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Circulating sclerostin is associated with bone mineral density independent of HIV-serostatus

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ABSTRACT

Background: Low bone mineral density (BMD) is commonly observed in people living with HIV (PLWH), however the cause for this BMD loss remains unclear. Sclerostin, a bone-derived antagonist to the Wnt/β-catenin-pathway, suppresses bone remodeling and is positively associated with BMD. The goal of the current study was to investigate associations between sclerostin and BMD in a cohort of HIV-seropositive and demographically-matched seronegative women.

Methods: This cross-sectional analysis used a subset of early postmenopausal women enrolled in the Women's Interagency HIV Study (WIHS). BMD was assessed at the lumbar spine, total hip, femoral neck, and distal and ultradistal radius via dual energy x-ray absorptiometry (DXA). Circulating sclerostin was assessed via commercial ELISAs. Univariate and multivariate linear regression modeling tested associations between sclerostin and BMD after adjusting for a variety of BMD-modifying variables.

Results: HIV-seropositive women had significantly reduced BMD at all skeletal sites compared to HIV-seronegative women. There was no difference in sclerostin levels according to HIV-serostatus (0.25 vs 0.27 ng/mL in HIV-seronegative and HIV-seropositive, respectively, $p = 0.71$). Circulating sclerostin was positively associated with BMD at all sites in both univariate and multivariate models adjusting for HIV status, age, BMI, and race, although the coefficients of association were attenuated in HIV-seropositive women. The positive association between sclerostin and BMD among seropositive women remained statistically significant after adjusting for ART or tenofovir disoproxil fumarate (TDF) use.

Conclusions: The current study suggests that circulating sclerostin is a biomarker for bone mass for both HIV seronegative and seropositive women using and not using ART. The lower coefficients of association between sclerostin and BMD by HIV status may suggest HIV-induced alternation in osteocyte function.

1. Introduction

Due to antiretroviral therapy (ART) advances, there has been a sharp increase in lifespan of people living with HIV (PLWH). In the past decade alone there has been a near four-fold increase in the number of PLWH over 50 years old in North America and Europe (Mahy et al., 2014) with further increases expected by 2030 (Smit et al., 2015). As a result, the prevalence of age-related HIV-associated comorbidities, such as osteoporosis, is increasing (Costagliola, 2014).

The prevalence of osteoporosis is higher in PLWH compared to uninfected controls, and ART use also appears to contribute to BMD loss (Brown and Qaqish, 2006). Despite well-described observational associations, mechanisms linking HIV infection, ART treatment, and bone loss remain uncertain. However, the Wnt/β-catenin signaling pathway

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is a critical mediator of bone mass (Zhong et al., 2014) that may be affected by HIV infection.

Skeletal Wnt/β-catenin signaling is regulated locally by osteocytes embedded within the bone matrix via production of sclerostin, a soluble antagonist to Wnt/β-catenin signaling (Ellies et al., 2006; Li et al., 2005; Semenov et al., 2005). Due to potent suppression of bone formation by sclerostin, antibody-based therapeutics targeting sclerostin have been developed as bone-building osteoporosis treatments (Ke et al., 2012). Despite sclerostin's role as a bone formation antagonist, sclerostin is positively associated with BMD in many diseases (Cejka et al., 2011; Thambiah et al., 2012; Malluche et al., 2014; Ishimura et al., 2014; He et al., 2014; Voskaridou et al., 2012). Sclerostin levels were reported to be positively associated with BMD in a cohort of PLWH (Erlandson et al., 2015). However, the previous study did not include an HIV-seronegative comparison group and thus could not assess whether the association between sclerostin and BMD is influenced by HIV-serostatus.

The goal of the current study was to determine associations between sclerostin and BMD in a cohort HIV-seropositive and HIV-seronegative women in early menopause, a population at particularly high risk for bone loss and osteoporosis. HIV-status has been reported to influence bone mass (Goh et al., 2018), as well as, the circulating levels of sclerostin (Almansouri et al., 2016; Mora et al., 2015). Additionally, ART use is associated with loss of bone mass, although the magnitude is dependent on the class of ART used (Brown and Qaqish, 2006). The effects of ART use on circulating sclerostin levels are not well known, although one study reported a mild increase in sclerostin after switching from tenofovir disoproxil fumarate (TDF) to abacavir (Negredo et al., 2015), which is likely a function of the increased bone mass noted after switching off of TDF (Negredo et al., 2014). Due to the strong associations between sclerostin and BMD and the previously reported influence of both HIV and ART on BMD, we hypothesize that the association between sclerostin and BMD are influenced by both HIV-serostatus and ART.

2. Materials and methods

2.1. Study participants

The current musculoskeletal (MSK) study was nested within the Women's Interagency HIV Study (WIHS) at the Bronx, San Francisco, and Chicago sites. The WIHS is an ongoing multisite longitudinal cohort study of women with and at risk for HIV infection that has recruited in waves since its initiation in 1993. As of 2015, a total of 4982 women have been recruited to participate in the cohort (Adimora et al., 2018). A total of 220 women (137 HIV-seropositive and 83 HIV-seronegative) were enrolled into the baseline MSK study between 2011 and 2014 and were included in this analysis. At the time of enrollment, all participants were between age 40–60, and either peri-menopausal or post-menopausal by self-report according to SWAN study definitions (Finkelstein et al., 2008): early perimenopause (at least 1 menstrual period in the last 3 months with some change in the regularity over last 12 months), late perimenopause (no bleeding in 3–11 of the last 12 months), or early postmenopausal (no bleeding for > 1 but < 5 years). Since Finkelstein et al. (2008) found that the rate of bone loss was similar in premenopause and early perimenopause, but lower than that of late perimenopause and early postmenopause, we categorized menopausal status into those in early perimenopause and those that were in postmenopause (late peri-menopause and early post-menopause), Additional study entry criteria included weight < 264 pounds and height 6′1″ or below (due to limitations of our DXA testing), and for HIV-seropositive women, the most recent CD4 count had to be > 100 cells/μL, and ART used for the prior year without missing > 2.5 months of therapy during that year.

2.2. Bone mineral density (BMD) assessment

Participants underwent whole body DXA. DXA scans performed on Lunar Prodigy densitometers (GE Medical Systems, Madison WI) at all study locations. Scans were read centrally at the Image Analysis Lab (New York, NY). Height and weight were measured using a stadiometer and balance beam scale.

2.3. Blood biomarkers

Blood samples were collected using sodium citrate coated tubes, separated into plasma aliquots, stored at −80 °C, then thawed and batch-analyzed at the Irving Columbia University Irving Medical Center Biomarker Laboratory and at Rush University Medical Center. We measured circulating sclerostin (TECOmedical ELISA, Sissach, Switzerland); N-terminal propeptide of procollagen type 1 (P1NP; RIA; IDS, Scottsdale, AZ); C-telopeptide of type 1 collagen (CTX, ELISA, IDS Scottsdale, AZ), tumor necrosis factor alpha (TNFα; ELISA, R&D Systems, Minneapolis, MN); and interleukin-6 (IL-6; ELISA, R&D Systems, Minneapolis, MN).

2.4. Additional participant characteristics

Cumulative ART exposure was categorized by class; (protease inhibitor (PI), nucleoside reverse transcriptase inhibitor (NRTI), non-nucleoside reverse transcriptase inhibitor (NNRTI), tenofovir disoproxil fumarate (TDF), and abacavir) use prior to MSK enrollment (in years) was determined from participant reported ART use. Body mass index (BMI) was calculated at MSK enrollment from objectively measured height and weight.

2.5. Statistical analysis

The primary outcomes of interest were areal BMD at the lumbar spine, total hip, femoral neck, distal radius and ultradistal radius, and the primary predictor of interest was circulating plasma sclerostin levels. Other covariates included age (continuous), race (Black, White, Other), WIHS study locations (Bronx, San Francisco, Chicago), menopause status at MSK enrollment, and the circulating levels of CTX and P1NP. Characteristics of HIV-seropositive vs. seronegative women were compared using exact tests for proportions and rank tests for continuous variables. Pearson's correlations quantified the association of sclerostin with CTX, P1NP, IL-6, and TNFα. Univariate linear regression models were fit between sclerostin and other variables of interest and BMD at each skeletal site. Hierarchical multivariate linear models were fit for each BMD site, with sclerostin as the primary predictor of interest and demographic and potential BMD-modifying variables as potential confounding variables. Due to a strong correlation between CTX and P1NP, and the significant association between CTX and sclerostin, only CTX was included in the multivariate models.

3. Results

3.1. Participant characteristics

The mean age was 49.3 for HIV-seronegative and 49.9 years for seropositive women. HIV seropositive and seronegative women did not statistically differ by race, study site, menopausal status at MSK enrollment, BMI, or hepatitis C infection, defined using HCV antibody and confirmed with HCV RNA (Table 1). Circulating levels of inflammatory cytokines (TNFα and IL-6) did not differ by HIV-serostatus. Compared to HIV-seronegative participants, HIV-seropositive women had both significantly lower BMD at each skeletal site measured and higher plasma levels of bone remodeling biomarkers, CTX and P1NP (Table 1). There were no significant differences between HIV-seropositive and HIV-seronegative women in the circulating levels of sclerostin.

Table 1
Characteristics of HIV seronegative and seropositive women.

	HIV uninfected	HIV infected	p-Value
Sample size (n)	83	137	
Age (yr)	49.3	49.9	0.18
[media (Q1, Q3)]	(43.9, 53.0)	(46.9, 53.3)	
Race (n)			
White	11 (13.25%)	22 (16.06%)	0.66
Black	60 (72.29%)	91 (66.42%)	
Other	12 (14.46%)	24 (17.52%)	
Enrollment site (n)			
Bronx NYC	32 (38.55%)	54 (39.42%)	0.95
San Francisco	35 (42.17%)	55 (40.15%)	
Chicago	16 (19.28%)	28 (20.44%)	
Menopausal status (n)			
Premenopausal/early perimenopause	34 (40.96%)	46 (33.58%)	0.27
Late perimenopause/post-menopausal	49 (59.04%)	91 (66.42%)	
BMI (kg/m2)			
< 18.5 = thin	2 (2.41%)	2 (1.46%)	0.42
18.5–24.9 = normal	13 (15.66%)	33 (24.09%)	
25.0–29.9 = overweight	24 (28.92%)	41 (29.93%)	
≥20.0 = obese	44 (53.01%)	61 (44.53%)	
Hepatitis C virus infection (n)			
Negative	62 (74.70%)	91 (66.42%)	0.087
Resolved infection			
(Ab & RNA negative)	3 (3.61%)	11 (8.03%)	
Ab & RNA unknown	3 (3.61%)	16 (11.68%)	
Active infection (RNA positive)	15 (18.07%)	19 (13.87%)	
Inflammatory cytokines (ng/mL)			
TNFα [mean (SD)]	1.29 (0.58)	1.51 (0.88)	0.10
IL-6 [mean (SD)]	0.04 (0.03)	0.04 (0.03)	0.11
Bone turnover markers (ng/mL)			
P1NP [mean (SD)]	0.52 (0.28)	0.60 (0.30)	**0.019**
CTX [mean (SD)]	0.30 (0.21)	0.35 (0.19)	**0.015**
Bone mineral density (g/cm^2)			
Weight bearing bones			
Lumbar spine [mean (SD)]	1.29 (0.21)	1.20 (0.21)	**0.004**
Total hip [mean (SD)]	1.08 (0.16)	1.02 (0.15)	**0.009**
Femoral neck [mean (SD)]	1.15 (0.18)	1.09 (0.17)	**0.027**
Non-weight bearing bones			
Distal radius [mean (SD)]	0.84 (0.11)	0.81 (0.14)	0.27
Ultradistal radius [mean (SD)]	0.45 (0.07)	0.42 (0.09)	**0.003**
Sclerostin (ng/mL) [mean (SD)]	0.25 (0.14)	0.27 (0.16)	0.71
CD4 count (cells/mL)	NA	566	NA
[Median, Q1, Q3]		(425, 762)	
CD4 nadir (cells/mL)	NA	248	NA
[Median, Q1, Q3]		(152, 367)	
HIV RNA viral load	NA	20	NA
[Median, Q1, Q3]		(20, 120)	
Cumulative PI (years)	NA	3	NA
[Median, Q1, Q3]		(0, 9.5)	
Cumulative NRTI (years)	NA	10	NA
[Median, Q1, Q3]		(3, 13.5)	
Cumulative NNRTI (years)	NA	2.5	NA
[Median, Q1, Q3]		(0, 6)	
Cumulative tenofovir disoproxil fumarate (TDF,years)	NA	2	NA
[Median, Q1, Q3]		(0, 6.5)	
Cumulative abacavir (years)	NA	11	NA
[Median, Q1, Q3]		(3.5, 15.5)	

Abbreviations: BMI - body mass index, Ab - antibody, TNFα - tumor necrosis factor α, IL-6 - interleukin 6, P1NP - Procollagen type 1 N-terminal propeptide, CTX – C-telopeptide of type I collagen.
Bolded values indicate $p < 0.05$.

Circulating sclerostin was positively associated with age (Coefficient: 0.03, $p = 0.002$) and this relationship was not affected by HIV-serostatus.

3.2. Relationship between sclerostin and BMD

Univariate modeling demonstrated significant positive associations ($p < 0.001$) between sclerostin and BMD at all skeletal sites, indicating women with higher levels of sclerostin tended to have higher BMD (Table 2). In nearly all skeletal sites, the associations between sclerostin and BMD were more significant than several known BMD-modifying variables, such as a HIV-serostatus, age, and BMI. Sclerostin was negatively associated with CTX ($\rho = -0.138$, $p = 0.041$) and positively associated with TNFα ($\rho = 0.181$, $p = 0.007$), but not statistically associated with P1NP and IL-6. Cumulative PI, NRTI, NNRTI, and abacavir exposure were not significantly associated with BMD (Table 3). The associations between cumulative TDF and BMD were statistically significant in the lumbar spine and the ultradistal radius. No class of ART, including TDF, was statistically associated with circulating sclerostin levels (Table 3).

The positive associations between sclerostin and BMD at each site remained statistically significant in fully adjusted multivariate models (Table 4). In each of the four models in Table 4, the sclerostin regression coefficients were larger in the weight bearing BMD sites such as lumbar spine, total hip and femoral neck when compared to the non-weight bearing sites, such as distal and ultradistal radius. The regression coefficients between sclerostin and BMD were greater in HIV-seronegative women (Model 2 in Table 4) compared to HIV-seropositives (Models 3 & 4 in Table 4) in the weight bearing skeletal sites of the lumbar spine, total hip and femoral neck, and unchanged in the distal and ultradistal radius. In models restricted to women with HIV, adding cumulative TDF exposure did not attenuate the associations between sclerostin and BMD.

4. Discussion

Our study investigated the relationship between circulating levels of sclerostin and BMD in HIV-seropositive and HIV-seronegative women. We demonstrated not only that higher circulating sclerostin is associated with higher BMD at several skeletal sites prone to fracture, but that these relationships are more statistically significant than are those of many known BMD-influencing factors, such as menopausal status and BMI. The association between sclerostin and BMD remained statistically significant in fully adjusted models, although the relationships were stronger in weight bearing skeletal sites when compared to non-weight bearing sites. Interestingly, the qualitatively attenuated model coefficients in HIV-seronegative (vs seropositive) women suggests a potential HIV-induced alteration in osteocyte expression of sclerostin, particularly in weight bearing skeletal sites.

Sclerostin is an osteocyte-derived antagonist to the Wnt/β-catenin signaling pathway (Li et al., 2005). Wnt/β-catenin signaling is inhibited due to HIV-infection (Al-Harthi, 2012) and HIV viral proteins inhibit osteoblast function, in part, through Wnt inhibition (Butler et al., 2013). Therefore, increased sclerostin expression and the subsequent inhibition of Wnt/β-catenin signaling may be a potential mechanism by which HIV infection could cause bone loss. However, the only other study to compare sclerostin levels according to HIV-serostatus reported that HIV-seropositive ART-naive men and women had lower circulating sclerostin than did HIV-seronegatives (Almansouri et al., 2016). In contrast to the results from Almansouri et al. (2016), we found no difference in the levels of circulating sclerostin when comparing HIV-seropositives to HIV-seronegatives. The reason for this discrepancy could be due to many factors. Our cohort included only women that were in peri- and early postmenopause and most of the HIV seropositive women were on ART. The average age of women in our study was 49 years compared to 38 years in the other (Almansouri et al., 2016); sclerostin levels are highly associated with age (Modder et al., 2011; Amrein et al., 2012), which we confirm in our study.

Despite the bone formation suppressing function of sclerostin (Balemans et al., 2001; Balemans et al., 2002; Loots et al., 2005), it has been well-established that higher circulating sclerostin levels are associated with increased BMD, including in a cohort of HIV-seropositive participants (Erlandson et al., 2015). The reason for this seeming contradiction may be due to the timing of the sclerostin measurements.

Table 2
Univariate linear regression associations with bone mineral density among HIV-seropositive and seronegative WIHS participants.

Variable	Weight bearing bones						Non-weight bearing bones			
	Lumbar spine		Total hip		Femoral neck		Distal radius		Ultradistal radius	
	Coefficient (95% CI)	p-Value	Coefficient (95% CI)	p-Value	Coefficient (95% CI)	p-Value	Coefficient (95% CI)	p-Value	Coefficient (95% CI)	p-Value
Sclerostin	0.47 (0.29, 0.64)	< 0.001	0.31 (0.17, 0.44)	< 0.001	0.34 (0.19, 0.49)	< 0.001	0.21 (0.10, 0.33)	< 0.001	0.14 (0.08, 0.22)	< 0.001
HIV Status	−0.09 (−0.15, −0.03)	0.002	−0.07 (−0.11, −0.02)	0.003	−0.06 (−0.11, −0.02)	0.010	−0.03 (−0.06, 0.01)	0.21	−0.04 (−0.06, −0.02)	0.002
Age (per 5-year)	−0.04 (−0.06, −0.01)	0.010	−0.02 (−0.04, 0.01)	0.064	−0.01 (−0.03, 0.01)	0.40	−0.01 (−0.02, 0.02)	0.77	−0.01 (−0.02, 0.01)	0.11
BMI (per 5-unit)	0.04 (0.01, 0.06)	0.003	0.05 (0.03, 0.07)	< 0.001	0.04 (0.02, 0.06)	< 0.001	0.03 (0.02, 0.05)	< 0.001	0.03 (0.02, 0.04)	< 0.001
Race: white vs black	−0.06 (−0.14, 0.02)	0.13	−0.05 (−0.11, 0.01)	0.10	−0.09 (−0.15, −0.02)	0.013	−0.07 (−0.12, −0.02)	0.010	−0.04 (−0.07, −0.01)	0.026
Race: other vs black	−0.11 (−0.19, −0.03)	0.006	−0.05 (−0.11, 0.02)	0.16	−0.05 (−0.12, 0.02)	0.13	−0.04 (−0.09, 0.01)	0.14	−0.01 (−0.04, 0.03)	0.78
Menopause	−0.11 (−0.17, −0.06)	< 0.001	−0.07 (−0.11, −0.02)	0.004	−0.04 (−0.09, 0.01)	0.10	−0.02 (−0.07, 0.01)	0.15	−0.03 (−0.05, −0.01)	0.032
IL-6	−0.10 (−0.96, 0.77)	0.83	0.12 (−0.54, 0.77)	0.73	0.26 (−0.47, 0.99)	0.48	0.23 (−0.32, 0.79)	0.41	0.24 (−0.11, 0.60)	0.18
TNFα	−0.01 (−0.04, 0.03)	0.65	0.01 (−0.02, 0.03)	0.80	−0.01 (−0.04, 0.02)	0.63	−0.01 (−0.03, 0.02)	0.85	−0.01 (−0.03, 0.01)	0.23
PINP	−0.18 (−0.27, −0.08)	< 0.001	−0.13 (−0.21, −0.06)	< 0.001	−0.11 (−0.19, −0.03)	0.005	−0.07 (−0.13, −0.01)	0.021	−0.07 (−0.11, −0.04)	< 0.001
CTX	−0.28 (−0.42, −0.14)	< 0.001	−0.23 (−0.33, −0.12)	< 0.001	−0.22 (−0.34, −0.10)	< 0.001	−0.17 (−0.26, −0.08)	< 0.001	−0.17 (−0.22, −0.11)	< 0.001

Table 3
Univariate associations between ART exposure and BMD and sclerostin among HIV positive participants.

Variable (per month)	Weight bearing bones						Non-weight bearing bones				Sclerostin	
	Lumbar spine		Total hip		Femoral Neck		Distal radius		Ultradistal radius			
	Coefficient (95% CI)	p-Value	Coefficient (95% CI)	p-Value	Coefficient (95% CI)	p-Value	Coefficient (95% CI)	p-Value	Coefficient (95% CI)	p-Value	Coefficient (95% CI)	p-Value
Cumulative PI	−0.02 (−0.09, 0.06)	0.66	0.01 (−0.04, 0.06)	0.66	−0.03 (−0.09, 0.02)	0.24	−0.04 (−0.09, 0.01)	0.12	−0.01 (−0.04, 0.03)	0.77	−0.02 (−0.09, 0.06)	0.66
Cumulative NRTI	−0.03 (−0.10, 0.03)	0.35	0.02 (−0.03, 0.07)	0.47	−0.03 (−0.08, 0.02)	0.26	−0.04 (−0.08, 0.01)	0.10	−0.02 (−0.04, 0.01)	0.29	−0.03 (−0.10, 0.03)	0.35
Cumulative NNRTI	−0.04 (−0.13, 0.05)	0.37	−0.03 (−0.09, 0.04)	0.43	−0.04 (−0.11, 0.04)	0.34	−0.04 (−0.10, 0.03)	0.27	−0.02 (−0.07, 0.01)	0.20	−0.04 (−0.13, 0.05)	0.37
Cumulative abacavir	−0.03 (−0.08, 0.03)	0.32	0.02 (−0.2 0.06)	0.33	−0.01 (−0.05, 0.05)	0.96	−0.01 (−0.05, 0.03)	0.52	0.01 (−0.02, 0.03)	0.66	0.02 (−0.02, 0.06)	0.38
Cumulative TDF	−0.13 (−0.23, −0.04)	**0.008**	−0.03 (−0.11, 0.04)	0.37	−0.07 (−0.15, 0.02)	0.11	−0.07 (−0.14, 0.01)	0.053	−0.05 (−0.09, −0.01)	**0.043**	−0.13 (−0.23, −0.04)	**0.008**

Bolded values indicate $p < 0.05$.

Morse et al. (Morse et al., 2012) investigated sclerostin concentrations in spinal cord injury (SCI) patients, a clinical situation where an incident injury triggers rapid bone loss, and proposed a phasic sclerostin reponse. In SCI, they suggested that there is an early acute elevation of sclerostin, which likely represents its mechanistic role in suppressing bone formation, followed by a chronic phase where sclerostin levels are lowered and become more indicative of sclerostin's role as a biomarker of BMD (Morse et al., 2012). After stabilization, sclerostin levels likely reflect the number of osteocytes (Cejka et al., 2011; Costa et al., 2013), the bone cell responsible for the production of sclerostin.

The potential reduction in the association between sclerostin and BMD in HIV-seropositive participants compared to HIV-seronegatives, while not statistically significant, may suggest HIV-induced alterations to osteocyte physiology. However, in the current study, we found no HIV-serostatus differences in the amount of circulating sclerostin, despite a clear reduction in bone mass in HIV-seropositives, which is generally associated with a reduction in sclerostin. Therefore, it is possible that HIV infection induces an increase in the amount of sclerostin produced per osteocyte. Mödder et al. (Mödder et al., 2011) came to a similar conclusion with respect to age when comparing the association between sclerostin and BMD in aging cohorts, suggesting that with aging, there is an increase in the production of sclerostin per individual osteocyte. Although HIV (Gibellini et al., 2008) and viral products (Cotter et al., 2007) have been shown to alter osteoblast physiology, it remains to be determined whether osteocytes, which are terminally differentiated osteoblasts, are similarly affected by HIV, especially in the ART era where there is maximum viral suppression and little to no-viral proteins secreted.

We also found that the association between sclerostin and BMD, at least qualitatively, varied according to the skeletal site assessed. Specifically, the associations were stronger in the weight bearing skeletal sites of the lumbar spine, total hip, and femoral neck, when compared to the non-weight bearing skeletal sites of the distal and ultradistal radius, a finding which is consistent with reports in a population cohort of men and women participants (Mödder et al., 2011). The skeletal sites that were most highly associated with sclerostin levels

Table 4
Multivariate associations between sclerostin and BMD at various skeletal sites.

	Weight bearing bones						Non-weight bearing bones			
	Lumbar spine		Total hip		Femoral neck		Distal radius		Ultradistal radius	
	Coefficient (95% CI)	p-Value	Coefficient (95% CI)	p-Value	Coefficient (95% CI)	p-Value	Coefficient (95% CI)	p-Value	Coefficient (95% CI)	p-Value
Total cohort (n = 212)										
Model 1	0.50 (0.33, 0.68)	< **0.001**	0.32 (0.19, 0.44)	< **0.001**	0.30 (0.15, 0.45)	< **0.001**	0.18 (0.07, 0.30)	**0.003**	0.15 (0.08, 0.21)	< **0.001**
HIV negative cohort (n = 80)										
Model 2	0.87 (0.54, 1.19)	< **0.001**	0.51 (0.24, 0.79)	< **0.001**	0.54 (0.22, 0.85)	**0.001**	0.17 (−0.09, 0.33)	0.27	0.14 (0.01, 0.26)	**0.023**
HIV positive cohort (n = 132)										
Model 3	0.36 (0.15, 0.57)	**0.001**	0.24 (0.10, 0.39)	< **0.001**	0.20 (0.02, 0.38)	**0.030**	0.21 (0.07, 0.36)	**0.005**	0.15 (0.06,0.23)	< **0.001**
Model 4	0.35 (0.14, 0.56)	**0.001**	0.25 (0.10, 0.39)	**0.001**	0.20 (0.02, 0.38)	**0.030**	0.21 (0.07, 0.36)	**0.005**	0.15 (0.06, 0.23)	**0.010**

Model 1 adjusts for HIV status, age, BMI, race, site and CTX. Model 2 adjusted for age, BMI, race, site, and CTX. Model 3 adjusts for age, BMI, race site, CTX, and cumulative Tenofovir.
Bolded values indicate $p < 0.05$.

were those with considerable trabecular bone, which may indicate that sclerostin levels are more sensitive to trabecular bone parameters, a finding consistent with published reports in haemodialysis patients (Cejka et al., 2011).

Initiation of ART is reported to cause bone mass losses (Brown and McComsey, 2006). TDF in particular, is associated with a greater reduction in BMD within the first year of initiation when compared to non-TDF containing ART regiments (Grant and Cotter, 2016). Switching from TDF to abacavir leads to increased circulating sclerostin levels (Negredo et al., 2015) and moderately increased BMD (Negredo et al., 2014). In the current study, TDF use was associated with reduced BMD, while the association between abacavir and BMD was not statistically significant. There were no associations of either TDF or abacavir with sclerostin. Due to the association between TDF and BMD, we included cumulative TDF in fully adjusted models and found no effect on the relationship between sclerostin and BMD, suggesting that in the current cohort, any effects of TDF on bone are independent of sclerostin.

This study is the first to assess the association between sclerostin and BMD according to HIV-serostatus. Among its strengths were that the cohort included postmenopausal women, a group at particularly high risk for osteoporosis. The study limitations include the use of a single sex cohort and therefore, the results may not be generalizable to men, and that sclerostin and bone density measures were only available at one time point so longitudinal trends could not be evaluated. There have been several studies comparing the validity of commercially available ELISA assays to measure circulating sclerostin and we chose the TECOmedical assay due to its consistently good performance characteristics (Costa et al., 2014; Piec et al., 2016). Additionally, the TECOmedical assay is able to detect sclerostin in the sodium citrate stabilized plasma samples used in this study. Although EDTA stabilized plasma is considered the preferred matrix for measuring circulating sclerostin, its performance in sodium citrate stabilized plasma has not been reported. Further, it is possible that the TECOmedical assay also detected sclerostin fragments, so we cannot confirm that all of the sclerostin measured is indeed biologically active (Costa et al., 2014).

As PLWH age, there is an increased prevalence of HIV-associated comorbidities, including low BMD. Understanding mechanism(s) that drive bone loss are critical to inform therapeutic strategies to ameliorate and/or reduce the risk of bone loss and associated fracture risks. These mechanisms may or may not be HIV-specific. Indeed, we report here that Wnt/β-catenin signaling as assessed by circulating sclerostin could be a potential biomarker for bone mass, independent of HIV status. However, the reduction in model estimate as a function of HIV-serostatus may point to an increased per cell production of sclerostin in HIV-seropositive women. As such, agents that activate Wnt/β-catenin signaling in bone may serve as viable therapeutic approach to reduce and/or protect against bone loss among aging individuals, including in PLWH.

Transparency document

The Transparency document associated with this article can be found, in online version.

CRediT authorship contribution statement

Ryan D. Ross: Conceptualization, Investigation, Data curation, Writing - original draft, Writing - review & editing, Project administration, Funding acquisition. **Anjali Sharma:**Conceptualization, Methodology, Writing - review & editing, Funding acquisition. **Qiuhu Shi:**Methodology, Data curation, Formal analysis, Writing - review & editing. **Donald R. Hoover:**Conceptualization, Methodology, Formal analysis, Writing - review & editing. **Kathleen M. Weber:**Conceptualization, Writing - review & editing, Funding acquisition. **Phyllis C. Tien:**Conceptualization, Writing - review & editing, Funding acquisition. **Audrey L. French:**Conceptualization, Writing - review & editing, Funding acquisition. **Lena Al-Harthi:**Conceptualization, Writing - review & editing, Funding acquisition. **Michael T. Yin:**Conceptualization, Methodology, Writing - review & editing, Project administration, Funding acquisition.

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References

Adimora, A.A., Ramirez, C., Benning, L., Greenblatt, R.M., Kempf, M.-C., Tien, P.C., et al., 2018. Cohort profile: the Women's Interagency HIV Study (WIHS). Int. J. Epidemiol. 47 (2) (393-4i).

Al-Harthi, L., 2012. Interplay between Wnt/beta-catenin signaling and HIV: virologic and biologic consequences in the CNS. J. NeuroImmune Pharmacol. 7 (4), 731–739 Dec.

Almansouri, A.Y., Abdulfatah, M.E., Baaqil, O.H., Bakheet, A.A., Turki, S.A., Kotb, M.M., et al., Feb 2016. Serum sclerostin levels in patients with human immunodeficiency virus infection and their association with bone turnover markers and bone mineral densitometry. J. Bone Metab. 23 (1), 16–22 (Epub 2016/03/17).

Amrein, K., Amrein, S., Drexler, C., Dimai, H.P., Dobnig, H., Pfeifer, K., et al., Jan 2012. Sclerostin and its association with physical activity, age, gender, body composition, and bone mineral content in healthy adults. J. Clin. Endocrinol. Metab. 97 (1), 148–154 (Epub 2011/10/14).

Balemans, W., Ebeling, M., Patel, N., Van Hul, E., Olson, P., Dioszegi, M., et al., 2001. Increased bone density in sclerosteosis is due to the deficiency of a novel secreted protein (SOST). HumMolGenet 10 (5), 537–543.

Balemans, W., Patel, N., Ebeling, M., Van Hul, E., Wuyts, W., Lacza, C., et al., Feb 2002. Identification of a 52 kb deletion downstream of the SOST gene in patients with van Buchem disease. J. Med. Genet. 39 (2), 91–97 (Epub 2002/02/12).

Brown, T.T., McComsey, G.A., 2006. Osteopenia and osteoporosis in patients with HIV: a review of current concepts. Curr. Infect. Dis. Rep. 8 (2), 162–170 Mar.

Brown, T.T., Qaqish, R.B., 2006. Antiretroviral therapy and the prevalence of osteopenia and osteoporosis: a meta-analytic review. AIDS 20 (17), 2165–2174 Nov 14.

Butler, J.S., Dunning, E.C., Murray, D.W., Doran, P.P., O'Byrne, J.M., 2013. HIV-1 protein induced modulation of primary human osteoblast differentiation and function via a Wnt/beta-catenin-dependent mechanism. J. Orthop. Res. 31 (2), 218–226 Feb.

Cejka, D., Jäger-Lansky, A., Kieweg, H., Weber, M., Bieglmayer, C., Haider, D.G., et al., 2011. Sclerostin serum levels correlate positively with bone mineral density and microarchitecture in haemodialysis patients. Nephrol. Dial. Transplant. 27 (1), 226–230.

Costa, A.G., Walker, M.D., Zhang, C.A., Cremers, S., Dworakowski, E., McMahon, D.J., et al., 2013. Circulating sclerostin levels and markers of bone turnover in Chinese-American and white women. J. Clin. Endocrinol. Metab. 98 (12), 4736–4743 (Epub 2013/09/13).

Costa, A.G., Cremers, S., Dworakowski, E., Lazaretti-Castro, M., Bilezikian, J.P., 2014. Comparison of two commercially available ELISAs for circulating sclerostin. Osteoporos. Int. 25 (5), 1547–1554 2014/05/01.

Costagliola, D., 2014. Demographics of HIV and aging. Curr. Opin. HIV AIDS 9 (4), 294–301 Jul.

Cotter, E.J., Malizia, A.P., Chew, N., Powderly, W.G., Doran, P.P., Dec 2007. HIV proteins regulate bone marker secretion and transcription factor activity in cultured human osteoblasts with consequent potential implications for osteoblast function and development. AIDS Res. Hum. Retrovir. 23 (12), 1521–1530 (Epub 2007/12/28).

Ellies, D.L., Viviano, B., McCarthy, J., Rey, J.P., Itasaki, N., Saunders, S., et al., 2006. Bone density ligand, Sclerostin, directly interacts with LRP5 but not LRP5G171V to modulate Wnt activity. J. Bone Miner. Res. 21 (11), 1738–1749.

Erlandson, K.M., O'Riordan, M., Hileman, C.O., Rapaport, E., Labbato, D., Campbell, T.B., et al., Jul 2015. Plasma sclerostin in HIV-infected adults on effective antiretroviral therapy. AIDS Res. Hum. Retrovir. 31 (7), 731–738.

Finkelstein, J.S., Brockwell, S.E., Mehta, V., Greendale, G.A., Sowers, M.R., Ettinger, B., et al., Mar 2008. Bone mineral density changes during the menopause transition in a multiethnic cohort of women. J. Clin. Endocrinol. Metab. 93 (3), 861–868 (Epub 2007/12/28).

Gibellini, D., De Crignis, E., Ponti, C., Cimatti, L., Borderi, M., Tschon, M., et al., 2008. HIV-1 triggers apoptosis in primary osteoblasts and HOBIT cells through TNFalpha activation. J. Med. Virol. 80 (9), 1507–1514 Sep.

Goh, S.S.L., Lai, P.S.M., Tan, A.T.B., Ponnampalavanar, S., 2018. Reduced bone mineral density in human immunodeficiency virus-infected individuals: a meta-analysis of its prevalence and risk factors: supplementary presentation. Osteoporos. Int. 29 (7), 1683 Jul. (Epub 2018/05/09).

Grant, P.M., Cotter, A.G., 2016. Tenofovir and bone health. Curr. Opin. HIV AIDS 11 (3), 326–332.

He, J., Zhang, H., Wang, C., Zhang, Z., Yue, H., Hu, W., et al., 2014. Associations of serum sclerostin and polymorphisms in the SOST gene with bone mineral density and markers of bone metabolism in postmenopausal Chinese women. J. Clin. Endocrinol. Metab. 99 (4) (E665-E73).

Ishimura, E., Okuno, S., Ichii, M., Norimine, K., Yamakawa, T., Shoji, S., et al., 2014. Relationship between serum sclerostin, bone metabolism markers, and bone mineral density in maintenance hemodialysis patients. J. Clin. Endocrinol. Metab. 99 (11), 4315–4320.

Ke, H.Z., Richards, W.G., Li, X., Ominsky, M.S., 2012. Sclerostin and dickkopf-1 as therapeutic targets in bone diseases. EndocrRev 33 (5), 747–783.

Li, X., Zhang, Y., Kang, H., Liu, W., Liu, P., Zhang, J., et al., 2005. Sclerostin binds to LRP5/6 and antagonizes canonical Wnt signaling. JBiolChem 280 (20), 19883–19887.

Loots, G.G., Kneissel, M., Keller, H., Baptist, M., Chang, J., Collette, N.M., et al., Jul 2005. Genomic deletion of a long-range bone enhancer misregulates sclerostin in Van Buchem disease. Genome Res. 15 (7), 928–935 (Epub 2005/06/21).

Mahy, M., Autenrieth, C.S., Stanecki, K., Wynd, S., 2014. Increasing trends in HIV prevalence among people aged 50 years and older: evidence from estimates and survey data. AIDS 28 (Suppl. 4), S453–S459 Nov.

Malluche, H.H., Davenport, D.L., Cantor, T., Monier-Faugere, M.-C., 2014. Bone mineral density and serum biochemical predictors of bone loss in patients with CKD on Dialysis. Clin. J. Am. Soc. Nephrol. 9 (7), 1254–1262.

Modder, U.I., Hoey, K.A., Amin, S., McCready, L.K., Achenbach, S.J., Riggs, B.L., et al., 2011. Relation of age, gender, and bone mass to circulating sclerostin levels in women and men. J. Bone Miner. Res. 26 (2), 373–379.

Mödder, U.I., Hoey, K.A., Amin, S., McCready, L.K., Achenbach, S.J., Riggs, B.L., et al., 2011. Relation of age, gender, and bone mass to circulating sclerostin levels in women and men. J. Bone Miner. Res. Off. J. Am. Soc. Bone Miner. Res. 26 (2), 373–379.

Mora, S., Puzzovio, M., Giacomet, V., Fabiano, V., Maruca, K., Capelli, S., et al., 2015. Sclerostin and DKK-1: two important regulators of bone metabolism in HIV-infected youths. Endocrine 49 (3), 783–790 Aug.

Morse, L.R., Sudhakar, S., Danilack, V., Tun, C., Lazzari, A., Gagnon, D.R., et al., 2012. Association between sclerostin and bone density in chronic spinal cord injury. J. Bone Miner. Res. Off. J. Am. Soc. Bone Miner. Res. 27 (2), 352–359.

Negredo, E., Domingo, P., Perez-Alvarez, N., Gutierrez, M., Mateo, G., Puig, J., et al., 2014. Improvement in bone mineral density after switching from tenofovir to abacavir in HIV-1-infected patients with low bone mineral density: two-centre randomized pilot study (OsteoTDF study). J. Antimicrob. Chemother. 69 (12), 3368–3371 Dec. (Epub 2014/08/16).

Negredo, E., Diez-Perez, A., Bonjoch, A., Domingo, P., Perez-Alvarez, N., Gutierrez, M., et al., Jul 2015. Switching from tenofovir to abacavir in HIV-1-infected patients with low bone mineral density: changes in bone turnover markers and circulating sclerostin levels. J. Antimicrob. Chemother. 70 (7), 2104–2107 (Epub 2015/03/15).

Piec, I., Washbourne, C., Tang, J., Fisher, E., Greeves, J., Jackson, S., et al., 2016. How accurate is your sclerostin measurement? Comparison between three commercially available sclerostin ELISA kits. Calcif. Tissue Int. 98 (6), 546–555 (Epub 2016/01/09).

Semenov, M., Tamai, K., He, X., Jul 22 2005. SOST is a ligand for LRP5/LRP6 and a Wnt signaling inhibitor. J. Biol. Chem. 280 (29), 26770–26775.

Smit, M., Brinkman, K., Geerlings, S., Smit, C., Thyagarajan, K., Sighem, A., et al., Jul 2015. Future challenges for clinical care of an ageing population infected with HIV: a modelling study. Lancet Infect. Dis. 15 (7), 810–818.

Thambiah, S., Roplekar, R., Manghat, P., Fogelman, I., Fraser, W.D., Goldsmith, D., et al., 2012. Circulating sclerostin and Dickkopf-1 (DKK1) in predialysis chronic kidney disease (CKD): relationship with bone density and arterial stiffness. Calcif. Tissue Int. 90 (6), 473–480.

Voskaridou, E., Christoulas, D., Plata, E., Bratengeier, C., Anastasilakis, A.D., Komninaka, V., et al., Nov 2012. High circulating sclerostin is present in patients with thalassemia-associated osteoporosis and correlates with bone mineral density. Hormone and Metabolic Research = Hormon- und Stoffwechselforschung = Hormones et metabolisme 44 (12), 909–913 (Epub 2012/05/15).

Zhong, Z., Ethen, N.J., Williams, B.O., Nov-Dec 2014. WNT signaling in bone development and homeostasis. Wiley Interdiscip. Rev. Dev. Biol. 3 (6), 489–500 (Epub 2014/09/30).