Published in final edited form as:
*J Acquir Immune Defic Syndr*. 2014 March 1; 65(3): 290–298. doi:10.1097/QAI.0000000000000005.

# Relationships between Inflammation, Immune Activation and Bone Health among HIV-Infected Adults on Stable Antiretroviral Therapy

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## Abstract

**Background—**To determine the association between bone health and inflammation, T-cell activation and monocyte activation among HIV-infected persons on stable antiretroviral therapy.

**Methods—**We performed a cross-sectional analysis of all subjects enrolling in the Stopping Atherosclerosis and Treating Unhealthy bone with RosuvastatiN in HIV (SATURN-HIV) trial with available skeletal assessments by dual-energy X-ray absorptiometry, inflammation, and immune activation markers. Analyses used Wilcoxon rank sum tests, Spearman correlation coefficients and linear regression.

**Results—**142 subjects were included: 78% male, 69% African-American, median age 46.3 years, CD4+ count 604 cells/μL, and 77% with undetectable HIV-1 RNA. 23% had osteopenia/osteoporosis at the hip; 21% at the lumbar spine. sVCAM-1 was correlated with hip (r=−0.22) and spine (r=−0.23 ) BMD, and bone turnover markers (r=0.20–0.33; all p <0.05). No significant correlations were observed between BMD and T-cell activation (%CD38HLA-DR on CD4+ or CD8+ T-cells), monocyte activation (CD14CD16, sCD14, sCD163), or inflammatory markers (IL-6, TNF-α, hs-CRP, d-dimer, RANKL, OPG, sTNF-RI and II). In regression models including traditional bone risk factors, hip BMD was associated with age, race, and body mass index; spine BMD was associated with race, family history of hip fracture, trunk fat, tenofovir, and HIV RNA; bone resorption (CTX) was associated with sICAM-1 and trunk fat; bone formation (P1NP) was associated with sVCAM-1, trunk and limb fat (p≤0.05).

**Conclusions—**Future studies should evaluate the longitudinal association of the adhesion molecules to further elucidate potential contributory mechanisms of bone loss among HIV-infected persons on stable ART.

### Keywords

osteopenia; immune activation; monocyte activation; inflammation; aging

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Clinical Trials Registration: NCT01218802

Conflicts: GAM has served as a scientific advisor or speaker for Bristol-Myers Squibb, Tibotec, Gilead, and Merck, has received research grants from Bristol-Myers Squibb, GlaxoSmithKline, and Gilead Sciences, and is currently serving as the DSMB Chair for a Pfizer-sponsored study

## INTRODUCTION

Low bone mineral density (BMD) and bone fractures are frequently observed among both younger and older HIV-infected persons, with up to three times the prevalence of osteoporosis and 30–70% higher fracture rates compared to HIV-uninfected controls [1]. The pathogenesis of low BMD appears to be multifactorial, with contribution of both traditional osteoporosis risk factors (i.e., cigarette smoking, low body weight, poor nutrition, heavy alcohol use, vitamin D deficiency, hypogonadism) and HIV-specific risk factors (i.e., antiretroviral therapy, low nadir CD4 count, hepatitis co-infection) [1–4].

Chronic inflammation is a well-established risk factor for bone loss. Bone resorption occurs through osteoclasts, highly specialized cells formed from the fusion of cells of monocyte/macrophage origin. Osteoclast differentiation and activation is regulated by RANKL (receptor activator of NF-kB ligand), a member of the tumor necrosis factor (TNF) family of cytokines and osteoprotegerin (OPG), a decoy for the RANKL receptor that opposes RANKL induced bone resorption [5,6]. In inflammatory conditions, RANKL is also expressed by activated T-cells and B-cells, resulting in increased osteoclast activity and bone loss [7,8]. Activated T-cell subsets have been associated with increased fracture risk among post-menopausal women [9]. Furthermore, in mice with T- and B-cell deficiency, injection of lipopolysaccharide can further induce bone loss, suggesting an additional role of monocyte activation in bone loss [10].

The role of the inflammation and immune activation in the development of HIV-related bone disease is not well understood. RANKL plasma concentrations correlate with HIV-1 RNA, and elevated RANKL concentrations with decreased OPG/RANKL ratio are found in HIV-infected persons with low BMD [11]. Similarly, infection with HIV-1 in a rodent model led to increased RANKL and decreased OPG [12]. The only published study to explore T-cell activation in HIV-related bone disease found no significant differences in CD8+T-cell activation (co-expression of CD38+ and HLA-DR+), TNF-α, RANKL, or OPG between subjects with low or normal BMD, however 25% of the subjects were antiretroviral therapy (ART)-naïve and the study was limited to 62 subjects (only 42 subjects had samples available for cytokine analysis)[13]. Given that increased inflammation, T-cell activation, and monocyte activation contribute to the pathogenesis of many HIV-related comorbidities [14–19], we hypothesized that low BMD and markers of bone turnover would be associated with increased inflammation, T-cell activation, and monocyte activation among HIV-infected persons on stable ART.

## METHODS

### Study Population

We performed a cross-sectional analysis of all subjects who underwent an initial comprehensive metabolic and skeletal assessment at the time of enrollment into the Stopping Atherosclerosis and Treating Unhealthy bone with RosuvastatiN in HIV (SATURN-HIV) trial. SATURN-HIV is a randomized double-blind placebo-controlled trial designed to measure the effect of rosuvastatin 10mg daily on cardiovascular disease risk and skeletal health. Enrollment spanned from March 2011 to August 2012. Eligible subjects were 18 years of age or older, without known coronary artery disease or diabetes, no known fragility fractures, receiving stable ART for at least 12 weeks with a cumulative duration of ART of at least 6 months, and an HIV-1 RNA ≤ 1,000 copies/mL. Additional entry criteria included fasting LDL-cholesterol of < 130 mg/dL. Subjects were excluded for an active or chronic inflammatory condition (besides HIV), receipt of systemic chemotherapy or steroids, uncontrolled diabetes mellitus or thyroid disease, or use of anabolic agents, growth hormone, >81 mg aspirin daily, bisphosphonates, or other agents for the treatment of

osteopenia/osteoporosis. The study was approved by the Institutional Review Board of University Hospitals Case Medical Center (Cleveland, OH). Written informed consent was provided by all participants.

### Clinical Evaluations

At the initial visit, self-reported demographics, medical history, family history of hip fracture, and HIV treatment history were obtained. Self-reported time since HIV diagnosis, nadir CD4 count, and antiretroviral treatment history were confirmed by medical records when available. Active hepatitis C was defined by presence of hepatitis C virus antibody and detectable hepatitis C RNA by PCR. Alcohol use was assessed using self-reported quantification by subjects. Daily calcium and vitamin D intake were estimated with the Block Calcium/Vitamin D Screener (Nutrition Quest, Berkeley, CA). Calorie and protein intake were estimated with the Block Brief 2000 Food Frequency Questionnaire (Nutrition Quest, Berkeley, CA). Physical activity was estimated through a self-reported questionnaire as minutes per two weeks [20]. HIV-1 RNA level and CD4 cell count were obtained as part of routine clinical care. Whole blood was collected in EDTA tubes and underwent processing for flow cytometry in real-time. Additional blood was collected for plasma and serum isolation and frozen at −70°C without prior thawing until analysis. For all laboratory assessments, laboratory personnel were blinded to clinical information.

### Dual-Energy X-ray Absorptiometry

Evaluations included whole body, lumbar spine (L1-4) and left hip dual-energy X-ray absorptiometry (DEXA). Total lean mass and fat distribution was measured by DEXA in anteroposterior view using Lunar Prodigy Advance (GE Healthcare) and derived peripheral fat depot (as limb fat) and central fat depots (as trunk fat) were used in the analysis. Technicians used the same machine on the same subject throughout the study. All DEXA scans were read at Case Medical Center by an experienced radiologist blinded to study information. Subjects were labeled with osteopenia if their t-score was ≤-1 and osteoporotic if t-score was ≤ −2.5 at either total hip or lumbar spine [21].

### Measurements of Bone Turnover Markers and Bone-specific Cytokines

Intact N-terminal propeptide of type 1 procollagen (PINP) was measured by radioimmunoassay (Immunodiagnostics Systems, Fountain Hills, AZ); osteocalcin and c-terminal collagen crosslinks (CTX) were determined by sandwich immunoassay (Roche, Indianapolis, IN). Interassay coefficients of variation (CV) ranged from 3.8–15.8%.

### Measurement of Soluble Markers in Plasma and Serum

RANKL was measured by singleplex immunoassay (Millipore, Billerica, MA) and OPG by sandwich enzyme-linked immunosorbent assays (ELISAs, Biomedica, Austria). Concentrations of soluble vascular cell adhesion molecule-1 (sVCAM-1), intracellular adhesion molecule-1 (sICAM-1), interleukin-6 (IL-6), and soluble TNF-α receptor (sTNFR)-I and -II were determined by quantitative sandwich ELISAs (R&D Systems, Minneapolis, MN). hs-CRP concentration was determined by particle enhanced immunonepholometric assays on a BNII nephelometer (Siemens, Munich, Germany). The D-dimer concentration was determined by an immunoturbidometric assay on a STA-R Coagulation Analyzer (Diagnostica Stago, Parsippany-Troy Hills, NJ, USA). Serum levels of soluble CD14 and soluble CD163 were measured as markers of monocyte immune activation using Quantikine ELISA kits (R&D Systems). All inter-assay variability were <12%, except for very low D-dimer values (<0.20 μg/mL) where the inter-assay CV was 21%. Concentrations of 25-hydroxy (OH) D were measured by ELISA (Immunodiagnostic

Systems Limited, Fountain Hills, AZ, USA) as per the manufacturer's product manual and tested in duplicate. Median intra-assay and inter-assay coefficients of variation were <12%.

### Flow Cytometric Assays

Monocyte and T-lymphocytes were identified by size, granularity, and expression of CD14 or CD3 and CD8, respectively. Cell surface molecule expression was monitored by staining cells with the following fluorochrome-labeled antibodies: anti-tissue factor fluorescein isothiocyanate (FITC; American Diagnostica, Stamford, CT), anti-CD14 Pacific Blue and anti-CD16 phycoerythrin (PE; BD Pharmingen, San Diego, CA). In order to assure that monocyte populations were not contaminated by lymphocytes, preliminary experiments using an exclusion gate that included anti-CD3 FITC, anti-CD20 FITC, and anti-CD56 FITC (BD Pharmingen) were performed.

T-cell activation was measured using anti-CD28 PE, anti-HLA-DR FITC, anti-CD3 peridinin-chlorophyll-protein complex (PerCP), anti-CD8 PerCP (BD Biosciences, San Diego, CA) and appropriate isotype control monoclonal antibodies.

Whole blood samples were incubated for 15 minutes on ice with FACS Lyse buffer (BD Biosciences) and then washed in wash buffer (phosphate-buffered saline with 1% bovine serum albumin and 0.1% sodium azide). Cells were then stained for 30 minutes in the dark on ice, washed in wash buffer and fixed in 1% formaldehyde. Monocytes were analyzed using a Miltenyi MACS Quant flow cytometer (Miltenyi Biotec, Bergisch Gladbach, Germany) and MACS QUANT software (version 2.21031.1; Miltenyi Biotec). T-lymphocytes were analyzed using an LSR II flow cytometer (BD Biosciences, San Jose, CA) and FACSDIVA software version 6.1.1.

### Study Design and Data Analysis

This study was a cross-sectional analysis of men and women receiving care for HIV-1 infection. The study population was described overall, and then by subgroups with and without combined osteopenia or osteoporosis based on hip BMD and spine BMD separately. Continuous measures are described with medians and interquartile ranges (IQRs), and categorical measures are described with frequencies and percents. Unadjusted relationships of selected variables with measures of BMD were estimated. Two-group non-parametric tests of significance (Wilcoxon rank sum tests) were used to test the difference in continuous variables between the osteopenia subgroups and Chi squared analysis or Fisher's exact tests were used for categorical variables. The relationship between continuous measures of markers of bone health and inflammation was estimated using Spearman correlation coefficients. We used multivariate regression methods to determine whether the relationship between sVCAM-1or ICAM-1 and BMD and bone turnover markers remained significant after adjusting for traditional bone risk factors. This approach was carried out in two steps. First, a series of screening models based on related variables known to impact BMD were constructed for each relationship of interest. These variables included demographics (age, race, sex, alcohol, smoking, family history of hip fracture, BMI, trunk and limb fat); nutrition (25(OH)D level, intake of vitamin D, calcium, calories, protein); physical activity; and HIV related variables (current CD4 count, HIV-1 RNA <50 copies/mL, current or cumulative tenofovir use). Any variable where $p <= 0.10$ was selected for inclusion in a final model. If none reached that level, the variable in that screening model with the lowest level of significance was chosen. Secondly, eight final models were constructed based on the screening results. We previously published using this approach [22].

The level of significance was set at $p = 0.05$; no correction for multiple comparisons were done, despite the large number of analyses. These analyses could be considered exploratory

and therefore a more liberal definition is appropriate as not to miss any potentially important relationships. Analyses were performed using SAS v 9.3 (The SAS Institute, Cary, NC).

# RESULTS

## Study Population

One-hundred and forty-two of 147 (97%) HIV-1-infected persons enrolled in SATURN met eligibility requirements and completed study procedures. Overall, participants were 78% male and 69% African American, with a median age of 46.3 years. The median CD4+ lymphocyte count was 604 cells/μL and 77% had an HIV-1 RNA < 50 copies/mL. Demographics and clinical characteristics of the study population are shown in Table 1.

Thirty-three (23%) subjects met criteria for osteopenia (N=32) or osteoporosis (N=1) at the hip and 30 (21%) met criteria for osteopenia (N=27) or osteoporosis (N=3) at the lumbar spine. 12 (8%) had osteopenia or osteoporosis at both sites; 89 (63%) had normal BMD at both sites. Osteopenia and osteoporosis were combined for the remainder of the analyses. Differences between hip and lumbar spine osteopenia and normal BMD groups are shown in Table 1.

## Cellular and Soluble Markers of Lymphocyte and Monocyte Activation and Association with Low BMD

As shown in Table 2, markers of lymphocyte and monocyte activation were not significantly different between subjects with osteopenia compared to subjects with normal hip BMD. Osteopenia at the lumbar spine was associated with greater CD4+ lymphocyte activation and trends towards greater CD8+ lymphocyte activation (Table 2).

## Association between Bone Health and Inflammation or Immune Activation

We then explored the correlation between bone health, as assessed by BMD and markers of bone turnover, with markers of inflammation and immune activation to identify those markers that may be associated with bone health, regardless of a diagnosis of osteopenia or osteoporosis. We detected a weak correlation between lower hip BMD in persons with higher levels of osteocalcin and lower hip and spine BMD in persons with higher 25(OH) vitamin D (Table 3). No significant correlations were detected between BMD or bone turnover markers and T-cell activation markers, soluble monocyte activation markers, or proportions of monocyte subpopulations. In contrast, higher sVCAM-1 had significant, albeit modest, correlations with lower hip and spine BMD, and with higher bone resorption and formation markers (Table 3). Higher sICAM-1 was correlated with lower bone resorption (CTX). No correlations were found with BMD or bone turnover and other inflammatory markers (Table 3).

## Association of sVCAM-1 and sICAM-1 with BMD and Bone Turnover

Lastly, we explored whether the correlation between sVCAM-1 or sICAM-1 with BMD and bone turnover markers persisted after adjusting for traditional bone risk factors. Results of the multivariate analysis are shown in Table 4. Both current tenofovir and cumulative tenofovir were evaluated separately with similar results. Only the model including current tenofovir is presented in Table 4. Greater bone resorption (CTX) was associated with lower sICAM-1 and greater bone formation (P1NP) with higher sVCAM-1; other associations between adhesion molecules and BMD or bone turnover did not remain significant after adjustment. Between models with sVCAM-1 and sICAM-1, greater T-score at the hip was associated with younger age, African American/black race, and greater BMI while greater T-score at the L-spine was associated with African American/black race, no family history of hip fracture, greater trunk fat, no current tenofovir use, and having an undetectable HIV-1

RNA level. Higher P1NP was associated with lower trunk fat and higher limb fat, while CTX was associated with lower trunk fat when sVCAM-1 was included in the model, and greater trunk fat when sICAM-1 was included in the model.

## DISCUSSION

We present the first study to comprehensively assess the relationship between bone health and markers of inflammation and monocyte and lymphocyte immune activation among HIV-infected persons on stable ART. Chronic inflammatory conditions (e.g., rheumatoid arthritis, inflammatory bowel disease, aging) are associated with increased bone loss and fracture risk [23–25]. Many of the inflammatory markers associated with bone loss in these diseases are known to be elevated and associated with poor clinical outcomes in HIV-infection [15,17]. Thus inflammation and activation are generally presumed to contribute to the higher prevalence of osteopenia and higher fracture risk among HIV-infected persons [26,27]. Interestingly, we found lower IL-6 among persons with osteopenia at the hip in our study population (Table 2), and we found no correlation between BMD and IL-6 or other soluble markers of inflammation. The lack of strong evidence supporting the inflammatory markers in HIV-related bone disease is evident in other study populations as well. Yin, et al found only a weak correlation ($r=0.18$, $p=0.03$) between IL-6 and lumbar spine BMD among post-menopausal women [28]. Brown, et al found a marginally significant association between higher baseline sTNFR-II and subsequent decline in total BMD with ART initiation, however the association was no longer significant after adjusting for baseline CD4 count [3]. A recent publication from a SMART Body Composition sub-study comparing intermittent versus continuous ART found increased RANKL, RANKL:OPG, IL-6, and TNF-α over 12 months in the intermittent ART group compared to those receiving continuous ART [29]. Higher RANKL and RANKL:OPG were associated with a counterintuitive increase in hip BMD. No association was found between increased IL-6 and TNF-α and BMD at the hip or spine by DXA, but higher TNF-α was associated with changes in spine BMD as measured by quantitative computed tomography. Furthermore, despite the higher inflammatory markers, the intermittent ART group actually had a gain in BMD compared to the continuous ART group [29].

The weak correlation between lower hip and spine BMD and higher levels of 25(OH) vitamin D may represent the effect of dietary vitamin D supplementation. As shown on Table 1, significantly higher levels of 25(OH) vitamin D among persons with lower hip BMD are seen in conjunction with significantly greater dietary intake of vitamin D. Alternatively, in our largely African American population, this finding could represent the lack of association between vitamin D and BMD among African Americans as previously reported [30].

Interestingly, despite the lack of association of BMD and bone turnover with TNF-α, D-dimer, hs-CRP, and sTNFR-I and II, we found significantly higher sVCAM-1 among persons with osteopenia at the hip or the spine and a strong correlation with BMD and with all bone turnover markers. sVCAM-1 and sICAM-1 are adhesion molecules released by the vascular endothelium, are increased in atherosclerotic plaques and tend to correlate with greater visceral adipose tissue [31–33]. Both sVCAM-1 and sICAM-1 are higher among HIV-infected populations and decline with ART, similar to other inflammatory/activation markers [31,34,35]. Although the importance of adhesion molecules in the pathogenesis of osteoporosis in HIV uninfected populations is unclear, bone microvasculature is closely linked to normal bone development, remodeling, and repair [36,37]. Both sVCAM-1 and sICAM-1 have been found to be expressed in the surface of osteoblasts and sICAM-1-bearing osteoblasts can induce osteoclast maturation and bone resorption [38,39]. One study found that estrogen deficiency resulted in an increase in sICAM-1 expression on osteoclast

precursors, and the authors suggested that this may be a mechanism underlying bone loss following the menopause or ovariectomy [40]. We have previously shown that sVCAM-1 and sICAM-1 levels were associated with carotid intima media thickness, a measure of vascular disease, in HIV-infected subjects, however, no assessment of skeletal health was included [41]. Given the association of sVCAM-1 with cardiovascular disease and adiposity, we expected the association with sVCAM-1 would be explained by the effects of fat or BMI. However, our findings show the opposite: sVCAM-1 was associated with lower BMD (Table 3), while greater BMI was associated with greater BMD at the hip and greater trunk fat was associated with greater BMD at the spine (Table 4). Thus another variable, or variables, included in our model appear to mediate the relationship between BMD and adhesion molecules.

Chronic immune activation is observed among persons on otherwise successful ART and may contribute to the earlier than expected appearance of some complications of aging and HIV-infected persons. Activated T-cells and B-cells increase both the production and expression of RANKL [7,8], and thus, chronic immune activation in HIV is hypothesized to induce osteoclastogenesis and contribute to the high prevalence of bone loss observed among HIV-infected persons [27]. Similar to other studies, we found higher RANKL among participants with osteopenia at the spine [11,42], although we were unable to detect significant differences at the hip. In contrast to our hypothesis, we found no difference in cellular or soluble activation markers at hip or spine with the exception of elevated CD4+ T-cell activation among persons with osteopenia at the spine. Similarly, across the range of BMD measured at hip or spine (Table 3), we found no correlation between BMD or bone turnover markers with any markers of immune activation. Higher immune activation in our population could have minimized differences between groups, however, the wide interquartile range of nearly 10% suggests that our study population had adequate variation in activation.

The main strength of our study is the carefully conducted comprehensive measurements of lymphocyte and monocyte cellular and soluble activation markers with inflammatory markers and site-specific BMD, measurements that have not previously been reported in HIV-infected or HIV-uninfected populations. We identified distinct associations with hip versus spine osteopenia, with age, race, BMI, and vitamin D or calcium intake significantly associated with hip osteopenia and race and family history of a hip fracture as more significant factors associated with spine osteopenia. Additionally, the study includes a relatively large sample size, both women and men, robust clinical data regarding traditional bone risk factors, and is restricted to persons on stable ART, with most participants on therapy for over 4 years (Table 1).

Our study did have limitations. First, less than 25% of our population had osteopenia at either the hip or spine and very few had osteoporosis, a low percentage compared with other reported populations [1,2], and likely reflective of the large proportion of African-American participants [43]. Due to the small sample of subjects with osteopenia or osteoporosis, we are unable to exclude that small differences may have been detected with a larger sample size. Only 22% of our cohort was female and the median age of the study population was less than 50 years of age. These characteristics may make the results of our study less generalizable to older, female, or largely Caucasian cohorts. We did not include an HIV-uninfected cohort for comparison. Lastly, our findings are limited to cross-sectional observations.

In conclusion, we found surprisingly little correlation between BMD and markers of inflammation and immune activation among persons on stable ART with the exception of an association with the adhesion molecules, and sVCAM-1 in particular. Adjusting for

traditional bone risk factors attenuated the relationship between adhesion molecules and bone health measures. Traditional bone risk factors including age, gender and race were strong predictors of low BMD and should be considered when identifying those with osteopenia or osteoporosis. Future studies should evaluate the longitudinal association of the adhesion molecules to further elucidate potential contributory mechanisms of bone loss among HIV-infected persons on stable ART.

## Acknowledgments

This project was supported by the National Institutes of Health [NR012642 to G.A.M. and AG040594 to K.M.E.].

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*J Acquir Immune Defic Syndr*. Author manuscript; available in PMC 2015 March 01.

## Table 1

Demographics and Clinical Characteristics of the Study Population

| | All (N=142) | Osteopenia Hip (N=33) | Normal BMD Hip (N=109) | *P-value* | Osteopenia Spine (N=30) | Normal BMD Spine (N=112) | *P-value* |
|---|---|---|---|---|---|---|---|
| *Demographics* | | | | | | | |
| Age (years) | 46.3 (39.8, 52.6) | 49.2 (41.9,55.9) | 45.5 (36.4, 51.3) | *0.02* | 47.5 (42.4, 55.3) | 45.9 (38.8, 51.7) | *0.21* |
| Male | 111 (78) | 29 (88) | 81 (74) | *0.14* | 25 (83) | 86 (77) | *0.44* |
| Race | | | | *<0.001* | | | *0.002* |
| African-American | 98 (69) | 14 (42) | 82 (78) | | 14 (47) | 84 (75) | |
| Other | 44 (31) | 19 (58) | 25 (42) | | 16 (53) | 28 (25) | |
| Smoker (current) | 91 (64) | 18 (55) | 72 (66) | *0.18* | 16 (53) | 75 (67) | *0.17* |
| Alcohol (current) | 65 (46) | 13 (39) | 51 (47) | *0.40* | 16 (53) | 49 (44) | *0.35* |
| *HIV-Related* | | | | | | | |
| CD4(cells/μL) | 604 (425, 834) | 614 (444, 857) | 607 (418, 831) | *0.57* | 494 (361, 666) | 635 (432, 834) | *0.12* |
| Nadir CD4(cells/μL) | 188 (425, 300) | 153 (81, 323) | 199 (89, 300) | *0.75* | 161 (36, 276) | 199 (94, 301) | *0.25* |
| HIV-1 <50 (copies/mL) | 109 (77) | 26 (24) | 82 (76) | *0.58* | 21 (19) | 88 (81) | *0.28* |
| Duration HIV (mo) | 137 (11, 211) | 151 (84, 213) | 132 (70, 208) | *0.31* | 118 (77, 213) | 147 (72, 210) | *0.86* |
| Duration ART (mo) | 56 (30, 109) | 71 (23, 121) | 54 (30, 106) | *0.52* | 50 (22, 121) | 62 (30, 107) | *0.82* |
| Current tenofovir | 127 (89) | 32 (97) | 94 (88) | *0.19* | 29 (97) | 98 (88) | *0.19* |
| Cumulative tenofovir (mo) | 40 (18, 62) | 49 (27, 83) | 39 (13, 60) | *0.07* | 41 (23, 69) | 40 (17, 62) | *0.91* |
| Duration PI (mo) | 30 (0, 74) | 30 (0, 79) | 20 (0, 72) | *0.30* | 22 (0, 79) | 30 (0, 74) | *0.80* |
| Active hepatitis C | 12 (9) | 4 (12) | 8 (7) | *0.48* | 2 (7) | 10 (9) | *1.0* |
| *Traditional Bone Risk Factors* | | | | | | | |
| BMI (kg/m$^2$) | 26.8 (23.5, 30.3) | 24.3 (22.3, 27.2) | 28.0 (24.3, 31.4) | *0.02* | 26.4 (23.2, 30.0) | 27.0 (23.5, 30.4) | *0.71* |
| 25(OH) Vitamin D (ng/mL) | 15.9 (11.7, 21.4) | 23.1 (14.7, 26.9) | 14.9 (11.6, 18.8) | *0.001* | 19.7 (13.0, 27.0) | 15.3 (11.7, 21.0) | *0.07* |
| Vitamin D intake (IU) | 158 (57, 423) | 233 (99, 450) | 143 (38, 427) | *0.03* | 140 (61, 440) | 188 (54, 432) | *0.94* |
| Calcium intake (mg) | 576 (303, 1150) | 969 (485, 1504) | 527 (291, 886) | *0.004* | 568 (343, 1323) | 576 (292, 1323) | *0.54* |
| Caloric intake (kcal) | 2034 (263, 3147) | 2081 (1502, 3161) | 1964 (1354, 3075) | *0.46* | 1926 (1505, 3161) | 2088 (1336, 3161) | *0.67* |
| Protein intake (g) | 15.4 (12.8, 17.8) | 16.4 (14.3, 18.3) | 15.1 (12.4, 17.7) | *0.08* | 15.0 (13.0, 17.5) | 15.6 (12.7, 17.5) | *0.73* |
| Physical activity (minutes/2 weeks) | 2495 (1010, 4610) | 2730 (910, 5040) | 2450 (1060, 4560) | *0.95* | 2393 (1380, 5200) | 2495 (925, 4550) | *0.48* |
| Family history hip fracture | 17 (12) | 7 (21) | 10 (9) | *0.12* | 8 (27) | 9 (8) | *0.01* |

Values presented as median (Q1, Q3) or frequency (%). BMD, bone mineral density; ART, antiretroviral therapy; BMI, body mass index.

**Table 2**

Comparison of Inflammatory and Activation Markers between Participants with Osteopenia/Osteoporosis, or Normal BMD, shown separately at Hip and Spine

| | All (N=142) | Osteopenia Hip (N=33) | Normal Hip BMD (N=109) | *P-value* | Osteopenia Spine (N=30) | Normal Spine BMD (N=112) | *P-value* |
|---|---|---|---|---|---|---|---|
| *Inflammatory Markers* | | | | | | | |
| IL-6 (pg/mL) | 2.7 (1.9, 4.6) | 2.4 1.6, 3.1) | 3.1 (2.0, 4.9) | *0.04* | 2.6 (2.0, 3.9) | 2.9 (1.0, 4.7) | *0.62* |
| hsCRP (μg/mL) | 1.7 (0.8. 4.9) | 1.5 (0.5, 2.5) | 2.0 (0.8, 5.2) | *0.10* | 1.6 (0.9, 3.5) | 2.0 (0.7, 5.3) | *0.67* |
| D-dimer (μg/mL) | 0.19 (0.11, 0.31) | 0.18 (0.11, 0.24) | 0.20 (0.11, 0.32) | *0.68* | 0.20 (0.13, 0.32) | 0.19 (0.11, 0.29) | *0.55* |
| RANKL (pg/mL) | 10.4 (2.9, 28.8) | 7.7 (3.4, 26.7) | 11.8 (2.4, 29.2) | *0.72* | 15.0 (7.7, 43.8) | 8.5 (2.0, 27.0) | *0.03* |
| OPG (pmol/L) | 3.8 (3.0, 4.8) | 3.7 (3.0, 4.9) | 3.9 (3.0, 4.8) | *0.77* | 3.8 (3.2, 4.4) | 3.9 (3.0, 4.8) | *0.79* |
| RANKL:OPG | 2.7 (0.8, 8.0) | 1.5 (0.8, 8.5) | 2.9 (0.8, 7.9) | *0.85* | 4.8 (1.4, 9.9) | 2.5 (0.7, 7.0) | *0.045* |
| sTNFR-I (pg/mL) | 1572 (1266, 2208) | 1630 (1341, 2426) | 1477 (1218, 2189) | *0.11* | 1597 (1341, 2441) | 1556 (1238, 2204) | *0.50* |
| sTNFR-II (pg/mL) | 2344 (1718, 2860) | 2368 (1737, 2924) | 2329 (1693, 2850) | *0.67* | 2451 (1993, 2900) | 2274 (1651, 2832) | *0.26* |
| sICAM-1 (ng/mL) | 237 (172, 315) | 225 (185, 297) | 243 (168, 317) | *0.83* | 232 (190, 329) | 237 (168, 305) | *0.56* |
| sVCAM-1 (ng/mL) | 660 (563, 805) | 798 (650, 844) | 635 (537, 722) | *0.005* | 707 (620, 837) | 640 (543, 776) | *0.03* |
| *Cellular T-cell Activation Markers* | | | | | | | |
| CD4+ CD38+HLA-DR+ (%) | 5.2 (3.6, 6.7) | 5.3 (3.4, 6.6) | 5.2 (3.7, 6.6) | *0.81* | 5.9 (4.5, 7.5) | 5.0 (3.4, 6.6) | *0.08* |
| CD4+CD38+HLA-DR+PD1+ (%) | 1.7 (1.3, 2.9) | 1.7 (1.3, 3.0) | 1.8 (1.3, 3.0) | *0.58* | 2.3 (1.7, 3.1) | 1.6 (1.2, 2.6) | *0.01* |
| CD8+ CD38+HLA-DR+ (%) | 12.3 (8.7, 17.5) | 12.3 (8.0, 17.6) | 12.2 (8.8, 17.6) | *0.96* | 13.7 (9.2, 22.2) | 12.0 (8.5, 17.3) | *0.21* |
| CD8+ CD38+HLA-DR+PD1+ (%) | 2.7 (1.7, 3.9) | 2.9 (1.7, 4.1) | 2.7 (1.7, 4.1) | *0.85* | 3.2 (2.1, 5.0) | 2.6 (1.6, 3.8) | *0.10* |
| *Soluble Monocyte Activation Markers* | | | | | | | |
| sCD14 (ng/mL) | 2126 (1725, 2455 | 2112 (1804, 2395) | 2157 (1708, 2518) | *0.96* | 2247 (1963, 2518) | 2063 (1673, 2439) | *0.14* |
| sCD163 (ng/mL) | 652 (475, 875) | 613 (470, 789) | 652 (482, 789) | *0.34* | 617 (555, 846) | 653 (460, 906) | *0.86* |
| *Monocyte Subpopulations* | | | | | | | |
| CD14dim CD16+ (%) | 11.3 (7.8, 14.9) | 11.1 (7.9, 14.1) | 11.2 (7.7, 31.0) | *0.68* | 12.1 (8.3,14.5) | 11.0 (7.8, 15.0) | *0.53* |
| CD14+CD16− (%) | 61.2 (52.4, 71.8) | 61.7 (50.9, 68.5) | 60.8 (53.1, 72.2) | *0.34* | 64.7 (52.1, 72.9) | 60.7 (52.9, 70.0) | *0.63* |

Values presented as median (Q1, Q3).

BMD, bone mineral density; IL-6, interleukin-6; hsCRP, highly sensitive C-reactive protein; RANKL, receptor activator of NF-kB ligand; OPG, osteoprotegerin; sTNFR, soluble tumor necrosis factor-α receptor; sICAM, soluble intracellular adhesion molecule; sVCAM, soluble vascular cell adhesion molecule-1 (sVCAM-1)

**Table 3**

Correlation between Markers of Bone Health and Markers of Inflammation and Immune Activation

| | | | | ***Bone Formation*** | | |
|---|---|---|---|---|---|---|
| | ***Hip BMD*** | ***Spine BMD*** | ***Bone Resorption* CTX** | **Osteocalcin** | **PINP** | **25-OH vitamin D** |
| *Bone Turnover* | | | | | | |
| CTX | −0.16 | −0.16 | -- | -- | -- | -- |
| Osteocalcin | **−0.18**[a] | −0.13 | **0.56**[c] | -- | -- | -- |
| PINP | −0.002 | −0.05 | **0.60**[c] | **0.59**[c] | -- | -- |
| 25(OH) vitamin D | **−0.27**[c] | **−0.17**[a] | −0.15 | 0.05 | **−0.26**[b] | -- |
| *Inflammatory Markers* | | | | | | |
| IL-6 | 0.13 | 0.08 | −0.06 | −0.04 | −0.15 | −0.11 |
| CRP | 0.11 | 0.09 | −0.11 | −0.08 | −0.06 | 0.02 |
| d-dimer | 0.05 | 0.13 | 0.01 | −0.04 | 0.072 | −0.10 |
| RANKL | 0.01 | 0.14 | 0.10 | 0.11 | 0.14 | −0.10 |
| OPG | 0.02 | 0.10 | −0.10 | −0.07 | −0.09 | 0.15 |
| RANKL:OPG | 0.003 | −0.13 | | | | −0.11 |
| sTNFR-I | −0.07 | −0.04 | −0.03 | 0.09 | −0.07 | 0.17 |
| sTNFR-II | −0.04 | −0.08 | 0.10 | 0.07 | 0.001 | 0.10 |
| sICAM-1 | −0.03 | −0.04 | **−0.20**[a] | −0.03 | −0.03 | 0.16 |
| sVCAM-1 | **−0.22**[b] | **−0.23**[b] | **0.23**[b] | **0.33**[c] | **0.20**[a] | **0.18**[a] |
| *Cellular T-cell Activation Markers* | | | | | | |
| CD4+ CD38/HLA-DR | 0.005 | −0.01 | 0.009 | 0.05 | 0.12 | −0.04 |
| CD4+CD38+HLA-DR+ PD1+ | 0.03 | −0.10 | 0.05 | 0.02 | 0.10 | −0.09 |
| CD8+CD38+HLA-DR+ | −0.02 | −0.04 | 0.03 | 0.04 | 0.08 | 0.002 |
| CD8+CD38+HLA-DR+ PD1+ | −0.04 | −0.12 | 0.01 | 0.02 | 0.02 | −0.005 |
| *Soluble Monocyte Activation Markers* | | | | | | |
| sCD14 | −0.10 | −0.05 | −0.002 | −0.020 | −0.02 | 0.13 |
| sCD163 | 0.04 | 0.05 | −0.16 | −0.04 | −0.14 | **0.24**[b] |

| | | | | *Bone Formation* | | |
|---|---|---|---|---|---|---|
| | ***Hip BMD*** | ***Spine BMD*** | ***Bone Resorption* CTX** | **Osteocalcin** | **PINP** | **25-OH vitamin D** |
| *Cellular Monocyte Activation Markers* | | | | | | |
| CD14dim CD16+ | 0.10 | 0.05 | −0.11 | −0.08 | −0.02 | −0.03 |
| CD14+ CD16/TF | 0.06 | 0.08 | −0.03 | −0.04 | 0.09 | **0.18**[a] |
| CD14dim CD16/TF | −0.02 | 0.05 | −0.05 | −0.03 | 0.05 | 0.13 |

All values represent Spearman correlation coefficients;

[a] p <0.05;

[b] p <0.01;

[c] p <0.001.

BMD, bone mineral density; CTX, c-terminal collagen crosslinks; PINP, intact N-terminal propeptide of type 1 procollagen; IL-6, interleukin-6; hsCRP, highly sensitive C-reactive protein; RANKL, receptor activator of NF-kB ligand; OPG, osteoprotegerin; sTNFR, soluble tumor necrosis factor-α receptor; sICAM, soluble intracellular adhesion molecule; sVCAM, soluble vascular cell adhesion molecule-1 (sVCAM-1).

**Table 4**

Linear Regression Evaluating the Association between VCAM and ICAM with BMD and Bone Turnover after Adjusting for Baseline Variables*

| | sVCAM-1 Multivariable Analyses | | | sICAM-1 Multivariable Analyses | | |
|---|---|---|---|---|---|---|
| **Covariate** | **β** | **SE** | ***P value*** | **β** | **SE** | ***P value*** |
| *Hip T-score* | | | | | | |
| sVCAM/ICAM | <0.001 | <0.001 | *0.30* | <0.001 | <0.001 | *0.61* |
| Age | **−0.02** | **0.009** | ***0.01*** | **−0.02** | **0.005** | ***0.03*** |
| Race | **0.66** | **0.19** | ***<0.001*** | **0.64** | **0.20** | ***0.002*** |
| Sex | −0.27 | 0.22 | *0.24* | −0.23 | 0.23 | *0.31* |
| Family history | −0.49 | 0.26 | *0.06* | −0.50 | 0.26 | *0.06* |
| BMI | **0.07** | **0.01** | ***<0.001*** | **0.06** | **0.01** | ***<0.001*** |
| Current tenofovir | −0.15 | 0.28 | *0.61* | −0.14 | 0.28 | *0.63* |
| 25-OH vitamin D | −0.002 | 0.01 | *0.82* | −0.004 | 0.01 | *0.73* |
| Calcium intake | --- | --- | --- | <−0.001 | <0.001 | *0.54* |
| *Spine T-score* | | | | | | |
| sVCAM/ICAM | <0.001 | <0.001 | *0.78* | <−0.001 | <0.001 | *0.61* |
| Race | **0.71** | **0.23** | ***0.002*** | **0.67** | **0.23** | ***0.004*** |
| Family history | **−0.80** | **0.32** | ***0.01*** | **−0.80** | **0.32** | ***0.01*** |
| Trunk fat | **<0.001** | **<0.001** | ***0.03*** | **<0.001** | **<0.001** | ***0.04*** |
| HIV RNA <50 | **0.54** | **0.25** | ***0.03*** | **0.52** | **0.25** | ***0.04*** |
| Current tenofovir | **−0.72** | **0.33** | ***0.03*** | **−0.73** | **0.33** | ***0.03*** |
| Physical activity | <−0.001 | <0.001 | *0.056* | **<−0.001** | **<0.001** | ***0.045*** |
| *CTX (bone resorption)* | | | | | | |
| sVCAM/ICAM | <0.001 | <0.001 | *0.12* | **<−0.001** | **<0.001** | ***0.04*** |
| Sex | 0.08 | 0.05 | *0.10* | 0.08 | 0.05 | *0.10* |
| Trunk fat | **<−0.001** | **<0.001** | ***0.045*** | **0.08** | **0.05** | ***0.05*** |
| Current tenofovir | 0.07 | 0.06 | *0.22* | 0.08 | 0.06 | *0.19* |
| Calcium intake | <−0.001 | <0.001 | *0.09* | --- | --- | --- |
| Caloric intake | --- | --- | --- | <0.001 | <0.001 | *0.18* |
| *P1NP (bone formation)* | | | | | | |
| sVCAM/ICAM | **0.01** | **0.007** | ***0.04*** | −0.003 | 0.02 | *0.87* |

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| | sVCAM-1 Multivariable Analyses | | | sICAM-1 Multivariable Analyses | | |
|---|---|---|---|---|---|---|
| **Covariate** | **β** | **SE** | ***P value*** | **β** | **SE** | ***P value*** |
| Age | −0.31 | 0.20 | *0.13* | −0.23 | 0.20 | *0.25* |
| Trunk fat | **−0.001** | **<0.001** | ***0.004*** | **−0.001** | **<0.001** | ***0.01*** |
| Limb fat | **0.001** | **<0.001** | ***0.02*** | <0.001 | <0.001 | *0.07* |
| HIV RNA <50 | −1.94 | 4.72 | *0.68* | −3.17 | 4.76 | *0.51* |
| Caloric intake | 0.001 | 0.001 | *0.29* | 0.002 | 0.001 | *0.21* |

*Baseline variables included in univariate analysis: age, race, sex, alcohol, smoking, family history of hip fracture, BMI, trunk fat, limb fat, current CD4 count, HIV-1 RNA <50 copies/mL, current tenofovir use, 25(OH)D, vitamin D intake, calcium intake, caloric intake, protein intake, physical activity.

BMD, bone mineral density; sVCAM, soluble vascular cell adhesion molecule-1 (sVCAM-1); sICAM, soluble intracellular adhesion molecule; BMI, body mass index; CTX, c-terminal collagen crosslinks; PINP, intact N-terminal propeptide of type 1 procollagen.