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ORIGINAL ARTICLE

Vitamin D deficiency in HIV-infected postmenopausal Hispanic and African-American women

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Abstract
Summary We evaluated vitamin D status in HIV+ and HIV− postmenopausal African-American (AA) and Hispanic women. Most women (74-78%) had insufficient 25-hydroxyvitamin D (25OHD) levels, regardless of HIV status. 25OHD was lower in AA women and women lacking supplement use, providing support for screening and supplementation. Among HIV+ women, 25OHD was associated with current CD4 but not type of antiretroviral therapy.
Introduction To evaluate vitamin D status and factors associated with vitamin D deficiency and insufficiency in HIV-infected (HIV+) postmenopausal minority women.
Methods In this cross-sectional study, 89 HIV+ and 95 HIV− postmenopausal women (33% AA and 67% Hispanic) underwent assessment of 25OHD, 1,25-dihydroxyvitamin D, parathyroid hormone, markers of bone turnover and bone mineral density by dual energy X-ray absorptiometry.
Results The prevalence of low 25OHD did not differ by HIV status; the majority of both HIV+ and HIV− women (74-78%) had insufficient levels (<30 ng/ml). Regardless of HIV status, 25OHD was significantly lower in AA subjects, and higher in subjects who used both calcium and multivitamins. In HIV+ women on antiretroviral therapy (ART), 25OHD was directly associated with current CD4 count (r= 0.32; p<0.01) independent of age, ethnicity, BMI, or history of AIDS-defining illness. No association was observed between $1,25(OH)_2D$ and CD4 count or between serum 25OHD, $1,25(OH)_2D$ or PTH and type of ART.
Conclusions In postmenopausal minority women, vitamin D deficiency was highly prevalent and associated with AA race and lack of supplement use, as well as lower current CD4 cell count. These results provide support for screening and repletion of vitamin D in HIV+ patients.

Keywords African-American · Hispanic · HIV+ postmenopausal women · Vitamin D

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Introduction

Antiretroviral therapy (ART) has dramatically increased life expectancy of HIV-infected (HIV+) individuals. By 2015, more than half of HIV+ individuals in the USA will be over age 50 [1]. Concomitant with improved survival, HIV+ patients have begun to develop degenerative cardiovascular, renal and neurological diseases [2], and frailty [3–5]. These conditions may begin at a younger age in HIV+ individuals than in the general population [6]. Osteoporosis [7–14] and fractures [12, 15, 16] are among the emerging medical problems that may disproportionately affect HIV+ patients.

Although older individuals are at much higher risk for osteoporotic fractures, few studies have evaluated skeletal status in older HIV+ men and postmenopausal women. Postmenopausal women may be particularly vulnerable to any adverse effects of HIV infection or ART on the skeleton by virtue of their estrogen deficiency. We therefore initiated

a longitudinal study to assess the prevalence, determinants and etiology of low bone mineral density (BMD) in HIV+ postmenopausal Hispanic and African-American women. This group is important to study because of the burgeoning rates of new infections among minority women in the USA [17]. We recently reported the baseline analyses of this study [18], finding that HIV+ minority women have lower BMD and a higher prevalence of certain types of fractures and higher biochemical bone turnover markers than HIV− controls. In a multivariate analysis, HIV+ status was independently and negatively associated with spine and hip BMD after adjustment for age, race/ethnicity, body mass index (BMI), and alcohol.

In the course of these analyses, we observed that a substantial proportion of the participants were vitamin D deficient. Although serum 25-hydroxyvitamin D (25OHD) was not an independent predictor of BMD in our subjects [18], known skeletal consequences of vitamin D deficiency include secondary hyperparathyroidism, bone loss, and fracture, as well as indirect effects on bone through increased muscle weakness and falls [19, 20]. Moreover, there are important non-skeletal associations of vitamin D deficiency [21, 22]. Particularly relevant to HIV+ patients, the active form of vitamin D, $1,25(OH)_2D$, is a potent modulator of both innate and adaptive immunity [23–25]. Thus, vitamin D deficiency, which has been described in several small studies of HIV+ patients [26–28], may be of particular consequence in HIV+ patients because of the potential for adverse effects on immune function.

Several commonly used antiretrovirals have been associated with dysregulation of vitamin D metabolism. Efavirenz induces cytochrome p450 enzymes and may increase the metabolism of 25OHD to inactive compounds through upregulation of the 24-hydroxylase [29, 30]. Initiation of efavirenz-containing regimens is associated with a decrease in serum 25OHD [30]. Protease inhibitors suppress 25- and 1-α hydroxylase, which could also lead to lower levels of $1,25(OH)_2D$ [31]. Tenofovir is associated with proximal renal tubular dysfunction resulting in phosphate wasting [32]. Since $1,25(OH)_2D$ increases renal and intestinal phosphate absorption as a compensatory mechanism, these effects may be exacerbated by vitamin D insufficiency.

In this report, we present a more detailed analysis of vitamin D status and risk factors for low serum 25OHD levels in postmenopausal minority women with and without HIV infection living in New York City. Given the northern latitude, we hypothesized that low serum 25OHD levels would be prevalent in both groups, but more common in African-American (AA) women in whom greater skin pigmentation reduces cutaneous vitamin D3 synthesis. We further hypothesized that in HIV+ women, low serum 25OHD levels would be associated with use of antiretrovirals that affect vitamin D metabolism. Finally, we investigated associations between serum 25OHD levels and measures of immune reconstitution in HIV+ women.

Methods

Subjects

Subjects were recruited at Columbia University Medical Center (CUMC) and the Bronx Lebanon Hospital Center (BLHC) in New York for an ongoing longitudinal study of changes in bone and mineral metabolism in HIV+ women. This prospective observational study tracks changes in bone density and microarchitecture and bone turnover in cohorts of HIV+ and HIV− postmenopausal women. A cross-sectional evaluation of bone mass and mineral metabolism in the cohort according to HIV status has recently been published [18]. The analyses presented here focus on HIV and vitamin D status and race/ethnicity. Eligible subjects were postmenopausal women over the age of 40, self-identified as Hispanic or AA. Subjects were excluded if they had metabolic bone disease, multiple myeloma or metastatic cancer, endocrinopathies (untreated hyper- or hypothyroidism, Cushing's syndrome or prolactinoma); renal insufficiency (creatinine >1.5 mg/dL), malabsorption, or were currently using medications that affect bone metabolism (glucocorticoids, anticonvulsants, current hormone replacement therapy, or bisphosphonates).

Study design

Subjects were evaluated at the Metabolic Bone Diseases Unit of CUMC. All subjects provided written informed consent and the Institutional Review Boards of CUMC and BLHC approved the study. Information on medical history, risk factors for osteoporosis, current and past medications, HIV and ART history was obtained by subject interview and confirmed by chart review. Control subjects, recruited from the clinic population of CUMC, included women who met the same eligibility criteria; enzyme-linked immunosorbent assay (ELISA) testing verified HIV-negative status.

Assays

All serum measurements were performed on fasting morning samples. Calcium and albumin were analyzed the day of collection by automated techniques; sera was archived at –80°C for batch analyses. Serum assays included: PTH (radioimmunoassay [RIA]; Corning-Nichols Laboratory, San Clemente, CA, USA), 25OHD (Diasorin RIA, Stillwater, MN, USA), $1,25(OH)_2D$ (Diasorin RIA, Stillwater, MN, USA), bone-specific alkaline

phosphatase (BAP; ELISA, Quidel Corp., San Diego, CA, USA), osteocalcin (OC; RIA, Immutopics, San Clemente, CA, USA), N-telopeptide of type I collagen (NTx, competitive-inhibition ELISA, Inverness Medical, Princeton, NJ, USA) and C-telopeptide of type 1 collagen (CTx; sandwich ELISA, Serum Crosslaps, IDS Ltd, Fountain Hills, AZ, USA). CD4 counts were measured by flow cytometry (FACS Calibur, Becton Dickinson, San Jose, CA, USA). HIV-1 RNA was quantified by polymerase chain reaction assay utilizing the AMPLICOR HIV-1 MONITOR Ultrasensitive Test (Version 1.5) with a linear range of 50-100,000 copies/ml (Roche Diagnostics, Indianopolis, IN, USA).

Bone mineral density

BMD of the lumbar spine (LS; L1-4), femoral neck, total hip, non-dominant 1/3 radius, and body composition were measured by dual energy X-ray absorptiometry on a QDR 4500 bone densitometer (Hologic Corp, Bedford, MA, USA). T-scores comparing subjects to normal individuals of the same gender and race with peak bone mass, and Z-scores comparing subjects to normal individuals of the same age, gender, and race were calculated from the manufacturer's database for Hispanics and African-Americans.

Statistical analysis

Data are described by mean±standard error or sample size and percentage by HIV-status group. Student's *t* test was used to assess continuous variable differences between groups defined by race/ethnicity, HIV-status, serum 25OHD, or antiretroviral therapy, while Fisher's exact test was used for categorical measures and analysis of continuous measures dichotomized into groups for purposes of examining differences surrounding clinically relevant cut-points. Pearson correlation analysis was used to estimate strength of association between continuous measures. Statistically significant correlations were further evaluated using partial correlation analysis to assess strength of association after removing the influence of potentially confounding variables. Between-group comparisons adjusted for continuous variables (e.g., BMD adjusted for age and BMI) used analysis of covariance. Analysis of variance was used to assess seasonal differences in 25OHD by coding month of serum collection as a fixed effect and entered with HIV-status (HIV− versus HIV+) and race/ethnicity (Hispanic versus AA). Spearman correlation and least-squares linear regression were used to assess the prediction of current CD4 count in the HIV+ by 25OHD concentration. Within-subject change in 25OHD and $1,25(OH)_2D$ was assessed with paired T-tests while difference in 1-year change in 25OHD and $1,25(OH)_2D$ by race or group defined by baseline 25OHD level used one-way ANOVA. Analyses were conducted with SAS version 9.1.3 (SAS Institute Inc., Cary, NC, USA) and two-sided p values<0.05 were considered to indicate statistical significance.

Results

Characteristics of the study population

Between April 2002 and October 2007, 92 HIV+ and 95 HIV− women enrolled in the study. Of those participants, 184 women (89 HIV+ and 94 HIV−) with complete 25OHD data were included in this analysis. Participants had a mean age of 59±8 years; 33% were AA and 67% Hispanic, the latter predominantly from the Dominican Republic (43%) and Puerto Rico (33%). Among HIV+ women, mean CD4 count at study evaluation was 494±20 cells/μl and nadir CD4 count was 217±21 cells/μl; 80% (71/89) were on ART. Seventy percent of those subjects on ART had HIV-1 plasma RNA level <50 copies/ml. Of the HIV+ women on ART, 51% (36/71) were on protease inhibitor (PI)-based regimens, 34% (24/71) on non-nucleoside reverse transcriptase inhibitor (NNRTI)-based regimens, 11% (8/71) on NRTI-only regimens, and 4% (3/71) on regimens that contained both PI and NNRTI. Among subjects on NNRTIs, 75% (18/24) were on efavirenz and 25% (6/24) were on nevirapine.

In Table 1, the characteristics of the study population are presented according to HIV status and race/ethnicity. On average, HIV+ women were 3-4 years younger than HIV− women, particularly among Hispanics. BMI was similar in Hispanic HIV+ and HIV− women, but significantly lower in AA HIV+ relative to HIV− women. Percent total and truncal fat were significantly lower in HIV+ women of both races. In general, subjects and controls were comparable in terms of chronic medical conditions. The most common in both HIV+ and HIV− women were type 2 diabetes, hypertension, hyperlipidemia, asthma, and hepatitis C. Hyperlipidemia and statin use were more common in the Hispanic HIV− than HIV+ and hypertension was more common in the African-American HIV− than HIV+ women. Hepatitis C was more common in HIV+ than HIV− Hispanic women, though not African-American women.

Using T-scores, which compare subjects to women of the same race/ethnicity at peak bone mass, the percentage of HIV+ subjects with low bone mass (T-score≤−1.0) at any site was 57% and the percentage of subjects with osteoporosis (T-score≤−2.5) was 30%. HIV− subjects had a similar prevalence of low bone mass (47%) and osteoporosis (22%). Further, there were no significant differences among Hispanic or AA subjects. Comparing T scores between groups may be misleading because of the younger age of the HIV+ women. Therefore, BMD Z-

Table 1 Characteristics of the study population by race/ethnicity and HIV status (mean±SE)

	Hispanic			African-American		
	HIV+ (n=55)	HIV− (n=69)	P value	HIV+ (n=34)	HIV− (n=26)	P value
Demographic and anthropomorphic characteristics						
Age (years)	56.3±0.9	60.3±0.8	0.001	55.2±1.1	57.9±1.2	0.10
Height (cm)	155±1	158±1	0.08	162±1	162±1	0.61
Weight (kg)	66.7±2.0	71.7±1.8	0.07	72.6±2.8	89.2±3.5	<0.001
BMI (kg/m^2)	26.1±0.8	27.5±0.7	0.33	27.5±1.1	34.3±1.4	<0.001
Body fat (%)	35.0±1.1	40.0±0.7	<0.001	34.6±1.5	41.6±1.2	<0.01
Trunk fat (%)	36.3±1.1	40.1±0.8	<0.01	34.2±1.3	42.1±1.4	<0.001
Bone mineral density[a]						
LS Z-score	−0.66±0.14	−0.05±0.15	<0.01/<0.01	−0.7±0.2	0.5±0.3	0.001/0.02
TH Z-score	−0.1±0.1	0.4±0.1	<0.01/0.001	−0.4±0.2	0.4±0.2	<0.01/0.21
FN Z-score	−0.2±0.1	0.2±0.1	0.03/0.05	−0.4±0.2	0.4±0.2	<0.01/0.26
1/3R Z-score	0.1±0.1	0.7±0.2	<0.01/0.01	1.1±0.2	1.4±0.2	0.27/0.90
Serum biochemistries and calciotropic hormones						
Creatinine	0.80±0.02	0.81±0.02	0.80	0.82±0.03	1.27±0.43	0.31
Calcium	9.1±0.1	9.1±0.0	0.69	9.1±0.1	9.2±0.1	0.75
iPTH	35.1±2.1	42.4±2.3	0.10	36.7±2.6	45.0±3.9	0.07
25OHD	25.1±2.2	24.9±1.5	0.94	20.0±2.1	17.1±1.9	0.34
1,25(OH)$_2$D	43.6±2.1	41.2±1.8	0.37	36.1±2.9	43.7±3.7	0.11
eGFR	84±3	81±3	0.62	98±4	94±9	0.68
Osteoporosis risk factors						
FH of osteoporosis (%)	22	29	0.36	18	12	0.72
Current smoker (%)	45	41	0.59	56	62	0.66
Cigarette pack-yrs	6.6±1.9	6.2±1.9	0.90	15.6±3.6	9.6±2.8	0.21
Alcohol (>1 drink/day;%)	27	26	0.88	53	35	0.20
IV drug use (ever; %)	5	0	0.08	29	23	0.77
Chronic medical conditions						
Hyperlipidemia (%)	26	54	<0.01	15	31	0.21
Asthma (%)	20	17	0.71	32	15	0.23
Hypertension (%)	35	49	0.10	35	62	<0.05
Cardiovascular disease (%)	11	6	0.30	3	0	1.00
Type 2 Diabetes (%)	27	30	0.70	12	8	0.69
Hepatitis C (%)	16	3	<0.01	32	15	0.23
Medications						
Levothyroxine (%)	2	6	0.24	3	0	1.00
Oral hypoglycemics (%)	22	30	0.28	9	8	1.00
Insulin (%)	7	12	0.42	3	0	1.00
Statins (%)	18	45	<0.01	6	19	0.22
Steroids (ever) (%)	15	9	0.31	21	4	0.12
Calcium supplements (%)	24	41	<0.05	21	31	0.38
Multivitamins (%)	53	28	<0.01	47	46	0.94

Normal ranges: *0.6-1.2 mg/dl* serum creatinine, *8.4-9.8 mg/dl* albumin-corrected calcium, *8-51 pg/ml* intact PTH, *20-57 ng/ml* 25OHD, *15-75 pg/ml* 1,25(OH)$_2$D

[a] For comparison of Z-scores between the groups, p values are presented unadjusted/adjusted for BMI

scores, which compare subjects to age- and race/ethnicity controls, were compared in order to control for the age differences between groups, and are presented in Table 1. Z-scores were significantly lower at all sites in Hispanic HIV+ than HIV− women, both before and after controlling for BMI. In HIV+ AA women, Z-scores were lower at the

spine and both hip sites, but not the forearm. However, differences at the hip sites were not significant after controlling for BMI.

Calciotropic hormones

The majority of both HIV+ and HIV− women (76%) had serum 25OHD concentrations below 30 ng/ml, a level currently considered optimal for skeletal health (Fig. 1): 36% had deficient 25OHD levels (<20 ng/ml) and 14% had severe deficiency (<10 ng/ml). The prevalence of low 25OHD levels did not differ by HIV status.

Mean serum 25OHD was in the insufficient range (25.0 ng/ml) in Hispanic women and the deficient range in AA women (18.7 ng/ml; Table 1), and was lower in AA women, regardless of HIV status (Fig. 2). This difference was more pronounced between HIV− (AA: 17.1±1.9 vs. Hispanic: 24.9±1.5 ng/ml; $p<0.01$) than HIV+ women (AA: 20.0±2.1 vs. Hispanic: 25.1.±2.2 ng/ml; $p=0.11$). Serum $1,25(OH)_2D$ did not differ by race/ethnicity or HIV status. PTH tended to be lower among HIV+ women of both races, but the difference between HIV+ and HIV− women was not significant in either group.

HIV+ women were significantly more likely to use multivitamins than HIV− women but less likely to take calcium supplements (Table 1). Only 17% of HIV+ and 20% of HIV− women reported taking both calcium and multivitamins at baseline; 44% of the HIV+ and 49% of the HIV− women reported daily vitamin D intakes below the daily recommended intake of 400 IU/d. Serum 25OHD concentrations were higher in women who reported use of calcium and multivitamins at the baseline visit than women who reported no supplement use (31.5±2.5 ng/ml vs 21.0±1.0, $p<0.0001$; Fig. 3). Notably, although mean 25OHD was higher in women who reported taking both multivitamins and calcium, the majority (70% of HIV+ and 69% of HIV−) still had levels below 30 ng/ml.

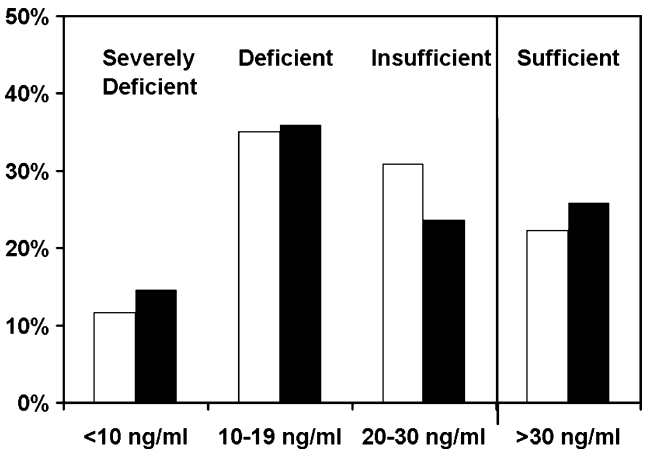

Fig. 1 Prevalence of vitamin D insufficiency and deficiency in HIV− (*open bars*) and HIV+ (*filled bars*) postmenopausal women

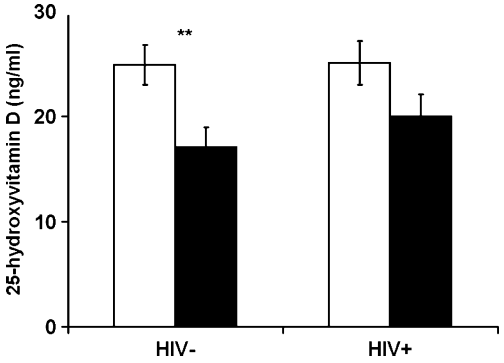

Fig. 2 Serum 25OHD levels (mean±SE) in HIV+ and HIV− women according to race/ethnicity: Hispanic (*open bars*) and African-American (*filled bars*); $^{**}p<0.01$

We observed no consistent association between 25OHD and BMI, nor did we detect any relationship between 25OHD and percent body fat, tobacco, or alcohol use in either group. Seasonal variation in 25OHD was not observed in the cohort as a whole, or in HIV+ and HIV− women assessed separately. Additionally, there was no seasonal variation in 25OHD when African-American and Hispanic women were examined separately.

Relationships between serum 25OHD and BMD, calciotropic hormones, and markers of bone turnover were examined according to HIV status and race. Among HIV+ subjects, serum 25OHD was weakly associated with LS BMD in Hispanic ($r=0.33$; $p=0.01$), but not AA subjects. We detected no association between 25OHD and BMD at other sites (data not shown).

We observed the expected pattern of relationships between serum levels of vitamin D, PTH, and calcium among HIV− women: serum 25OHD was inversely

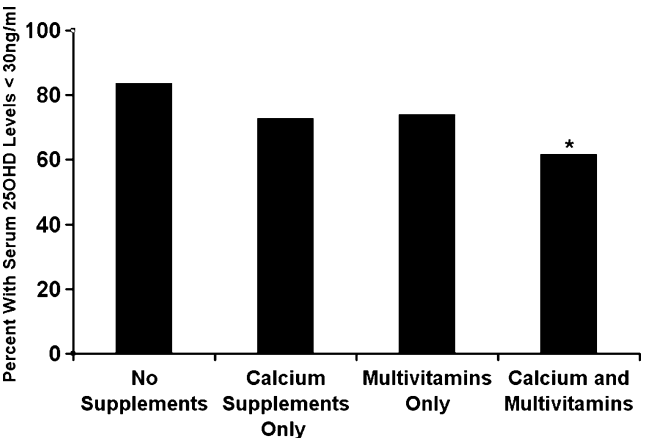

Fig. 3 Proportion of HIV+ and HIV− postmeonpausal women with serum 25OHD levels <30 ng/ml according to self-reported supplementation at the baseline evaluation $^{*}p<0.05$ compared to group without supplementation

associated with PTH (r=−0.28; p<0.01) and directly associated with serum 1,25$(OH)_2$D (r=0.22; p=0.04) and calcium (r=0.23, p=0.03). Among HIV+ women, serum 25OHD was inversely associated with PTH (r=−0.31; p< 0.01) and there was a non-significant positive association with 1,25$(OH)_2$D (r=0.19; p=0.08) but not with serum calcium. The inverse association between 25OHD and PTH was found among HIV+ Hispanic and AA subjects as well as HIV− Hispanic subjects; however, among HIV− AA subjects, no association between 25OHD and PTH was observed. Among HIV− subjects, 25OHD was inversely and weakly associated with the bone formation marker, BAP (r=−0.24; p=0.02). No relationship was observed between 25OHD and other markers of bone turnover.

Associations between 25OHD and HIV-related parameters

Among HIV+ women, higher 25OHD was associated with higher current CD4 count in women on ART (r=0.32; p< 0.01; Fig. 4), but not in women currently off ART (r=0.09, p=0.72). The correlation between 25OHD and current CD4 count among HIV+ women on ART remained significant (r=0.27, p=0.03) after adjustment for factors associated with 25OHD (age, race/ethnicity), current CD4 (history of AIDS-defining illness) and general health (BMI) as detailed in Table 2. The association between higher 25OHD and nadir CD4 count was not significant in women on ART (r=0.20, p=0.09) or off ART (r=0.11, p=0.69). No association was observed between 1,25$(OH)_2$D and current or nadir CD4 count.

Serum 25OHD and 1,25$(OH)_2$D concentrations did not differ in women on or off ART, or on different ART regimens (Table 3). Serum 25OHD and 1,25$(OH)_2$D concentrations were also similar in women on ritonavir-boosted versus unboosted nelfinavir or indinavir-based regimens (Table 3). Among subjects on NNRTI- or PI-based regimens, mean 25OHD was not different among the subjects on tenofovir compared to other NRTIs (data not shown).

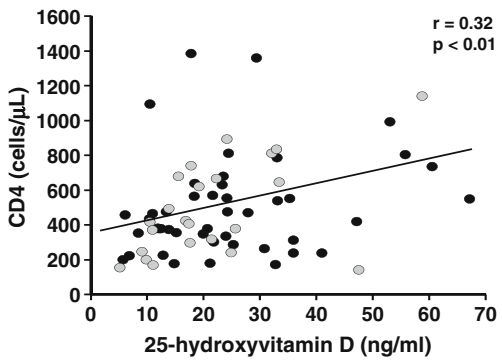

Fig. 4 Association between serum 25OHD and current CD4 count in HIV+ postmenopausal women currently taking antiretroviral therapy (N=68; r=0.32, p<0.01). Hispanic subjects are represented by *filled circles*, AA subjects by *open circles*

Evolution of serum 25OHD concentrations

At enrollment and throughout their entire participation, all subjects were provided with calcium and vitamin D supplements which contained 500 mg calcium carbonate/100 IU vitamin D3 (Viactiv, McNeil Nutritionals LLC, Ft. Washington, PA, USA) and advised to take two chews daily plus a multivitamin that contained 400 IU of vitamin D. To determine the effects of this clinical recommendation on vitamin D status, changes in 25OHD and 1,25$(OH)_2$D were assessed in 45 HIV+ women who had completed 1 year of observation. Serum 25OHD levels decreased significantly over 1 year (−5.5±2.3 ng/ml; p=0.02) while 1,25$(OH)_2$D remained stable (+2.1±2.7 pg/ml; p=0.46). The observed change in 25OHD did not differ between those with serum 25OHD levels above or below 20 ng/ml at baseline or between African-Americans and Hispanics (data not shown).

Discussion

This study confirms and extends previously available information on racial and ethnic differences in vitamin D status among HIV+ and HIV− individuals, and provides new data on risk factors for, and consequences of vitamin D deficiency in postmenopausal minority women. The inclusion of a control group of HIV− postmenopausal minority women allowed us to assess whether vitamin D status differed between HIV+ and HIV− women who were similar with regard to other chronic medical conditions. We found that prevalence of vitamin D insufficiency and deficiency, as defined by serum 25OHD levels, was similar between HIV+ and HIV− postmenopausal minority women. We also found no difference in 1,25$(OH)_2$D levels, which were normal in both groups. In contrast, serum PTH tended to be lower among HIV+ women. Factors associated with lower 25OHD levels were AA race and lack of calcium and multivitamin use. Among HIV+ women, 25OHD levels were similar in those receiving protease inhibitors and efavirenz. In HIV+ women on ART, lower serum 25OHD was weakly associated with lower current CD4 counts. In HIV+ women, serum 25OHD declined over 1 year of observation.

The prevalence of vitamin D deficiency was very high in our subjects, but was comparable between HIV+ and HIV− subjects. Although most studies reporting 25OHD levels in HIV+ subjects have not included a control group, a recent controlled study found similar results [26]. In a group of 57 HIV+ outpatients, prevalence of vitamin D insufficiency (25OHD<32 ng/ml; 74%) and deficiency (25OHD<20 ng/

Table 2 Correlation between CD4 and 25OHD

	ART+ (n=68[a])	ART− (n=18)
Unadjusted	r=0.32	r=0.09
	p<0.01	p=0.72
Adjusted for age, BMI, ethnicity and AIDS criteria	r=0.26	r=0.02
	p<0.05	p=0.93

[a] Excludes three subjects missing CD4 count data on the day of study visit

ml; 37%) was similar to our findings [28]. In other recent studies, 29% of HIV+ subjects had 25OHD <10-14 ng/ml [27] and 87% of HIV+ adolescents and young adults, the majority of whom were AA, had levels below 15 ng/ml [26]. Other studies have found lower 25OHD [33] and 1,25 $(OH)_2D$ [33, 34] in HIV+ patients compared to non-infected controls; however, HIV+ patients in these studies may have been more ill than those included in our cohort, and our control population of postmenopausal minority women with chronic diseases may have been more ill than typical control subjects in the literature. It has been postulated that poorer general health and antiretroviral medications, specifically protease inhibitors, may interfere with conversion of 25OHD to 1,25$(OH)_2D$ [35].

As expected, serum 25OHD concentrations were lower among AA women compared to Hispanic women, regardless of HIV status. Lower 25OHD levels among African-Americans compared to other races have been demonstrated in several studies [36–39], including elderly adults living in Boston [39], as well as HIV+ adolescents [26] and adults [27]. The relationship between 25OHD and BMD differed by race. The direct association that we observed between lower 25OHD and lower spine BMD in Hispanic women is of potential importance given the higher prevalence of vertebral fractures recently reported in HIV+ women [15]. However, we did not observe similar findings at other sites or in the AA group. We found an inverse association between 25OHD and bone alkaline phosphatase among HIV− women, perhaps indicative of a compensatory increase in bone turnover in response to low 25OHD.

We also found that PTH was lower among HIV+ subjects, although the difference between groups was not significant possibly because of our small sample size. The phenomenon of lower PTH among HIV+ individuals has been reported previously [34, 40], and has been thought due to direct effects of the virus or autoantibodies against CD4 on the parathyroid glands [40–42]. We did not find a difference in PTH according to race. While several investigators have found higher mean PTH in AA compared to Caucasian subjects [39, 43, 44], others have not [45, 46]. We may not have observed this phenomenon because we were comparing our AA subjects not to Caucasians but to Hispanics from the Caribbean, in whom there may be considerable racial admixture. Whether the PTH-25OHD axis in subjects from the Caribbean resembles that of AA or Caucasian subjects is unknown. As expected, we found an inverse association between 25OHD and PTH, although not among HIV− AA women. Again, it is possible that disparate PTH and vitamin D metabolism among AA women accounted for this difference.

Initiation of efavirenz-containing ART was associated with an approximate 5 ng/ml decrease in 25OHD within 6-12 months, whereas no significant change in 25OHD was observed with initiation of other, predominantly PI-based regimens [30]. In studies of HIV+ patients on established ART, 25OHD levels tended to be lower in patients on NNRTI- than PI-based ART [27, 28]; however, differences were greater in white subjects who had higher overall 25OHD and not significant in non-white HIV+ subjects [27]. The women in our study were either Hispanic or African-American and the mean ART exposure in the efavirenz group was 4.5±0.6 years. The impact of efavirenz on 25OHD metabolism may be less significant in non-white women because 25OHD levels are already lower and additionally the impact of efavirenz may diminish over time as nutritional and overall health status improve with antiretroviral therapy.

Table 3 Mean CD4, 25OHD and 1,25$(OH)_2D$ by ART treatment type

	ART- N=18	PI-based ART N=36	NNRTI-based ART N=24	PI with ritonavir N=22	PI without ritonavir N=14	Efavrirenz N=18
CD4	478±301	419±1901	551±366	364±166	508±200	593±413
25OHD	22.7±18.9	22.2±15.1	23.6±13.3	21.5±13.9	23.4±17.3	23.1±12.9
1,25$(OH)_2D$	41.9±19.0	38.4±14.6	42.0±15.7	40.9±14.1	34.1±15.0	40.4±16.7

Comparisons of CD4, 25OHD, and 1,25OHD by treatment type were not significant.

ART not on antiretroviral therapy, *PI-based ART* protease inhibitor plus nucleoside reverse transcriptase inhibitors (NRTIs), *NNRTI-based ART* non-nucleoside reverse transcriptase inhibitor plus NRTIs, *PI with ritonavir* ritonavir-boosted PIs plus NRTIs, *PI without ritonavir* unboosted nelfinavir or indinavir plus NRTIs, *efavirenz* efavirenz plus NRTIs

We found that use of calcium and multivitamins was one of the strongest predictors of 25OHD level. Low dietary intake of vitamin D [28] and lack of supplement use [26] have been associated with lower 25OHD in other populations of HIV+ subjects. While mean 25OHD levels in women who used both calcium and multivitamins (daily intake ~600-800 IU) were close to 30 ng/ml, a large proportion of these women and of women who used either calcium or multivitamins had 25OHD levels below 30 ng/ml, suggesting that an intake of greater than 800 IU per day would be necessary for sufficiency. Serum 25OHD levels generally increase by approximately 1 ng/ml for every 100 IU of vitamin D intake [47] and have been shown to be inadequate even in subjects receiving 1,000 IU daily [48], Thus, it is not surprising that levels did not increase in women who were provided calcium carbonate and vitamin D supplementation of 1,000 mg/200 IU daily, even though the majority also received 400 IUs of vitamin D in a multivitamin. However, it is of interest that levels actually declined in HIV+ women taking this amount of vitamin D (~600 IU daily). These observations indicate that much higher vitamin D intakes may be necessary for our subjects even to maintain relatively low 25OHD levels. This may be because they reside in an inner city environment in the northeastern USA, receive relatively low amounts of sunlight exposure, and may have less cutaneous synthesis of vitamin D3 for a given amount of sunlight than individuals with less darkly pigmented skin [49]. It could also be because they receive medications that interfere with vitamin D metabolism.

Seasonal variations in 25OHD, with higher levels in summer months have been reported, typically by studies with predominantly or uniformly Caucasian populations [50, 51]. In a group of male veterans, there was no association between sun exposure and 25OHD among AA, although a relationship was observed in Caucasians [45]. One study of HIV+ subjects, predominantly AAs, found a seasonal variation in 25OHD, but included subjects residing in Florida. We may not have observed seasonal variation in 25OHD, perhaps because of factors specific to our study population, including increased skin pigmentation and travel habits in our subjects from the Caribbean who commonly return to their countries of origin in the winter.

We did not observe a consistent association between 25OHD and BMI or body fat in our cohort, in contrast to reports in Caucasians and those of mixed racial backgrounds [36, 50, 52–57]. This association may be less pronounced among Hispanic [36] and AA [37] cohorts. In a recent analysis of women from NHANES III,% body fat was related to 25OHD in all Caucasian women, but only in AA women under 50 years old [37]. Perhaps we did not observe an association due to the older age of our subjects. It is also conceivable that our sample size precluded our finding this association.

Recent evidence has shown that vitamin D modulates immune function by promoting innate immunity, activating T lymphocytes and monocyte/macrophages, and attenuating adaptive immunity [33, 35, 58]. Through its effects on the innate immune system, vitamin D may protect against bacterial infections and tuberculosis [24, 59] and modulate HIV-1 replication [60]. When macrophages are activated by specific toll-like receptors, the mitochondrial enzyme, CYP27B1, converts 25OHD to $1,25(OH)_2D$. Higher circulating 25OHD levels would provide more substrate for local 1α-hydroxylation and thus could be associated with better innate immunity to HIV [58]. Our observation that higher serum 25OHD was weakly associated with higher current but not nadir CD4 cell count in women on ART could reflect greater immune competence in patients with higher 25OHD resulting in better immune reconstitution. In all likelihood, however, the correlation with CD4 counts reflects better overall health in those with higher serum 25OHD, with more critically ill individuals having lower levels. It is conceivable that HIV+ individuals who are more severely ill spend less time out of doors and have diets containing lower amounts of vitamin D. De Luis et al. found a similar positive association between vitamin D intake by questionnaires and CD4 count after adjustment for age, sex, energy and protein intake, and ART [61]. In contrast, a study of mostly male HIV+ middle aged adults found no association between CD4 count and 25OHD and no association between baseline vitamin D status and CD4 recovery rate after initiation of ART; however, the definition of vitamin D insufficiency used was much lower than current standards (<25-35 nmol/L or 10-14 ng/ml) [27]. Similarly, Arpadi et al. found no difference in change in absolute CD4 count or CD4% in perinatally infected HIV+ children and adolescents randomized to high-dose cholecalciferol supplementation or placebo [62]. The discrepancy with our findings may be due to differences in the age, gender, and general health of our study populations.

We did not find a direct association between $1,25(OH)_2D$ and CD4 count, as reported by other authors [33, 34, 63]; however, 25OHD is the most abundant vitamin D metabolite and a better indicator of vitamin D status than $1,25(OH)_2D$ [64]. It is conceivable that an association between circulating $1,25(OH)_2D$ and CD4 count was not observed because immune competence in these patients was related to local conversion of 25OHD to $1,25(OH)_2D$ where it may act in a paracrine fashion. This conversion occurs in many extrarenal sites, including monocytes, dendritic cells, T and B lymphocytes [58].

Our study has some limitations. Our sample size was relatively small and we may not have observed some associations because of inadequate power. Other limitations include the cross-sectional design and the potential selection bias as we used a convenience sample and recruited subjects from the New York area. Our focus on HIV+

postmenopausal minority women enabled us to explore a group that has not been well described previously but also may have limited the generalizability of our results. We did not specifically evaluate sun exposure in our subjects or ask about recent travel; both factors may have affected 25OHD levels in our population, and contributed to the lack of seasonal variation in 25OHD that we observed. In addition, vitamin D and calcium supplements were provided as a courtesy to participants and we did not assess participant adherence to supplementation. However, this approach allowed us to demonstrate that merely recommending calcium and vitamin D supplements is inadequate in terms of ensuring vitamin D sufficiency and that a more aggressive approach is required. Our study also has important strengths. To our knowledge, this is the first in-depth investigation of vitamin D status in postmenopausal minority women with HIV. Second, the presence of a control group of the same racial and menopausal background and similar prevalence of common chronic medical conditions of aging allowed us to assess those factors that were related to vitamin D status in AA and Hispanic postmenopausal women and those related to HIV infection.

In conclusion, in a population of minority postmenopausal women living in New York, prevalence of vitamin D insufficiency and deficiency was very high but comparable between HIV+ and HIV− women. Some factors associated with poor vitamin D status, such as African-American race, are not modifiable. The association between supplement use and higher serum 25OHD suggests that these subjects may be able to achieve sufficient 25OHD levels with adequate supplementation. However, it is clear that vitamin D supplementation greater than 600-800 IU daily is necessary for sufficiency, especially in postmenopausal African-Americans. Lower 25OHD was associated with lower current CD4 cell count in HIV+ women on ART. In all likelihood, the correlation with CD4 counts reflects better overall health in those with higher serum 25OHD levels. However, given emerging data on vitamin D and the immune system, it is intriguing and warrants further investigation. A randomized interventional trial of vitamin D administration would be necessary to evaluate the association between low 25OHD and CD4 count. Vitamin D insufficiency and osteoporosis are two of many medical problems that have been recognized as the HIV+ population survives longer and ages. Given the importance of vitamin D for skeletal health and potential beneficial effects on extra-skeletal processes including immune function, we conclude that all HIV+ patients should be screened and treated for vitamin D deficiency.

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Conflicts of interest None.

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