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Short Communication: Association of Vitamin D Insufficiency and Protective Tenofovir Diphosphate Concentrations with Bone Toxicity in Adolescent Boys and Young Men Using Tenofovir Disoproxil Fumarate/Emtricitabine for HIV Pre-Exposure Prophylaxis

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Abstract

We examined associations of 25-hydroxy vitamin D (25-OHD), tenofovir disoproxil fumarate (TDF), and bone toxicity. We studied TDF/emtricitabine (FTC) HIV pre-exposure prophylaxis (PrEP) in young men who have sex with men (YMSM). Bone toxicity was predefined using bone mineral density/content change from baseline to week 48. Baseline serum 25-OHD was dichotomized as <20 ng/mL (insufficient/deficient) versus ≥20 (sufficient), and week 48 dried blood spot tenofovir diphosphate (TFV-DP) as >700 fmol/punch (protective against HIV acquisition) versus ≤700. Associations were examined by univariate and multivariable logistic regression, reporting crude and adjusted odds ratios (ORs), with 95% confidence intervals (CIs). Of 101 enrolled, 69 had complete bone assessments and 25-OHD; of these, 59 had week 48 TFV-DP data. Median (Q1–Q3) age was 20 (18–21) years; 54% were black/African American. In univariate analysis, participants with baseline 25-OHD <20 ng/mL (OR = 5.4; 95% CI = 1.9–16.5) and blacks (OR = 4.9; 95% CI = 1.7–15.2) had greater odds of bone toxicity than those with 25-OHD ≥20 or other races. TFV-DP was not associated with bone toxicity (OR = 1.6; 95% CI = 0.5–5.5). In multivariable analysis, compared with those with 25-OHD ≥20 and TFV-DP ≤700, those with 25-OHD ≥20 and TFV-DP >700 (OR = 11.5; 95% CI = 1.4–169.6), 25-OHD <20 and TFV-DP ≤700 (OR = 19.4; 95% CI = 3.0–228.7), and 25-OHD <20 and TFV-DP >700 (OR = 32.3; 95% CI = 3.3–653.6) had greater odds of bone toxicity after adjusting for race. In multivariable models, 25-OHD insufficiency, protective TFV-DP concentrations, and black race were significantly associated with bone toxicity after 48 weeks of TDF/FTC PrEP in YMSM.

Clinical Trials Registration: NCT01769469.

Keywords: tenofovir disoproxil fumarate, bone mineral density, HIV pre-exposure prophylaxis, 25-hydroxy vitamin D

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Introduction

Tenofovir disoproxil fumarate combined with emtricitabine (TDF/FTC), when used daily for HIV pre-exposure prophylaxis (PrEP), protects against HIV acquisition.[1] TDF decreases dual-energy X-ray absorptiometry (DXA)-measured bone mineral density (BMD) in adults with[2] and without[3] HIV. In persons without HIV receiving TDF/FTC for PrEP, BMD decrease is most pronounced in those with highest drug exposure.[3,4]

In youth receiving TDF/FTC for PrEP, BMD decrease is associated with changes in endocrine control of calcium and phosphate metabolism, including increased parathyroid hormone (PTH).[4] Vitamin D insufficiency/deficiency, defined as serum 25-hydroxy vitamin D (25-OHD) concentration <20 ng/mL,[5] is also associated with BMD loss and increased PTH in non-HIV-infected populations. TDF-associated increased PTH occurs in the presence[6,7] and absence[8] of 25-OHD insufficiency, suggesting that TDF exposure and 25-OHD insufficiency are important in TDF-associated endocrine perturbations, and potentially BMD change.

Using data from recently completed trials of PrEP in young men who have sex with men (YMSM) at high risk of HIV acquisition, this report analyzes the association of 25-OHD insufficiency and TDF exposure with bone toxicity.

Materials and Methods

Overview

Adolescent Medicine Trials Network for HIV/AIDS Interventions (ATN) 117[4] was a metabolic substudy of ATN 113[9] (ages 15–17 years) and ATN 110[10] (ages 18–22 years). These were prospective PrEP demonstration and safety studies in HIV-uninfected YMSM conducted at ATN sites in the United States. All studies were approved by each center's institutional review board and required participants' written consent before enrollment.

Parent studies: visits and data

Participants were provided coformulated TDF/FTC [Truvada®] and were advised to take 1 tablet by mouth daily. Study visits occurred at baseline and weeks 4, 8, 12, 24, 36, and 48. Dried blood spot specimens for tenofovir diphosphate (TFV-DP) concentrations[9–11] were collected at each visit after baseline. Spine, hip, and whole body DXA scans were performed at baseline and weeks 24 and 48, and analyzed centrally.[9,10] Four sites had scanners manufactured by GE/Lunar (Madison, WI), and eight sites had Hologic devices (Waltham, MA). With very few exceptions, participants were scanned on the same instrument throughout the study.

The initial 48-week study had prespecified endpoints identifying participants with bone or renal toxicity who would be studied another 48 weeks in an ''extension phase'' to determine whether the toxicity reversed when PrEP was discontinued. Bone toxicity criteria for extension phase inclusion were based on change from baseline to week 48 in DXA results as follows: (1) for ages <20 years, when bone growth is expected,[12] no increase in whole-body bone mineral content (BMC) or spine BMD; (2) for ages ≥20 years, decrease of ≥1% in whole-body BMC or BMD in total hip, femoral neck, or spine; (3) all ages, any decrease in BMD Z-score ≥0.5 in total hip, femoral neck, or spine. Similar criteria were established for renal toxicity but no ATN 117 participant met any renal toxicity criteria.

ATN 117 substudy data

Blood samples were collected after ≥8-h fast, processed at study sites, and sent frozen for batch analysis of serum 25-OHD at the U.S. Department of Agriculture, Agricultural Research Service, Western Human Nutrition Research Center, Davis, CA, as previously described.[4,13] Baseline and week 48 questionnaires assessed lifestyle and dietary variables.[4]

Exposure and outcome categorization for this secondary analysis

Drug exposure was categorized as TFV-DP >700 fmol/punch (protective against HIV acquisition[1,9,10]) versus ≤700 fmol/punch. TFV-DP concentrations below the limit of quantitation were set to 0.[9,10] 25-OHD concentrations were categorized as insufficient/deficient (<20 ng/mL) and sufficient (≥20 ng/mL) based on Institute of Medicine criteria.[5]

The outcome variable was eligibility for the extension phase, based on meeting one or more prespecified bone or renal criteria. Since all participants qualified for the extension phase based on bone, not renal, criteria, the outcome variable is termed ''bone toxicity'' (met extension phase criteria), or ''no bone toxicity'' (did not meet criteria). This analysis reports data from the first 48 weeks of study. Extension phase data are reported in a separate article.

Statistical analysis

Continuous variables are reported as median (Q1, Q3) and categorical variables as frequency and percentage. The distribution of 25-OHD and TFV-DP by bone toxicity and race was compared using Wilcoxon rank-sum test. Associations of TFV-DP, 25-OHD, race, ethnicity, body mass index (BMI), and alcohol use with bone toxicity were examined using univariate and multivariable logistic regression, reporting crude and adjusted odds ratios (ORs), respectively, with corresponding 95% confidence intervals (CIs). Regression model performance was assessed using maximum rescaled r-square, c-statistic and Hosmer–Lemeshow goodness of fit-test. Firth's penalized likelihood approach was used to account for potential bias from small sample sizes. Effect measure modification was examined by stratified analysis of dichotomized TFV-DP and 25-OHD. Dichotomized TFV-DP and 25-OHD were combined (four categories) to examine the simultaneous effect of both variables; overall association was assessed using the type 3 likelihood ratio test. Statistical significance was set at 0.05 (two-tailed). Analyses were conducted using SAS statistical software, version 9.3 (Cary, NC).

Results

From the initial 101 participants in ATN 117, 69 had DXA data at baseline and week 48 and 25-OHD data at baseline and 59 of those had TFV-DP data at week 48. Median (Q1, Q3) age of this analysis cohort was 20 (18, 21) years; 54% were black/African American (black/AA) and 36% Hispanic, distributions similar to the initial study. From the 69 participants with baseline and week 48 DXA results, 47 (68%) met

one or more prespecified extension phase inclusion criteria (7 participants met two criteria). There was no difference in age, but black/AA race was overrepresented in the group with bone toxicity compared to without: 31/47 (66%) versus 6/22 (27%), respectively ($p = .003$). Black/AA, who may finish bone accrual at an earlier age than youth of other races,[12] were not overrepresented in the bone toxicity group based on lack of bone accrual at age <20 years (data not shown). There were no differences in ethnicity (''A'' in Table 1), BMI, or alcohol use by bone toxicity outcome (data not shown).

Vitamin D status at baseline

Overall, baseline serum 25-OHD concentration was 17 (10, 23) ng/mL, and 44 (64%) participants had 25-OHD <20 ng/mL (''A'' in Table 1). Supplemental calcium or vitamin D use was reported in 23% and 25% of participants, respectively. Median calcium intake was 1,158 (627, 1,720) mg/day and vitamin D intake was 160 (82, 374) IU/day. There were no significant differences in baseline calcium or vitamin D intake by bone toxicity category.

Baseline 25-OHD concentrations were 16 (10, 20) and 21 (13, 24) ng/mL in groups with and without evidence of bone toxicity, respectively ($p = .038$). Baseline 25-OHD concentration was significantly lower in black/AA than other races: 13 (9, 18) versus 21 (16, 24) ng/mL, respectively ($p < .001$). Calcium or vitamin D intake did not differ by race (data not shown).

Drug exposure at week 48

Of the 59 participants with week 48 TFV-DP data, TFV-DP concentration was 483 (0, 1,032) fmol/punch, suggesting use of 2–3 tablets of TDF/FTC per week on average.[1] TFV-DP concentrations trended lower in black/AA than in other races: 211 (0, 921) versus 620 (258, 1,207) fmol/punch, respectively ($p = .066$). Week 48 TFV-DP concentrations were 488 (45, 1,081) and 368 (0, 973) fmol/punch in those with and without evidence of bone toxicity, respectively ($p = .396$). Of the 41 participants with bone toxicity for whom week 48 TFV-DP data were available, 39% had TFV-DP >700 fmol/punch (''A'' in Table 1).

Effects of vitamin D status and drug exposure on bone toxicity

Analysis using 25-OHD as a continuous variable showed a significantly higher odds of bone toxicity at lower 25-OHD concentrations (''A'' in Table 1). In univariate categorical analyses, black/AA and participants with 25-OHD <20 ng/mL had greater odds of bone toxicity. TFV-DP >700 fmol/punch was not associated with bone toxicity (''A'' in Table 1).

In stratified analysis, the 25-OHD effect was greatest in participants with TFV-DP ≤700, and the TFV-DP effect was greatest in participants with 25-OHD ≥20 (''B'' in Table 1). In multivariable analysis, both variables were significantly associated with bone toxicity, but the association of 25-OHD remained stronger than that of TFV-DP (''C'' in Table 1, model 1); a multiplicative interaction term added to this model was not significant ($p = .29$; not shown). Addition of race improved the model r-square and c-statistics, whereas the relationship of 25-OHD and TFV-DP with bone toxicity remained unchanged (''C'' in Table 1, model 2). A 4-category model showed a gradient of increasing odds of bone toxicity at each level of TFV-DP and 25-OHD (type 3 analysis, $p = .009$; ''A'' in Table 1). Associations of 25-OHD and TFV-DP with bone toxicity remained significant even after adjusting for race (type 3 analysis, $p = .038$; ''C'' in Table 1, model 3).

Discussion

This analysis showed that during 48 weeks of TDF/FTC PrEP in high-risk YMSM, bone toxicity was associated with both 25-OHD insufficiency/deficiency and protective TFV-DP concentrations in stratified and multivariable models. The presence of 25-OHD <20 ng/mL had a stronger effect than that of TFV-DP >700 fmol/punch, but both were significantly associated with bone toxicity. The odds of bone toxicity increased from lowest to highest along a gradient of risk: (1) vitamin D replete with poor TDF adherence/exposure, (2) vitamin D replete with high TDF exposure, (3) vitamin D insufficient/deficient with low TDF exposure, and (4) vitamin D insufficient/deficient with high TDF exposure. Vitamin D insufficiency is a readily modifiable threat to bone health that should be considered when starting PrEP with TDF/FTC.

These data suggest that vitamin D insufficiency should be treated in persons taking TDF/FTC for PrEP. Several regimens are effective in increasing serum 25-OHD concentration. The Endocrine Society recommends a 25-OHD treatment target of ≥30 ng/mL in individuals whose clinical profile includes a threat to bone health,[14] whereas the Institute of Medicine suggests that a 25-OHD concentration of ≥20 is adequate for bone health in healthy populations.[5] A study of youth with HIV treated with TDF-containing combination antiretroviral therapy (TDF-cART) showed increased lumbar spine BMD for 48 weeks with high-dose vitamin D3 supplementation (50,000 IU monthly), reaching a serum 25-OHD concentration of 36.9 (30.5, 42.4) ng/mL, but not with lower dose supplements (400 IU daily), which yielded 25-OHD concentration of 20.6 (14.4, 25.8) ng/mL,[15] independent of initial 25-OHD concentration. Another 48-week study in youth with HIV, 89% treated with TDF-cART, also showed benefit of high-dose vitamin D3 supplementation (2,000 or 4,000 IU daily), reaching 25-OHD ≥30 ng/mL in 80% of participants, with increased lumbar spine BMD Z-score in the high-dose group that was not seen with lower doses.[16] Another study showed that the decrease in BMD with initiation of TDF-cART was attenuated in adults with coadministration of high-dose vitamin D3 (4,000 IU/day) and calcium carbonate (1,000 mg/day) for 48 weeks.[17]

Although a recommendation to administer vitamin D supplementation to all persons treated with TDF could be made based on those studies in persons with HIV infection,[15–17] the data in this report in seronegative individuals suggest but do not prove that there would be benefit of vitamin D supplementation for persons using FTC/TDF for PrEP who have 25-OHD ≥20 ng/mL. Given the added toxicity of TDF even in persons with 25-OHD ≥20, a prospective randomized study of vitamin D supplementation in this setting is warranted.

The finding of increased rate of bone toxicity in black/AA was surprising, particularly given the trend to lower TFV-DP levels in this group. Although we observed significantly

Table 1. Univariate and Multivariable Analyses Examining Association of Serum 25-Hydroxy Vitamin D,[A] Red Blood Cell Tenofovir Diphosphate,[B] and Race with Evidence of Bone Toxicity[C] in the ATN-117 Participants

Variable	N	*Bone toxicity,* n *(%)*	*R-square (%)*[d]	*C-statistic (%)*[e]	*Odds ratio (95% CI)*[f]	p[g]
A. Univariate analysis[h]						
25-OHD (ng/mL), 1 unit decrease	69	47 (68)	8.8	65.4	1.1 (1.0–1.2)	.040
25-OHD (ng/mL)	69	47 (68)	19.6			
<20	44	36 (82)			5.4 (1.9–16.5)	.002
≥20*	25	11 (44)			Reference	
TFV-DP (fmol/punch), 10 units increase	59	41 (69)	3.2	57.0	1.01 (1.00–1.02)	.280
TFV-DP (fmol/punch)	59	41 (69)	1.5	55.6		
>700	21	16 (76)			1.6 (0.5–5.5)	.432
≤700*	38	25 (66)			Reference	
4-Category model of 25-OHD (ng/mL) and TFV (fmol/punch)	59	41 (69)	37.9	79.5		
25-OHD ≥20 and TFV-DP >700	11	10 (91)			8.2 (1.2–99.0)	.033
25-OHD <20 and TFV-DP ≤700	29	24 (83)			25.2 (4.4–276.8)	<.001
25-OHD <20 and TFV-DP >700	10	6 (60)			39.7 (4.6–719.1)	<.001
25-OHD ≥20 and TFV-DP ≤700*	9	1 (11)			Reference	
Race	69	47 (68)	17.4	69.3		
Black	37	31 (84)			4.9 (1.7–15.2)	.003
Other race[i]*	32	16 (50)			Reference	
Ethnicity	69	47 (68)	5.3	60.1		
Hispanic	25	14 (56)			0.4 (0.2–1.2)	.108
Non-Hispanic*	44	33 (75)			Reference	
B. Stratified analysis[j]						
1. Participants with TFV-DP >700 (fmol/punch)	21	16 (76)	17.3	71.3		
25-OHD (ng/mL): <20	11	10 (91)			4.8 (0.7–57.0)	.116
≥20*	10	6 (60)			Reference	
2. Participants with TFV-DP ≤700 (fmol/punch)	38	25 (66)	45.7	78.8		
25-OHD (ng/mL): <20	29	24 (83)			25.2 (4.4–276.8)	<.001
≥20*	9	1 (11)			Reference	
1. Participants with 25-OHD <20 (ng/mL)	40	34 (85)	1.0	56.4		
TFV-DP (fmol/punch): >700	11	10 (91)			1.6 (0.3–16.7)	.639
≤700*	29	24 (83)			Reference	
2. Participants with 25-OHD ≥20 (ng/mL)	19	7 (37)	30.2	76.2		
TFV-DP (fmol/punch): >700	10	6 (60)			8.2 (1.2–99.0)	.033
≤700*	9	1 (11)			Reference	
C. Multivariable analysis[k]						
Model 1 (H-L test, $p = .57$)	59	41 (69)	36.9	79.5		
25-OHD (ng/mL): <20 vs. ≥20*					15.4 (3.8–90.56)	<.001
TFV-DP (fmol/punch): >700 vs. ≤700*					4.5 (1.0–28.9)	.045
Model 2[l] (H-L test, $p = .38$)	59	41 (69)	45.7	85.0		
25-OHD (ng/mL): <20 vs. ≥20*					10.6 (2.4–65.6)	.001
TFV-DP (fmol/punch): >700 vs. ≤700*					5.5 (1.1–40.6)	.033
Race: black vs. other race*					4.7 (1.2–20.5)	.024
Model 3: (H-L test, $p = .62$)	59	41 (69)	46.9	84.1		
25-OHD ≥20 and TFV-DP >700					11.5 (1.4–169.6)	.021
25-OHD <20 and TFV-DP ≤700					19.4 (3.0–228.7)	.001
25-OHD <20 and TFV-DP >700					32.3 (3.3–653.6)	.002
25-OHD ≥20 and TFV-DP ≤700*					Reference	
Race: Black					5.0 (1.3–23.6)	.021
Other race*					Reference	

[a]Baseline value assessed at the time of study enrollment.
[b]Assessed at week 48 after the study enrollment.
[c]See ''Materials and Methods'' section for definition.
[d]Nagelkerke's Max-rescaled R-square reported in PROC LOGISTIC procedure of SAS.
[e]Area under the receiver operating characteristic curve reported in PROC LOGISTIC procedure of SAS.
[f]Profile penalized likelihood CI using Firth's bias correction.
[g]Penalized likelihood ratio test using Firth's bias correction.
[h]Unconditional logistic regression model reporting crude OR.
[i]Included white = 18, American Indian = 10, Pacific Islander = 1, unknown = 3.
[j]Unconditional logistic regression model reporting stratified OR.
[k]Unconditional logistic regression model reporting adjusted OR.
[l]Interaction terms of race with 25-OHD ($p = .33$) and TFV-DP ($p = .32$) when added to Model 2 were not statistically significant (model not shown).
*Reference category.
25-OHD, 25 hydroxy vitamin D; ATN, Adolescent Medicine Trials Network for HIV/AIDS Intervention; CI, confidence interval; H-L: Hosmer–Lemeshow goodness-of-fit test; OR, odds ratio; TFV-DP, tenofovir diphosphate.

lower serum 25-OHD concentrations among black/AA participants, as has been observed previously,[18] multivariable analysis revealed that this group had significantly higher odds of bone toxicity, independent of 25-OHD or TFV-DP concentrations. As the multivariable analyses show, inclusion of race did not change the primary findings of the study.

The current results stem from a secondary analysis, with small number of participants in some categories, so caution should be used in interpretation. Although we collected data on physical activity, tobacco, and alcohol use,[4] these factors were not included in the analysis presented here because of resultant small number in subcategories of exposure. Findings in this cohort of YMSM without HIV infection may not be generalizable to older populations, women, or persons with HIV infection treated with TDF-cART.[7] The predefined bone toxicity criteria for extension phase inclusion were broad and intended to capture all participants showing any evidence of bone loss or less-than-expected bone accrual during adolescence and early adulthood, a period of expected continuing bone growth.[12]

In summary, in multivariable analysis, 25-OHD insufficiency, protective concentrations of TFV-DP, and black/AA race showed statistically significant associations with bone toxicity after 48 weeks of PrEP use in YMSM. Based on these results and published studies of vitamin D supplementation in HIV-infected persons receiving TDF-containing regimens, for persons starting TDF-PrEP, consideration should be given to prospective randomized studies of vitamin D supplementation at doses large enough to reach a serum 25-OHD concentration of ≥30 ng/mL.

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References

1. Grant RM, Anderson PL, McMahan V, *et al.*: Uptake of pre-exposure prophylaxis, sexual practices, and HIV incidence in men and transgender women who have sex with men: A cohort study. Lancet Infect Dis 2014;14:820–829.
2. McComsey GA, Kitch D, Daar ES, *et al.*: Bone mineral density and fractures in antiretroviral-naive persons randomized to receive abacavir-lamivudine or tenofovir disoproxil fumarate-emtricitabine along with efavirenz or atazanavir-ritonavir: AIDS clinical trials group A5224s, a substudy of ACTG A5202. J Infect Dis 2011; 203:1791–1801.
3. Mulligan K, Glidden DV, Anderson PL, *et al.*: Effects of emtricitabine/tenofovir on bone mineral density in HIV-negative persons in a randomized, double-blind, placebo-controlled trial. Clin Infect Dis 2015;61:572–580.
4. Havens PL, Stephensen CB, Van Loan MD, *et al.*: Decline in bone mass with tenofovir disoproxil fumarate/emtricitabine is associated with hormonal changes in the absence of renal impairment when used by HIV-uninfected adolescent boys and young men for HIV preexposure prophylaxis. Clin Infect Dis 2017;64:317–325.
5. Institute of Medicine: *Dietary Reference Intakes for Calcium and Vitamin D.* National Academies Press, Washington, DC, 2011.
6. Rosenvinge MM, Gedela K, Copas AJ, *et al.*: Tenofovir-linked hyperparathyroidism is independently associated with the presence of vitamin D deficiency. J Acquir Immune Defic Syndr 2010;54:496–499.

7. Childs K, Welz T, Samarawickrama A, Post FA: Effects of vitamin D deficiency and combination antiretroviral therapy on bone in HIV-positive patients. AIDS 2012;26: 253–262
8. Havens PL, Stephensen CB, Hazra R, *et al.*: Vitamin D3 decreases parathyroid hormone in HIV-infected youth being treated with tenofovir: A randomized, placebo-controlled trial. Clin Infect Dis 2012;54:1013–1025.
9. Hosek SG, Landovitz RJ, Kapogiannis B, *et al.*: Safety and feasibility of antiretroviral preexposure prophylaxis for adolescent men who have sex with men aged 15 to 17 years in the United States. JAMA Pediatr 2017;05:1063–1071.
10. Hosek SG, Rudy B, Landovitz R, *et al.*: An HIV preexposure prophylaxis demonstration project and safety study for young MSM. J Acquir Immune Defic Syndr 2017; 74:21–29.
11. Zheng JH, Rower C, McAllister K, *et al.*: Application of an intracellular assay for determination of tenofovir-diphosphate and emtricitabine-triphosphate from erythrocytes using dried blood spots. J Pharm Biomed Anal 2016; 122:16–20.
12. Zemel BS, Kalkwarf HJ, Gilsanz V, *et al.*: Revised reference curves for bone mineral content and areal bone mineral density according to age and sex for black and non-black children: Results of the bone mineral density in childhood study. [Erratum appears in J Clin Endocrinol Metab 2013;98:420]. J Clin Endocrinol Metab 2011;96: 3160–3169.
13. Stephensen CB, Zerofsky M, Burnett DJ, *et al.*: Ergocalciferol from mushrooms or supplements consumed with a standard meal increases 25-hydroxyergocalciferol but decreases 25-hydroxycholecalciferol in the serum of healthy adults. J Nutr 2012;142:1246–1252.
14. Holick MF, Binkley NC, Bischoff-Ferrari HA, *et al.*: Evaluation, treatment, and prevention of vitamin D deficiency: an Endocrine Society clinical practice guideline. [Erratum appears in J Clin Endocrinol Metab 2011;96: 3908]. J Clin Endocrinol Metab 2011;96:1911–1930.
15. Havens PL, Stephensen CB, Van Loan MD, *et al.*: Vitamin D3 supplementation increases spine bone mineral density in adolescents and young adults with HIV infection being treated with tenofovir disoproxil fumarate: a randomized, placebo-controlled trial. Clin Infect Dis 2017;64:317–325.
16. Eckard AR, O'Riordan MA, Rosebush JC, *et al.*: Effects of vitamin D supplementation on bone mineral density and bone markers in HIV-infected youth. J Acquir Immune Defic Syndr 2017;11:11.
17. Overton ET, Chan ES, Brown TT, *et al.*: Vitamin D and calcium attenuate bone loss with antiretroviral therapy initiation: a randomized trial. Ann Intern Med 2015;162: 815–824.
18. Gutiérrez OM, Farwell WR, Kermah D, Taylor EN: Racial differences in the relationship between vitamin D, bone mineral density, and parathyroid hormone in the National Health and Nutrition Examination Survey. Osteoporos Int 2011;22:1745–1753.

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