for the daily doses that was assessed by pill count was calculated as the number of daily doses taken by the participant divided by the number of daily doses expected.

Outcome definitions

HIV disease progression was defined as any increase in WHO HIV disease stage during follow-up from the WHO HIV stage at randomization, or death (20). Participant BMI (in kg/m^2) was calculated at randomization and all follow-up visits. We examined the effect of vitamin D_3 supplementation on mean BMI over follow-up, the incidence of wasting (BMI <18.5), and weight loss of >10% from randomization based on the definition of HIV-related wasting (21). At every study visit, participant symptoms in the past 28 d were assessed based on self-report of a list that included nausea or vomiting, cough, fever, diarrhea, oral thrush, skin rashes or lesions, neuropathy, and genital discharge or sores. An unsuppressed HIV-1 viral load was defined as >1000 copies/mL (22). The HSCL-25 was used to assess symptoms of depression and anxiety at 6 and 12 mo of follow-up. The HSCL-25 has previously been validated among Tanzanian women living with HIV and, based on the validation study results, we defined symptoms consistent with depression as an average score >1.06 for 8 questions (23). In the validation study, this cutoff demonstrated 88% sensitivity and 89% specificity compared with a Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition (DSM-IV) diagnosis of major depressive disorder. Nevertheless, this cutoff has not been validated among men living with HIV in Tanzania and therefore we also assessed HSCL-25 scores continuously and conducted a sensitivity analysis among all participants that defined symptoms consistent with depression using the standard HSCL-25 cutoff of 1.75 (19).

Statistical analysis

An intent-to-treat analysis was used as the analytic strategy for all analyses. For time-to-event analysis of HIV disease progression, >10% weight loss from ART initiation, and incident wasting, the log-rank test was used to evaluate differences between the treatment groups. Proportional hazard regression models were also used to produce HRs. The effect of vitamin D_3 supplementation on BMI at monthly clinic visits was examined using linear mixed-effects models with a random intercept, a compound symmetric covariance structure, and robust SEs. We examined the effect of vitamin D_3 supplementation on postrandomization symptoms reported to nurses at monthly clinic visits (repeated events) using generalized estimating equations with an exchangeable working covariance and the log link to produce population-averaged RR estimates (24). Log-binomial regression models were used to assess the RRs of the binomial outcomes

TABLE 1 Baseline characteristics of HIV-infected adult men and nonpregnant women with insufficient serum 25(OH)D concentrations (<30 ng/mL) at ART initiation enrolled in the trial, by vitamin D_3 and placebo treatment groups[1]

	Vitamin D_3 ($n = 2001$)	Placebo ($n = 1999$)
Sex		
Female	1367 (68)	1368 (68)
Male	634 (32)	631 (32)
Age, y	38.6 ± 9.8	38.8 ± 10.0
Education		
No formal education	308 (15)	327 (16)
Primary	1294 (64)	1294 (65)
Secondary/advanced	398 (20)	377 (19)
BMI, kg/m^2		
<18.5	440 (22)	404 (20)
18.5–24.9	1038 (52)	1064 (53)
≥25.0	521 (26)	531 (27)
CD4 T-cell count, cells/μL		
<200	866 (43)	845 (42)
200–349	461 (23)	445 (22)
350–499	300 (15)	333 (17)
≥500	284 (14)	178 (14)
Missing	90 (5)	98 (5)
WHO HIV disease stage		
I/II	744 (37)	760 (38)
III	1161 (58)	1143 (57)
IV	96 (5)	96 (5)
Baseline pulmonary tuberculosis	189 (10)	175 (9)
HSCL-25 score consistent with depression	982 (49)	953 (48)
ART regimen		
Efavirenz/lamivudine/tenofovir	1940 (97)	1943 (97)
Other ART regimen	61 (3)	56 (3)
25(OH)D concentrations at screening visit, ng/mL		
Insufficient (20.0–30.0)	955 (48)	972 (49)
Moderate deficiency (10.0–19.9)	920 (46)	865 (43)
Severe deficiency (0–9.9)	126 (6)	162 (8)

[1] $n = 4000$. Values are n (%) or mean ± SD. ART, antiretroviral therapy; HSCL-25, Hopkins Symptom Checklist-25; vitamin D_3, cholecalciferol; 25(OH)D, 25-hydroxyvitamin D.

of symptoms consistent with depression at 6 and 12 mo of follow-up and unsuppressed (>1000 copies/mL) and detectable viral load (>50 copies/mL) after 6 mo of ART. Linear regression models with a robust empirical variance structure were used to analyze continuous HSCL-25 scores. All models included a fixed effect for study clinic to account for stratified randomization. Sensitivity analyses that adjusted for baseline 25(OH)D concentration and CD4 T-cell count, which showed some degree of imbalance between treatment groups based on a $P < 0.20$, were conducted (17). All statistical analyses were done with SAS version 9.3 (SAS Institute Inc., Cary, NC).

Results

Figure 1 presents the trial flow diagram. A total of 6250 HIV-infected individuals initiating ART consented to serum 25(OH)D screening, of which 4848 (77.7%) had 25(OH)D concentrations <30 ng/mL and were eligible for randomization. Among the eligible individuals, 4000 participants were enrolled and randomly assigned in the trial (2001 to vitamin D_3 and 1999 to placebo). The baseline characteristics were similar between the randomly assigned groups (Table 1). The percentage of trial participants with baseline 25(OH)D concentrations that indicated vitamin D insufficiency (20–30 ng/mL) was 48.2%, 44.6% of participants had mild vitamin D deficiency [25(OH)D = 10–20 ng/mL], and only 7.2% of participants had 25(OH)D concentrations that indicated moderate or severe vitamin D deficiency (<10 ng/mL).

During the trial, 415 deaths (10.4%) were recorded, 19 (0.5%) participants withdrew consent to continue participating, and 94 (2.4%) women became pregnant and were censored. Loss to follow-up was low in both arms (2.1% in the vitamin D_3 group and 1.7% in the placebo group). Adherence to the trial supplements was high in both groups; 81.6% and 81.4% of participants took all the weekly doses in the vitamin D_3 and placebo groups, respectively. The mean adherence for the daily regimen supplements was 80.8% for the vitamin D_3 group and 80.6% for the placebo group.

HIV progression or death

During the trial, 345 of 2001 participants (17.2%) in the vitamin D_3 and 371 of 1999 participants (18.6%) in the placebo group experienced HIV disease progression or death and there was no difference in risk between the randomly assigned groups ($P = 0.22$) (Table 2).

HIV-1 viral suppression

The proportion of participants with an unsuppressed viral load (>1000 copies/mL) after 6 mo of ART was 12.4% in the vitamin D_3 group and 11.3% in the placebo group and there was no difference in risk between the randomly assigned groups (RR: 1.10; 95% CI: 0.87, 1.41) (Table 2).

Morbidities

Table 3 presents the effect of vitamin D_3 supplementation on participant-reported morbidities at monthly follow-up visits. There was no effect of vitamin D_3 on the risk of nausea or vomiting, cough, fever, diarrhea, oral thrush, skin rashes or lesions, neuropathy, and genital discharge or sores during follow-up (P values >0.05).

Weight change, >10% weight loss, and incident wasting

The mean BMI of participants increased from ART initiation to 12 mo of treatment in both randomly assigned groups (Figure 2). The mean ± SD BMIs at baseline and the 12-mo endline visit were 22.6 ± 5.7 and 24.6 ± 5.2 in the vitamin D_3 group and 22.6 ± 5.2 and 24.6 ± 5.3 in the placebo group, respectively. There was no difference in change in BMI over the course of follow-up between the randomly assigned groups (P value for difference in trajectory = 0.99). There was also no difference in the risk of >10% weight loss from baseline (HR: 1.05; 95% CI: 0.87, 1.28) or incident wasting (HR: 0.84; 95% CI: 0.66, 1.08) between the randomly assigned groups (Table 2).

Depression

There was no effect of vitamin D_3 on the risk of symptoms consistent with depression at 6 and 12 mo of follow-up using the Tanzania-adapted HSCL-25 cutoffs (P values >0.05) (Table 2). In a sensitivity analysis, there was also no effect on symptoms consistent with depression using the standard HSCL-25 cutoff at 6 mo (RR: 1.07; 95% CI: 0.94, 1.23) or 12 mo of follow-up (RR: 0.83; 95% CI: 0.61, 1.12).

TABLE 2 Effect of vitamin D_3 supplementation on HIV disease progression or death, viral suppression, nutritional outcomes, and depression as compared with placebo among HIV-infected adult men and nonpregnant women with insufficient serum 25-hydroxyvitamin D concentrations (<30 ng/mL) at ART initiation enrolled in the trial[1]

	Vitamin D_3 *n* events/ *n* participants at risk (%)	Placebo *n* events/ *n* participants at risk (%)	HR (95% CI)	*P* value
HIV disease progression or death[2]	345 / 2001 (17.2)	371 / 1999 (18.6)	0.91 (0.79, 1.06)	0.22
Unsuppressed viral load >1000 copies/mL after 6 mo of ART[3]	123 / 993 (12.4)	107 / 951 (11.3)	1.10 (0.87, 1.41)	0.43
>10% weight loss from ART initiation[4]	213 / 1844 (11.6)	204 / 1847 (11.0)	1.05 (0.87, 1.28)	0.60
Incident wasting (BMI < 18.5 kg/m^2)[5]	113 / 1559 (7.3)	137 / 1595 (8.6)	0.84 (0.66, 1.08)	0.17
HSCL-25 scores consistent with depression at 6 mo of ART[6]	368 / 1385 (26.6)	395 / 1387 (28.5)	0.94 (0.83, 1.06)	0.29
HSCL-25 scores consistent with depression at 12 mo of ART[6]	228 / 1232 (18.5)	242 / 1261 (19.2)	0.96 (0.82, 1.12)	0.60

[1] ART, antiretroviral therapy; HSCL-25, Hopkins Symptom Checklist-25; vitamin D_3, cholecalciferol.
[2] HIV disease progression defined as any increase in WHO HIV disease stage or death.
[3] Among participants who had HIV-1 viral load assessed after 6 mo of ART.
[4] Among participants who had baseline weight assessed and ≥1 postbaseline weight assessment.
[5] Among participants who had baseline weight assessed, a baseline BMI ≥18.5 kg/m^2, and ≥1 postbaseline weight assessment.
[6] Tanzania-adapted HSCL-25 cutoff used to define depression.

Sensitivity analyses

In sensitivity analyses, there was no change in the findings after adjusting for potential baseline imbalances in 25(OH)D and CD4 T-cell count between the treatment groups (**Supplemental Tables 1** and **2**).

Discussion

In this randomized, double-blind, placebo-controlled trial, vitamin D_3 supplementation did not affect secondary trial outcomes of HIV disease progression or death, viral load suppression, the incidence of morbidities, weight change, wasting, or depressive symptoms among adults living with HIV that were initiating ART in Tanzania.

We found no effect of vitamin D_3 supplementation on the composite outcomes of HIV disease progression or death. These findings are consistent with our null findings on the coprimary trial outcomes of all-cause mortality and PTB (17). Overall, our trial findings are not consistent with our prior observational cohort studies in Tanzania as well as studies from high-income settings, which have found low concentrations of vitamin D to be associated with increased risk of mortality, disease progression, and poor treatment outcomes among people living with HIV (5, 6, 11). In a prior observational cohort study in Tanzania in adults initiating ART, we found that vitamin D deficiency defined by 25(OH)D concentrations <20 ng/mL at ART initiation was associated with twice the risk of mortality as compared with individuals who were vitamin D sufficient [25(OH)D concentrations >30 ng/mL] (5). Further, in the EuroSIDA cohort study in people living with HIV in Europe, Israel, and Argentina, individuals with 25(OH)D concentrations <12 ng/mL had an increased risk of mortality or AIDS events as compared with individuals with 25(OH)D concentrations >12 ng/mL (11). In addition, we found no effect of vitamin D_3 supplementation on viral suppression, which is an important risk factor for HIV disease progression or death. This finding is also in contrast to several observational studies that have found an association between low vitamin D and greater HIV-1 viral load (25, 26). In addition, a small randomized trial of vitamin D_3 supplementation among 58 children and young adults living with HIV in the United States found no effect on HIV-1 RNA detection but found some indication that vitamin D reduced absolute viral concentrations among individuals with detectable viral loads (27). As a result, our randomized trial results are not aligned with evidence from observational studies and may be due to low concentrations of vitamin D in blood in observational studies acting as a marker of disease severity or

TABLE 3 Effect of vitamin D_3 supplementation on participant-reported morbidities during the prior month collected at monthly follow-up clinic visits as compared with placebo among HIV-infected adult men and nonpregnant women with insufficient serum 25-hydroxyvitamin D concentrations (<30 ng/mL) at antiretroviral therapy initiation enrolled in the trial[1]

	Vitamin D_3 *n* visits morbidity reported/ *n* follow-up clinic visits (%)	Placebo *n* visits morbidity reported/ *n* follow-up clinic visits (%)	RR (95% CI)	*P* value
Weakness or fatigue	593 / 15,049 (3.9)	598 / 14,996 (4.0)	0.98 (0.85, 1.11)	0.71
Nausea or vomiting	343 / 15,049 (2.3)	305 / 14,996 (2.0)	1.11 (0.92, 1.34)	0.27
Cough	489 / 15,049 (3.3)	490 / 14,996 (3.3)	0.99 (0.85, 1.16)	0.89
Fever	460 / 15,049 (3.1)	436 / 14,996 (2.9)	1.04 (0.89, 1.21)	0.65
Diarrhea	181 / 15,049 (1.2)	157 / 14,996 (1.1)	1.13 (0.87, 1.47)	0.35
Oral thrush	59 / 15,049 (0.4)	48 / 14,996 (0.3)	1.21 (0.81, 1.80)	0.35
Skin rashes or lesions	527 / 15,049 (3.5)	479 / 14,996 (3.2)	1.09 (0.93, 1.27)	0.29
Neuropathy	262 / 15,049 (1.7)	242 / 14,996 (1.6)	1.06 (0.84, 1.34)	0.61
Genital discharge or sores	260 / 15,049 (1.7)	245 / 14,996 (1.6)	1.05 (0.83, 1.31)	0.70

[1] Vitamin D_3, cholecalciferol.

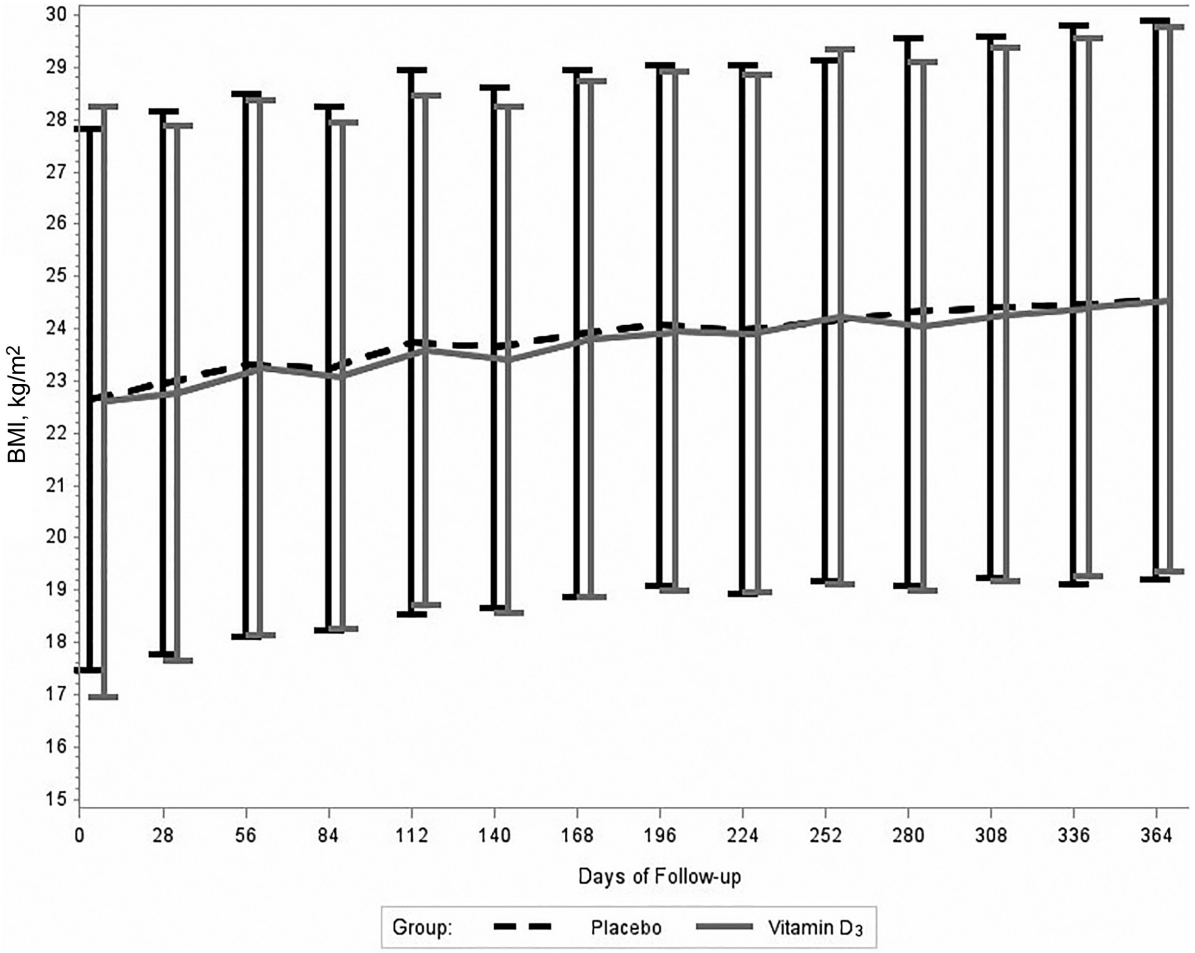

FIGURE 2 Effect of vitamin D_3 supplementation on participant BMI at monthly follow-up clinic visits as compared with placebo among HIV-infected adult men and nonpregnant women with insufficient serum 25-hydroxyvitamin D concentrations (<30 ng/mL) at antiretroviral therapy initiation enrolled in the trial. Values presented are means with bars indicating SDs. *P* value for difference in BMI trajectory between treatment groups = 0.99. Vitamin D_3, cholecalciferol.

other factors rather than indicating a causal effect of vitamin D on HIV disease progression or viral suppression. Therefore, the association of vitamin D status with HIV disease progression and viral load suppression in observational studies may be attributable to unmeasured or residual confounding or other biases.

We also found no effect of vitamin D_3 supplementation on participant-reported morbidities. In a prior cohort study in Tanzania, we found that vitamin D deficiency at ART initiation was associated with increased risk of oral thrush, but there was no association with participant-reported cough, diarrhea, rash, neuropathy, nausea or fatigue, or genital ulcers (12). As a result, the null findings in the trial are partially aligned with the observational data. Nevertheless, randomized trials of vitamin D supplementation, conducted among individuals not living with HIV, have found an overall protective effect on the incidence of ARIs (9). As a result, the effect of vitamin D on ARIs and other comorbidities may differ for people living with HIV, whose risk of ARIs and other opportunistic infections is largely associated with the degree of immunosuppression (28).

We also found no effect of vitamin D_3 supplementation on nutritional outcomes including change in BMI and incidence of >10% weight loss or wasting. In a prior observational cohort study in Tanzania, we found that vitamin D deficiency at ART initiation was associated with increased risk of >10% weight loss from ART initiation and wasting. It was hypothesized that vitamin D supplementation may reduce the incidence of comorbidities, including PTB, which are in turn associated with weight loss. In our randomized trial, we previously reported no overall effect of vitamin D supplementation on the incidence of PTB and we also found no effect on the incidence of other comorbidities or weight change in this study. Nevertheless, there is some observational evidence that vitamin D supplementation may play a role in weight loss or obesity in populations not affected by HIV, but randomized trial evidence is needed (29).

In addition, we found no effect of vitamin D_3 supplementation on the risk of depression at 6 or 12 mo after ART initiation. Similar to other outcomes, prospective observational studies have found low serum 25(OH)D to be a risk factor for depression but this relation has not been replicated in randomized trials (14). Our null findings are aligned with the results of the recent large VITAL-DEP (VITamin D and OmegA-3 TriaL-Depression Endpoint Prevention) trial conducted among a general population of US men and women over the age of 50 y that found no effect of vitamin D supplementation on the incidence or recurrence of depression as well as depressive symptoms (30). Our findings are also consistent with other randomized trials of vitamin D_3 supplementation that have found no effect on depressive symptoms (31, 32). Therefore, the role of vitamin D supplementation in depression remains

unclear, but the current evidence from randomized trials suggests limited to no effect.

There are several limitations to our study. Less than 5% of participants had 25(OH)D concentrations <10 ng/mL at baseline and therefore our findings may not be generalizable to populations with a greater prevalence of severe vitamin D deficiency. In addition, there was likely some degree of misclassification of participant-reported morbidities that are likely nondifferential to the randomly assigned treatment group and therefore would bias estimates toward the null. Last, we used the Tanzania-adapted HSCL-25 cutoff which was validated among pregnant women living with HIV, and as a result there may have been some degree of misclassification for men and nonpregnant women in our study (23). Nevertheless, there was no effect of vitamin D_3 on depression in a sensitivity analysis of depression defined by the standard HSCL-25 cutoff.

In conclusion, vitamin D_3 supplementation did not affect the risk of HIV progression or death, viral suppression, comorbidities, weight change, or depression outcomes. Taken together with the null findings of the trial on the primary outcomes of death and incidence of PTB, our findings do not support the routine use of vitamin D_3 supplementation for adults living with HIV initiating ART during the first year of ART. Alternative strategies are needed to improve treatment outcomes and mental health for adults living with HIV in Tanzania and similar settings.

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Data Availability

Reasonable requests for de-identified individual participant data described in this article may be directed to the corresponding author. Data sharing requests will be subject to ethical approval and data transfer agreements.

References

1. Nair R, Maseeh A. Vitamin D: the "sunshine" vitamin. J Pharmacol Pharmacother 2012;3(2):118–26.
2. Mogire RM, Mutua A, Kimita W, Kamau A, Bejon P, Pettifor JM, et al. Prevalence of vitamin D deficiency in Africa: a systematic review and meta-analysis. Lancet Glob Health 2020;8(1):e134–e42.
3. Brown TT, McComsey GA. Association between initiation of antiretroviral therapy with efavirenz and decreases in 25-hydroxyvitamin D. Antivir Ther 2010;15(3):425–9.
4. Cozzolino M, Vidal M, Arcidiacono MV, Tebas P, Yarasheski KE, Dusso AS. HIV-protease inhibitors impair vitamin D bioactivation to 1,25-dihydroxyvitamin D. AIDS 2003;17(4):513–20.
5. Sudfeld CR, Wang M, Aboud S, Giovannucci EL, Mugusi FM, Fawzi WW. Vitamin D and HIV progression among Tanzanian adults initiating antiretroviral therapy. PLoS One 2012;7(6):e40036.
6. Havers F, Smeaton L, Gupte N, Detrick B, Bollinger RC, Hakim J, et al. 25-Hydroxyvitamin D insufficiency and deficiency is associated with HIV disease progression and virological failure post-antiretroviral therapy initiation in diverse multinational settings. J Infect Dis 2014;210(2):244–53.
7. Jolliffe DA, Griffiths CJ, Martineau AR. Vitamin D in the prevention of acute respiratory infection: systematic review of clinical studies. J Steroid Biochem Mol Biol 2013;136:321–9.
8. Laaksi I, Ruohola J-P, Tuohimaa P, Auvinen A, Haataja R, Pihlajamäki H, et al. An association of serum vitamin D concentrations < 40 nmol/L with acute respiratory tract infection in young Finnish men. Am J Clin Nutr 2007;86:714–17.
9. Martineau AR, Jolliffe DA, Hooper RL, Greenberg L, Aloia JF, Bergman P, et al. Vitamin D supplementation to prevent acute respiratory tract infections: systematic review and meta-analysis of individual participant data. BMJ 2017;356:i6583.
10. Vescini F, Cozzi-Lepri A, Borderi M, Re MC, Maggiolo F, De Luca A, et al. Prevalence of hypovitaminosis D and factors associated with vitamin D deficiency and morbidity among HIV-infected patients enrolled in a large Italian cohort. J Acquir Immune Defic Syndr 2011;58(2):163–72.
11. Viard JP, Souberbielle JC, Kirk O, Reekie J, Knysz B, Losso M, et al. Vitamin D and clinical disease progression in HIV infection: results from the EuroSIDA study. AIDS 2011;25(10):1305–15.
12. Sudfeld CR, Giovannucci EL, Isanaka S, Aboud S, Mugusi FM, Wang M, et al. Vitamin D status and incidence of pulmonary tuberculosis, opportunistic infections, and wasting among HIV-infected Tanzanian adults initiating antiretroviral therapy. J Infect Dis 2013;207(3):378–85.
13. Li H, Sun D, Wang A, Pan H, Feng W, Ng CH, et al. Serum 25-hydroxyvitamin D levels and depression in older adults: a dose–response meta-analysis of prospective cohort studies. Am J Geriatr Psychiatry 2019;27(11):1192–202.
14. Parker GB, Brotchie H, Graham RK. Vitamin D and depression. J Affect Disord 2017;208:56–61.
15. Bernard C, Dabis F, de Rekeneire N. Prevalence and factors associated with depression in people living with HIV in sub-Saharan Africa: a systematic review and meta-analysis. PLoS One 2017;12(8):e0181960.
16. Regan M, Muhihi A, Nagu T, Aboud S, Ulenga N, Kaaya S, et al. Depression and viral suppression among adults living with HIV in Tanzania. AIDS Behav 2021;25(10):3097–105.
17. Sudfeld CR, Mugusi F, Muhihi A, Aboud S, Nagu TJ, Ulenga N, et al. Efficacy of vitamin D_3 supplementation for the prevention of pulmonary tuberculosis and mortality in HIV: a randomised, double-blind, placebo-controlled trial. Lancet HIV 2020;7(7):e463–e71.
18. Sudfeld CR, Mugusi F, Aboud S, Nagu TJ, Wang M, Fawzi WW. Efficacy of vitamin D_3 supplementation in reducing incidence of pulmonary tuberculosis and mortality among HIV-infected Tanzanian adults initiating antiretroviral therapy: study protocol for a randomized controlled trial. Trials 2017;18(1):66.
19. Derogatis LR, Lipman RS, Rickels K, Uhlenhuth EH, Covi L. The Hopkins Symptom Checklist (HSCL): a self-report symptom inventory. Behav Sci 1974;19(1):1–15.
20. World Health Organization. Antiretroviral therapy for HIV infection in adults and adolescents: recommendations for a public health approach: 2010 revision. Geneva, Switzerland: WHO; 2010.
21. Polsky B, Kotler D, Steinhart C. HIV-associated wasting in the HAART era: guidelines for assessment, diagnosis, and treatment. AIDS Patient Care STDs 2001;15(8):411–23.
22. World Health Organization. Consolidated guidelines on the use of antiretroviral drugs for treating and preventing HIV infection: recommendations for a public health approach. Geneva, Switzerland: WHO; 2016.
23. Kaaya SF, Fawzi MC, Mbwambo JK, Lee B, Msamanga GI, Fawzi W. Validity of the Hopkins Symptom Checklist-25 amongst HIV-positive pregnant women in Tanzania. Acta Psychiatr Scand 2002;106(1):9–19.
24. Diggle PJ, Heagerty P, Liang K-Y, Zeger S. Analysis of longitudinal data. Oxford, United Kingdom: Oxford University Press; 2002.
25. Flauzino T, Simao ANC, de Almeida ERD, Morimoto HK, Oliveira SR, Alfieri DF, et al. Association between vitamin D status, oxidative stress biomarkers and viral load in human immunodeficiency virus type 1 infection. Curr HIV Res 2017;15(5):336–44.
26. Bearden A, Abad C, Gangnon R, Sosman JM, Binkley N, Safdar N. Cross-sectional study of vitamin D levels, immunologic and virologic outcomes in HIV-infected adults. J Clin Endocrinol Metab 2013;98(4):1726–33.

27. Stallings VA, Schall JI, Hediger ML, Zemel BS, Tuluc F, Dougherty KA, et al. High-dose vitamin D_3 supplementation in children and young adults with HIV: a randomized, placebo-controlled trial. Pediatr Infect Dis J 2015;34(2):e32–e40.
28. Benito N, Moreno A, Miro JM, Torres A. Pulmonary infections in HIV-infected patients: an update in the 21st century. Eur Respir J 2012;39(3):730–45.
29. Pourshahidi LK. Vitamin D and obesity: current perspectives and future directions. Proc Nutr Soc 2015;74(2):115–24.
30. Okereke OI, Reynolds CF, 3rd, Mischoulon D, Chang G, Vyas CM, Cook NR, et al. Effect of long-term vitamin D_3 supplementation vs placebo on risk of depression or clinically relevant depressive symptoms and on change in mood scores: a randomized clinical trial. JAMA 2020;324(5):471–80.
31. de Koning EJ, Lips P, Penninx B, Elders PJM, Heijboer AC, den Heijer M, et al. Vitamin D supplementation for the prevention of depression and poor physical function in older persons: the D-Vitaal study, a randomized clinical trial. Am J Clin Nutr 2019;110(5):1119–30.
32. Bot M, Brouwer IA, Roca M, Kohls E, Penninx B, Watkins E, et al. Effect of multinutrient supplementation and food-related behavioral activation therapy on prevention of major depressive disorder among overweight or obese adults with subsyndromal depressive symptoms: the MooDFOOD randomized clinical trial. JAMA 2019;321(9):858–68.