Vitamin D Deficiency and Metabolism in HIV-Infected and HIV-Uninfected Men in the Multicenter AIDS Cohort Study

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Abstract

We evaluated associations of serum 25-hydroxyvitamin D (25[OH]D) and 1,25-dihydroxyvitamin D (1,25[OH]₂D) levels in a cohort of HIV-infected and HIV-uninfected men at risk for infection in the United States. Stored samples collected between 1999 and 2008 were tested for vitamin D metabolites between 2014 and 2015. Vitamin D deficiency was defined as a serum concentration of 25[OH]D <20 ng/ml. Multivariate models were used to assess associations of various demographic and clinical factors with vitamin D status. HIV-infected men on effective antiretroviral therapy ($n=640$) and HIV-uninfected men ($n=99$) had comparable levels of 25[OH]D and 1,25[OH]₂D, and prevalences of vitamin D deficiency were 41% in HIV-infected and 44% in HIV-uninfected men, respectively. Self-reported black or other non-white race, obesity, and normal kidney function were significant predictors of vitamin D deficiency regardless of HIV serostatus. Lower CD4⁺ T cell count was associated with vitamin D deficiency in HIV-infected men, while current ritonavir use was protective. Self-reported black race was the only factor significantly associated with higher levels of 1,25[OH]₂D (vs. whites; $\beta=4.85$ pg/ml, $p=.003$). Levels of 1,25[OH]₂D and 25[OH]D were positively correlated in HIV-infected men ($\beta=0.32$ pg/ml, $p<.001$), but not in uninfected men ($\beta=-0.09$ pg/ml, $p=.623$; $p<.05$ for interaction). Vitamin D deficiency was prevalent regardless of HIV serostatus in this cohort, suggesting that HIV infection did not confer additional risk of deficiency in this cohort of well-treated HIV-infected men. However, HIV infection and race may have implications for vitamin D metabolism and 1,25[OH]₂D levels.

Keywords: vitamin D, vitamin D deficiency, 25[OH]D, 1,25[OH]₂D, HIV-infected, HIV-uninfected

Introduction

VITAMIN D PLAYS A VITAL ROLE in calcium homeostasis and other physiological processes in human health.¹ Vitamin D deficiency is associated with an increased risk for numerous comorbidities, including osteoporosis, frailty, metabolic syndrome, cardiovascular disease, diabetes, cognitive function, and certain cancers, in diverse populations.^{2–8} As the life expectancy of HIV-infected persons has increased due to

highly active antiretroviral therapy (HAART), a higher risk of comorbid diseases has been noted compared to the general population, potentially the result of immune system dysfunction.⁹ Vitamin D deficiency may be particularly deleterious in this population as it might interfere with immune restoration following HAART and exacerbate HIV-related disease complications.¹⁰ A positive association between vitamin D levels and CD4⁺ T cell count has been repeatedly noted¹¹ and a lower absolute CD4⁺ T cell recovery following HAART was found

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among HIV infected with vitamin D deficiency.¹² Vitamin D deficiency has also been linked to the occurrence of opportunistic infections, wasting, HIV disease progression, and death among HIV-untreated individuals.^{10,13,14}

Vitamin D deficiency, often defined clinically as serum 25-hydroxyvitamin D (25[OH]D) <20 ng/ml,¹⁵ is common in both the general population and the HIV-infected population.^{2,16} The SUN study reported a 30% prevalence of vitamin D deficiency among 672 HIV-infected middle-age participants compared with 39% in the US adult population adjusted for age, race, and sex.¹⁶ However, the WIHS found a 60% prevalence of vitamin D deficiency in 1268 HIV-infected mostly minority women using the same definition.¹⁷

25[OH]D is the vitamin D metabolite standardly used to assess nutritional vitamin D status.¹⁸ 1,25-dihydroxyvitamin D (1,25[OH]₂D) is the active metabolite of vitamin D, but is infrequently measured due to its short half-life and tight regulation within the human body.¹ Measuring 1,25[OH]₂D in addition to 25[OH]D could help identify disorders of 1 α -hydroxylation, the main driver for conversion of 25[OH]D into its active form in the kidney, or abnormal production of 1,25[OH]₂D in extrarenal tissues (i.e., sarcoidosis, hematologic malignancies, and systemic infections).^{18,19}

Established risk factors for 25[OH]D deficiency include low sun exposure, black race, winter season, and low dietary intake of vitamin D.^{13,14,20} Although these risk factors are well established in the general population, few studies have compared the risk factor profile for deficiency between HIV-infected and HIV-uninfected groups.¹⁶ Among HIV-infected persons, certain antiretroviral drugs may contribute to vitamin D deficiency through interference with vitamin D metabolism.²¹ Furthermore, the inflammatory milieu associated with proinflammatory cytokines may affect vitamin D metabolism.²² Yet, little is known about the distribution of 1,25[OH]₂D levels among HIV-infected compared to HIV-uninfected individuals and the degree to which chronic HIV infection or antiretroviral therapies impact the production of 1,25[OH]₂D.

In this study, we examined factors associated with 25[OH]D and 1,25[OH]₂D levels in a sample of HIV-infected and HIV-uninfected men from the Multicenter AIDS Cohort Study (MACS).

Materials and Methods

Study sample

The MACS is an ongoing prospective cohort study of HIV-infected and HIV-uninfected men who have sex with men that started in 1984. The study has followed 7,317 men in four sites in the United States (Baltimore, MD; Chicago, IL; Pittsburgh, PA; and Los Angeles, CA).²³ Semiannual data are collected through interviewer-administered questionnaires, physical examinations, and laboratory testing of biological specimens.

Using stored specimens, the MACS vitamin D ancillary study measured 25[OH]D and 1,25[OH]₂D pre-HAART and post-HAART concentrations in 640 HIV-infected men, with the aim of evaluating the impact of viral suppression on the change in vitamin D status. Post-HAART vitamin D measurements were taken after 1 year to allow for viral suppression to occur. The study also included a small comparison group of 99 HIV-uninfected men with available serum samples, matched to the HIV-infected men on sam-

pling time points. The MACS and vitamin D substudy were approved by the institutional review boards at all sites and the study has been performed in accordance with the World Medical Association Declaration of Helsinki. Written informed consent from all study participants has been obtained.

For the present study examining correlates of vitamin D status and metabolism, we included all post-HAART 25[OH]D and 1,25[OH]₂D measurements from the 640 HIV-infected men and corresponding measurements in the 99 HIV-uninfected men. Post-HAART measurements were within 3.5 years of HAART initiation.

Measurement of vitamin D

Serum samples for present study were collected and stored in the repository between 1999 and 2008, and then assayed for vitamin D metabolites between October 2014 and February 2015, using immunoaffinity liquid chromatography–tandem mass spectrometry. Testing was completed at the University of Washington, yielding sensitive (limit of quantitation [LOQ] <1 ng/ml) and precise (coefficient of variation [CV] <5%) measurement of 25[OH]D, and sensitive (LOQ <4 pg/ml) and precise (CV <12%) measurement of 1,25[OH]₂D from the same assay.^{24,25} For every batch of specimens, two quality control samples were tested along with experimental samples. Vitamin D metabolites measured included 25[OH]D₃, 25[OH]D₂, 1,25[OH]₂D₃, and 1,25[OH]₂D₂. Total 25[OH]D and 1,25[OH]₂D levels were obtained by adding together their D₂ and D₃ forms, respectively. Consistent with previous studies,²⁶ we defined vitamin D deficiency as total 25[OH]D <20 ng/ml, and severe vitamin D deficiency as total 25[OH]D <10 ng/ml.

Measurement of covariates

Covariates were assessed using information collected with standardized protocols at the time of the vitamin D measurement. HIV serostatus was determined using enzyme-linked immunosorbent assays (ELISA) and confirmed with Western blot.²⁷ Site was the location where participants were enrolled and was included to adjust for differences in sun exposure due to variability in latitude—an important determinant of 25[OH]D level. The season of serum sample collection was categorized into four periods (spring: March, April, and May; summer: June, July, and August; fall: September, October, and November; winter: December, January, and February).

Race, employment, income, education, alcohol consumption, smoking, and current antiretroviral drug use were self-reported. BMI was categorized as normal (BMI <25.0 kg/m²), overweight (25.0 ≤ BMI <29.9 kg/m²), and obese (BMI ≥30.0 kg/m²). Only seven individuals had BMI <18.5 kg/m² and so they were included in the normal BMI category. Hepatitis C virus (HCV) positivity was defined as having a positive plasma RNA test. The presence of depressive symptoms was defined as a score of ≥16 on the Center for Epidemiologic Studies Depression (CESD) scale.²⁸ Kidney function was dichotomized into normal (estimated glomerular filtration rate [GFR] from the CKD-EPI creatinine equation²⁹ >90 ml/min/1.73 m²) and low (GFR ≤90 ml/min/1.73 m²), based on established chronic kidney disease staging.³⁰

Suppressed plasma HIV RNA (viral load) was defined as HIV-1 RNA <400 copies/ml, measured mostly using the Roche assays (27% Roche second generation, LOQ <50

copies/ml; 68% Roche COBAS TaqMan, LOQ <20 copies/ml). CD4⁺ T lymphocyte cell levels were quantified using standardized flow cytometry³¹ and categorized as <200, 200–499, and ≥500 cells/mm³. HAART use was defined using the DHHS/Kaiser Panel guidelines.³² As participants enrolled in the MACS after 2001 were demographically and socioeconomically different from those enrolled before 2001,³³ enrollment after 2001 was used as a covariate to further examine the effect of unmeasured socioeconomic factors on vitamin D status.

Missing covariate values (22% GFR, 8% BMI, 1% HIV-1 RNA levels, 1% CD4⁺ T cell count, 1% current efavirenz use, 1% current tenofovir use, and 1% current ritonavir use) were imputed 10 times using Markov Chain Monte Carlo and assuming multivariate normality.³⁴

Statistical analyses

The continuous and categorical characteristics of the study population by HIV serostatus were compared using Wilcoxon and chi-square tests, respectively. Multivariate linear and logistic regression was used to assess associations of vitamin D metabolite levels or deficiency with HIV and other hypothesized risk factors. Potential risk factors for inclusion in multivariate models were selected based on univariate models ($p < .10$) and literature review.^{16,17} Assessed covariates included HIV serostatus, age >50 years, race, employment, yearly income, education, drinking, smoking, BMI, HCV infection, depressive symptom, GFR >90 ml/min/1.73 m², geographic location, and season. The final model included HIV serostatus, demographics (enrollment after 2001, age >50 years, and race), geographic location, season, and clinical covariates (BMI, GFR >90 ml/min/1.73 m², and HCV serostatus).

Associations of risk factors with vitamin D metabolite levels were examined in the overall cohort, and in HIV-infected and HIV-uninfected participants separately. Among HIV-infected participants, additional HIV-related variables were examined, including CD4⁺ T cell count, suppressed viral load (suppressed vs. unsuppressed), and current tenofovir, efavirenz, and ritonavir use. Models examining risk factors for 1,25[OH]₂D levels were also adjusted for 25[OH]D. To test for differential effects of covariates on vitamin D metabolites by HIV serostatus, interaction terms were added to models in the overall cohort. p -values <.05 were used to determine statistical significance of associations. All statistical analyses were performed using SAS 9.3 (SAS Institute, Cary, NC).

Results

Characteristics of study population

A total of 640 HIV-infected men on HAART and 99 HIV-uninfected men contributed 739 serum samples (one sample per person) to the analysis. The post-HAART samples in HIV-infected men were collected 1.9 median years (interquartile range [IQR] 1.3–2.3) after HAART initiation. HIV-infected men were more likely to be younger, non-white, have a normal BMI, and to have samples collected in spring and winter compared with HIV-uninfected men. They were similar in all other factors assessed at the time of vitamin D metabolite measurements. Among HIV-infected men, CD4⁺ T cell count was <200 cells/mm³ in 7% of men and 200–499

cells/mm³ in 40%. The HIV-infected men mostly achieved virological suppression following HAART initiation (80%) and 11%, 23%, and 16% were currently using tenofovir, efavirenz, and ritonavir, respectively (Table 1).

Prevalence of vitamin D deficiency and levels of vitamin D metabolites

The overall median 25[OH]D level was 22.4 ng/ml (IQR 14.5–29.2 ng/ml) and medians were similar across strata of HIV serostatus (HIV infected: 22.5, HIV uninfected: 21.4 ng/ml, $p = .292$). The prevalence of vitamin D deficiency and severe deficiency was 41% and 10%, respectively, in HIV-infected men versus 44% and 12%, respectively, in HIV-uninfected men. Distributions of 1,25[OH]₂D levels also did not differ by HIV serostatus with a median 1,25[OH]₂D level 45.0 pg/ml in HIV infected and 45.1 pg/ml in HIV uninfected (Table 1).

Determinants of 25[OH]D status

In the overall cohort, several factors were significantly associated with increased odds of vitamin D deficiency, after controlling for geographic location, season, enrollment after 2001, and age >50 years old. Factors including self-reported black race, other self-reported non-white race, obesity, and having normal estimated kidney function were associated with increased odds of vitamin D deficiency (Table 2). HIV serostatus and HCV infection were not significant predictors of 25[OH]D deficiency, and we found no meaningful differences in any of the predictors of 25[OH]D deficiency by HIV serostatus when interaction terms were added to the model.

Among HIV-infected men, we found that having a CD4⁺ T cell count ≥200 cells/mm³ was associated with an approximate 70% reduction in the odds of vitamin D deficiency compared to men with lower counts, but there was no further reduction in the risk of deficiency with a higher CD4 threshold (CD4⁺ T cell count ≥500 cells/mm³). Ritonavir was the only antiretroviral drug significantly associated with vitamin D deficiency, with an estimated 42% lower odds among current users. No significant independent associations were found for current efavirenz or tenofovir use, or a suppressed HIV viral load (Table 2).

We also modeled 25[OH]D as a continuous outcome but found inferences remained consistent in terms of important determinants of 25[OH]D level. Therefore, we only present results for the dichotomous outcome of vitamin D deficiency.

Determinants of 1,25[OH]₂D levels

Few factors were significant predictors of 1,25[OH]₂D level. Controlling for geographic location, season, enrollment after 2001, and age, only self-reported black race was positively associated with 1,25[OH]₂D such that blacks were estimated to have a mean level 4 pg/ml higher than whites. Among HIV-infected men, none of the HIV-related factors were significantly associated, although a CD4⁺ T cell count between 200 and 499 cells/mm³ (compared to <200 cells/mm³) had a similar effect size as the black race and was marginally significant ($p = .093$) (Table 3). Interactions with HIV serostatus were explored and none were found to be significant in adjusted models, with the exception of the effect of 25[OH]D on 1,25[OH]₂D as described below.

TABLE 1. CHARACTERISTICS OF STUDY POPULATION BY HIV SEROSTATUS

<i>Characteristics</i>	<i>HIV-uninfected</i>	<i>HIV-infected</i>	<i>p-Value</i>
<i>N</i>	99	640	
Enrollment after 2001, <i>n</i> (%)	50 (50.5)	257 (40.2)	.052
Age ≥50 years, <i>n</i> (%)	41 (41.4)	175 (27.3)	.004
Race, <i>n</i> (%)			
White	60 (60.6)	381 (59.5)	.034
Black	33 (33.3)	164 (25.6)	
Other non-white	6 (6.1)	95 (14.8)	
Employment, <i>n</i> (%)	62 (63.9)	414 (66.5)	.624
Yearly income, <i>n</i> (%)			
<19,999	34 (37.4)	223 (37.4)	.875
20,000–49,999	29 (31.9)	204 (34.2)	
>50,000	28 (30.8)	170 (28.5)	
Education, <i>n</i> (%)			
High school	19 (19.2)	156 (24.4)	.484
Any college	58 (58.6)	340 (53.1)	
Graduate school	22 (22.2)	144 (22.5)	
Drinking, <i>n</i> (%)			
0/week	19 (19.4)	133 (21.1)	.558
1–3/week	45 (45.9)	318 (50.5)	
4–13/week	27 (27.6)	150 (23.8)	
>13/week	7 (7.1)	29 (4.6)	
Smoking, <i>n</i> (%)			
Never smoking	28 (28.6)	159 (25.2)	.662
Former smoking	33 (33.7)	240 (38)	
Current smoking	37 (37.8)	233 (36.9)	
BMI, <i>n</i> (%)			
Normal (<25.0 kg/m ²)	41 (46.1)	311 (52.8)	<.001
Overweight (25.0–29.9 kg/m ²)	23 (25.8)	211 (35.8)	
Obese (≥30.0 kg/m ²)	25 (28.1)	67 (11.4)	
HCV infection, <i>n</i> (%)	13 (13.3)	50 (7.8)	.072
Depressive symptom (CESD ≥16), <i>n</i> (%)	31 (33)	166 (27)	.231
GFR >90 ml/min/1.73 m ² , <i>n</i> (%)	47 (51.1)	296 (61.5)	.061
Site, <i>n</i> (%)			
Baltimore	23 (23.2)	183 (28.6)	.234
Chicago	30 (30.3)	175 (27.3)	
Pittsburgh	33 (33.3)	165 (25.8)	
Los Angeles	13 (13.1)	117 (18.3)	
Season, <i>n</i> (%)			
Spring	25 (25.3)	178 (27.8)	.024
Summer	33 (33.3)	153 (23.9)	
Fall	29 (29.3)	156 (24.4)	
Winter	12 (12.1)	153 (23.9)	
Year of sampling, [median (IQR)]	2007 (2005–2008)	2003 (1999–2006)	<.001
CD4 cell count (cells/mm ³), <i>n</i> (%)			
<200	—	47 (7.4)	
200–499	—	255 (40.3)	
≥500	—	330 (52.2)	
Nadir CD4 cell count (cells/mm ³), <i>n</i> (%)			
<200	—	179 (28.0)	
200–499	—	350 (54.8)	
≥500	—	110 (17.2)	
Duration of HAART, [median (IQR)], years	—	1.9 (1.3–2.3)	
Suppressed viral load (<400 copies/ml), <i>n</i> (%)	—	505 (79.5)	
Current tenofovir use, <i>n</i> (%)	—	67 (10.5)	
Duration of tenofovir use up to current visit, [median (IQR)], years	—	0 (0–0.8)	
Current efavirenz use, <i>n</i> (%)	—	146 (23.1)	
Duration of efavirenz use up to current visit, [median (IQR)], years	—	0 (0–1.4)	
Current ritonavir use, <i>n</i> (%)	—	99 (15.6)	
Duration of ritonavir use up to current visit, [median (IQR)], years	—	0 (0–0.5)	
25[OH]D <20 ng/ml, <i>n</i> (%)	44 (44.4)	263 (41.1)	.529
25[OH]D <10 ng/ml, <i>n</i> (%)	12 (12.1)	66 (10.3)	.586
25[OH]D [median (IQR)], ng/ml	21.4 (13.8–28.3)	22.5 (14.8–29.3)	.292
1,25[OH] ₂ D [median (IQR)], pg/ml	45.1 (36.4–53.4)	45.0 (35.6–55.0)	.892

1,25[OH]₂D, 1,25-dihydroxyvitamin D; 25[OH]D, 25-hydroxyvitamin D; CESD, Center for Epidemiologic Studies Depression scale; GFR, glomerular filtration rate; HAART, highly active antiretroviral therapy; HCV, hepatitis C virus; IQR, interquartile range.

TABLE 2. MULTIVARIATE LOGISTIC REGRESSION FOR VITAMIN D DEFICIENCY

Covariates	Overall cohort (n = 739)		HIV-uninfected ^a (n = 99)		HIV-infected ^b (n = 640)		HIV-infected ^c (n = 640)	
	OR (95% CI)	p-Value	OR (95% CI)	p-Value	OR (95% CI)	p-Value	OR (95% CI)	p-Value
HIV positive	0.93 (0.56,1.54)	.775						
White	Ref	—	Ref	—	Ref	—	Ref	—
Black	6.4 (4.02,10.2)	<.001	6.41 (4.02,10.21)	<.001	6.46 (3.9,10.71)	<.001	6.91 (4.09,11.67)	<.001
Other non-white race	2.74 (1.51,4.98)	.001	2.73 (1.5,4.96)	.001	2.44 (1.3,4.58)	.006	2.74 (1.43,5.26)	.002
Thin-normal	Ref	—	Ref	—	Ref	—	Ref	—
Overweight	1.01 (0.69,1.48)	.966	1.01 (0.69,1.48)	.975	0.92 (0.62,1.38)	.697	0.97 (0.64,1.48)	.902
Obese	2.74 (1.56,4.84)	.001	2.77 (1.58,4.86)	<.001	2.61 (1.34,5.08)	.005	2.6 (1.33,5.09)	.005
GFR >90 ml/ min/1.73 m ²	1.8 (1.21,2.68)	.004	1.8 (1.21,2.67)	.004	1.71 (1.11,2.64)	.015	1.74 (1.11,2.72)	.016
HCV infection	0.78 (0.41,1.46)	.43	0.78 (0.41,1.46)	.435	0.77 (0.38,1.54)	.455	0.76 (0.37,1.53)	.438
Enrollment after 2001	1.35 (0.91,2.01)	.14	1.36 (0.91,2.02)	.131	1.28 (0.84,1.96)	.249	1.25 (0.8,1.96)	.322
Age >50 years	1.12 (0.74,1.69)	.584	1.13 (0.75,1.7)	.558	1.04 (0.67,1.63)	.859	1.08 (0.68,1.71)	.742
Baltimore	Ref	—	Ref	—	Ref	—	Ref	—
Chicago	0.92 (0.58,1.44)	.71	0.92 (0.59,1.45)	.723	0.87 (0.54,1.42)	.586	0.88 (0.53,1.45)	.609
Pittsburgh	0.79 (0.5,1.27)	.337	0.8 (0.5,1.28)	.347	0.9 (0.54,1.49)	.674	0.94 (0.56,1.58)	.817
Los Angeles	0.34 (0.19,0.61)	<.001	0.34 (0.19,0.61)	<.001	0.41 (0.22,0.76)	.005	0.41 (0.22,0.77)	.006
Summer	Ref	—	Ref	—	Ref	—	Ref	—
Spring	2.86 (1.77,4.61)	<.001	2.84 (1.77,4.58)	<.001	2.54 (1.52,4.26)	<.001	2.61 (1.55,4.41)	<.001
Fall	1.38 (0.84,2.27)	.197	1.38 (0.84,2.27)	.2	1.34 (0.78,2.32)	.287	1.35 (0.78,2.34)	.282
Winter	3.02 (1.83,4.99)	<.001	3 (1.82,4.94)	<.001	2.76 (1.62,4.7)	<.001	2.95 (1.71,5.07)	<.001
CD4 ≤ 199							Ref	—
CD4 200–499							0.31 (0.14,0.69)	.004
CD4 ≥ 500							0.27 (0.12,0.61)	.001
Suppressed viral load							1.41 (0.85,2.36)	.187
Current ritonavir use							0.58 (0.35,0.99)	.044
Current tenofovir use							0.75 (0.41,1.4)	.369
Current efavirenz use							1.13 (0.72,1.77)	.601

^aThe HIV-uninfected and HIV-infected models had the same set of adjusted variables for comparison.

^cThe second HIV-infected model accounted for additional HIV-related variables, including CD4, viral load, and different types of antiretroviral therapies.

CI, confidence interval; OR, odds ratio.

Relationship between 25[OH]D and 1,25[OH]₂D levels

The association between 25[OH]D levels and 1,25[OH]₂D levels was stronger among HIV-infected men than among HIV-uninfected men ($p < .05$ for interaction). In the stratified multivariate models, among HIV-infected men, a 1 ng/ml increase of 25[OH]D was significantly associated with an estimated 0.32 pg/ml increase in 1,25[OH]₂D ($p < .001$). By contrast, among HIV-uninfected men, the same increase of 25[OH]D was associated with only a negligible and nonsignificant change in 1,25[OH]₂D ($\beta = -0.09$ pg/ml, $p = .623$). After restricting this analysis to whites ($n = 441/739$), this pattern still existed, but the positive association between 25[OH]D and 1,25[OH]₂D among HIV-infected individuals was attenuated and became marginally significant ($\beta = 0.15$ pg/ml, $p = .07$). In contrast, the association between 25[OH]D and 1,25[OH]₂D among HIV-infected individuals was much stronger after restricting the analysis to blacks ($n = 197/739$, $\beta = 0.71$ pg/ml, $p < .001$) (Fig. 1).

Discussion

Three primary findings emerged from our analysis of 25[OH]D and 1,25[OH]₂D levels in this large cohort of HIV-infected men and a comparable HIV-uninfected sample of men at risk for infection. First, vitamin D deficiency was

highly prevalent in both serostatus groups, but was not different from that reported in the general population. HIV-infected men had comparable distributions of 25[OH]D and 1,25[OH]₂D to HIV-uninfected men, suggesting that HIV infection does not play a large role in altering vitamin D status, and risk factor profiles were similar. Second, blacks had higher levels of 1,25[OH]₂D than whites despite having lower levels of 25[OH]D, regardless of HIV serostatus. Third, a significant association between 1,25[OH]₂D and 25[OH]D was seen only in HIV-infected men.

The vitamin D deficiency prevalence of 41% observed among HIV-infected men in this sample of MACS lies in the middle of a very wide range of prevalence estimates (10%–83%) reported in other studies of HIV-infected individuals^{16,20,35–37} and is consistent with the 39% prevalence reported in the general population.¹⁶ The wide range of vitamin D deficiency prevalence estimates in HIV infected likely results from differences in demographics, geographic location, seasonality, and other study population characteristics as well as varying definitions of vitamin D deficiency. For example, Adeyemi *et al.* reported 60% prevalence of vitamin D deficiency, 20% higher than our result, using the same definition (<20 ng/ml) in a cohort of predominantly non-white HIV-infected women with lower average levels of income and education.¹⁷ Viard *et al.* found a 24% prevalence

TABLE 3. MULTIVARIATE LINEAR REGRESSION FOR 1,25[OH]₂D

Covariates	Overall cohort (n = 739)		HIV-uninfected ^a (n = 99)		HIV-infected ^b (n = 640)		HIV-infected ^c (n = 640)	
	Mean difference (95% CI)	p-Value	Mean difference (95% CI)	p-Value	Mean difference (95% CI)	p-Value	Mean Difference (95% CI)	p-Value
HIV positive	0.02 (-3.28,3.32)	.991						
25[OH]D	0.29 (0.16,0.41)	<.001						
White	Ref	—	-0.09 (-0.44,0.26)	.623	0.32 (0.19,0.45)	<.001	0.3 (0.17,0.44)	<.001
Black	4.85 (1.69,8.01)	.003	6.98 (0.05,13.92)	.048	4.36 (0.84,7.87)	.015	4.09 (0.54,7.63)	.024
Other non-white race	2.53 (-1.53,6.58)	.221	4.01 (-7.36,15.39)	.49	1.85 (-2.52,6.22)	.407	1.27 (-3.14,5.67)	.573
Thin-normal	Ref	—						
Overweight	1.19 (-1.29,3.68)	.346	-1.42 (-8.63,5.79)	.697	1.47 (-1.17,4.11)	.275	1.47 (-1.23,4.16)	.286
Obese	2.83 (-0.84,6.49)	.131	-1.24 (-7.96,5.48)	.717	3.06 (-1.4,7.53)	.178	3.3 (-1.16,7.77)	.146
GFR >90 ml/min/1.73 m ²	1.67 (-1.15,4.5)	.243	4.42 (-0.93,9.78)	.105	1.32 (-1.89,4.52)	.416	1.39 (-1.82,4.6)	.393
HCV infection	-1.42 (-5.45,2.6)	.488	-1.75 (-9.46,5.96)	.657	-1.41 (-6.01,3.2)	.549	-1.41 (-6.01,3.18)	.547
Enrollment after 2001	3.56 (0.94,6.18)	.008	-0.27 (-6.17,5.64)	.93	3.76 (0.87,6.64)	.011	3.3 (0.32,6.28)	.03
Age >50 years	2.32 (-0.28,4.92)	.08	0.86 (-4.66,6.38)	.76	2.66 (-0.25,5.57)	.073	2.16 (-0.76,5.07)	.147
Baltimore	Ref	—						
Chicago	-2.31 (-5.27,0.64)	.125	-6.45 (-13.23,0.33)	.062	-1.92 (-5.16,1.33)	.247	-1.86 (-5.11,1.38)	.261
Pittsburgh	-3.15 (-6.21, -0.09)	.044	-6.75 (-13.48, -0.02)	.049	-2.98 (-6.38,0.41)	.085	-3.14 (-6.53,0.25)	.07
Los Angeles	0.16 (-3.49,3.81)	.932	-7.94 (-16.47,0.59)	.068	1.12 (-2.9,5.15)	.585	0.82 (-3.21,4.84)	.69
Summer	Ref	—						
Spring	-0.37 (-3.44,2.7)	.813	-3.06 (-9.9,3.77)	.38	0.03 (-3.38,3.43)	.988	0.03 (-3.36,3.41)	.988
Fall	-2 (-5.07,1.07)	.202	-6.28 (-12.26, -0.3)	.04	-1.14 (-4.6,2.31)	.517	-1.01 (-4.45,2.43)	.565
Winter	-0.95 (-4.17,2.27)	.563	-8.89 (-17.8,0.03)	.051	-0.07 (-3.56,3.42)	.969	-0.13 (-3.62,3.37)	.943
CD4 ≤ 199								
CD4 200–499								
CD4 ≥ 500								
Suppressed viral load								
Current ritonavir use								
Current tenofovir use								
Current efavirenz use								

^aThe HIV-uninfected and HIV-infected models had the same set of adjusted variables for comparison.^cThe second HIV-infected model accounted for additional HIV-related variables, including CD4, viral load, and different types of antiretroviral therapies.

FIG. 1. Correlation between serum concentrations of 25[OH]D and 1,25[OH]₂D among the overall cohort ($n=739$, top left), whites ($n=441$, bottom left), and blacks ($n=197$, bottom right). 1,25[OH]₂D, 1,25-dihydroxyvitamin D; 25[OH]D, 25-hydroxyvitamin D. The red dot and line represent HIV-infected group and the blue dot and line refer to HIV-uninfected group.

of vitamin D deficiency in a group of mostly HIV-infected men using a threshold of 25[OH]D <10 ng/mL.¹⁴ With this cutoff, we observed a prevalence of 10% among HIV-infected men in the MACS.

Our findings were consistent with past reports of increased risk for vitamin D deficiency in blacks.³⁸ However, our results also showed that blacks have higher average levels of 1,25[OH]₂D, suggesting that lower 25[OH]D levels in blacks do not necessarily indicate lower levels of the active metabolite 1,25[OH]₂D. It has been reported that blacks have lower levels of vitamin D binding protein, which could cause higher circulating levels of the free-form 25[OH]D available for conversion to 1,25[OH]₂D.³⁹ However, whether reported

racial differences in vitamin D-binding protein level are the result of true biological differences or differential measurement error (by race) resulting from immunoassay affinity variability is debatable.⁴⁰ Higher 1,25[OH]₂D levels might also result from racial differences in 1 α -hydroxylation regulated by plasma parathyroid hormone, serum calcium, and phosphorus levels.¹⁵ Variability in vitamin D metabolism by race may have implications for the assessment of vitamin D status and associations with long-term health risks. It has been noted that blacks, despite lower 25[OH]D levels, have higher bone mineral density and a lower risk of fragility fracture compared to whites,⁴¹ suggesting that measurement of 25[OH]D may be not enough for assessing vitamin D

status in this group. Our findings could suggest that measuring 1,25[OH]₂D in addition to 25[OH]D would provide a more accurate depiction of vitamin D status for assessing long-term comorbidity risk, but further study is needed to determine the merits of 1,25[OH]₂D measurement, given its short half-life and additional assay cost.

Similar to the noted difference by race, our results suggest that the association between 25[OH]D and 1,25[OH]₂D levels may also be altered in HIV-infected compared to HIV-uninfected individuals. Differences in this relationship by HIV serostatus may reflect the impact of greater levels of immune activation associated with HIV infection on vitamin D metabolism. Despite achieving viral suppression, HIV-infected individuals experience residual inflammation,⁴² which may influence vitamin D metabolism. Some *in vitro* studies showed that elevated TNF- α could downregulate the 1- α hydroxylase directly or through inhibition of parathyroid hormone.^{43,44} Inflammation may also induce the extrarenal production of 1,25[OH]₂D although this is less likely to significantly contribute as the amount of 1,25[OH]₂D produced through the extrarenal pathway is relatively small.⁴⁵ Few studies⁴⁶ have examined the relationship between levels of 25[OH]D and 1,25[OH]₂D. Further studies are needed to investigate the effect of inflammation and immune activation on vitamin D metabolism.

Antiretroviral therapies may also impact vitamin D metabolism. Both protease inhibitors (PIs) and non-nucleoside reverse transcriptase inhibitors may impair vitamin D metabolic pathways.⁴⁷ Current ritonavir use was associated with higher levels of 25[OH]D and a lower risk of vitamin D deficiency in our study. PIs can inhibit vitamin D 1 α - and 25 α -hydroxylation *in vitro*^{47,48} and the reduced conversion of 25[OH]D may explain the increased 25[OH]D levels that we found among ritonavir users. Evidence regarding effects of efavirenz use on 25[OH]D levels is not conclusive,^{10,49,50} and our results showed only a weak association. A prior study found that HIV-infected individuals with normal 25[OH]D levels on tenofovir had increased levels of 1,25[OH]₂D compared to those not on tenofovir.⁵¹ We did not find an association between tenofovir and either vitamin D metabolite in our data.

One surprising result was the association between normal estimated kidney function and a higher risk of 25[OH]D deficiency. However, this cross-sectional inverse relationship between 25[OH]D and estimated GFR has been previously noted and likely results from the serum creatinine-based estimation of kidney function as higher 25[OH]D can increase creatinine production.⁵²

The strengths of our study include the large sample size of HIV-infected men with measures of vitamin D metabolites using a robust assay. Although we measured 1,25[OH]₂D in addition to 25[OH]D, yielding a more complete picture of vitamin D status, we did not have data on other important biomarkers of vitamin D metabolism, including serum calcium and phosphorus, parathyroid hormone, and vitamin D binding protein, which would allow for a more accurate and complete evaluation of differences in vitamin D metabolism between HIV-infected and HIV-uninfected individuals. Our sample of HIV-uninfected men was relatively small, and thus, estimates of association were less precise in this strata of serostatus. Finally, we examined cross-sectional associations and did not look at predictors of future vitamin D status, so temporality of associations cannot be established.

In conclusion, vitamin D deficiency is prevalent regardless of HIV serostatus. However, both race and factors associated with HIV infection, such as inflammation and antiretroviral therapies, may influence vitamin D metabolism. Establishing the association of 1,25[OH]₂D with long-term comorbidity risk would provide some rationale for its measurement in clinical settings. Future studies should examine whether and how inflammatory cytokines play a role in vitamin D metabolism.

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Authors' Contributions

L.Z. contributed to the analysis and composing the manuscript. A.G.A. helped to improve the analysis and contributed to the drafting of the manuscript. A.G.A., L.P.J., T.T.B., and J.B.M. were responsible for the design and conduct of the study. A.N.H. carried out the measurement of serum samples. A.T., L.P.J., M.D.W., F.J.P., L.A.K. provided critical intellectual contribution to revisions of the manuscript.

Author Disclosure Statement

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