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Body Composition, Soluble Markers of Inflammation, and Bone Mineral Density in Antiretroviral Therapy-Naïve HIV-1 Infected Individuals

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Abstract

Objective—To determine the association between bone mineral density (BMD), inflammatory markers, and alterations in fat and lean mass in untreated HIV-infected individuals.

Design—Cross-sectional analysis of antiretroviral therapy (ART)-naïve persons enrolled into a randomized clinical trial

Methods—Dual energy x-ray absorptiometry (DXA) for BMD, lean and fat mass, and a laboratory assessment were performed. Soluble biomarkers included adipocytokines (leptin, adiponectin), inflammatory markers (hsCRP, IL-6), and markers related to bone metabolism (osteoprotegerin (OPG)), receptor activator of NFκB Ligand (RANKL)). BMD at the lumbar spine, total hip, and femoral neck was expressed as a Z-score (number of standard deviations away from an age-, race-, sex-matched reference population).

Results—331 subjects had a median (Q_1, Q_3) age of 36 (28,45) years, were 89% male, and 44% white. The prevalence of low BMD (Z-score ≤ −2 at any of the 3 sites) was 10%. No associations were detected between Z-scores and hsCRP, IL-6, or RANKL (P≥0.1). In a linear model adjusting for age, gender, race, and total fat mass, lower lumbar spine Z-scores were associated with lower total lean mass, higher serum adiponectin, and lower OPG. Results at the total hip or femoral neck were similar.

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Conflicts of Interest

Dr Brown has served as a consultant for BMS, GSK, Merck, Abbott, Gilead, ViiV Healthcare and has received research funding from Merck and GSK. Dr. Currier has served as a consultant for Gilead and has received research funding from Merck. Dr. Murphy has served as a consultant for Gilead and serves on a Data Safety Monitoring Board for Gilead. Dr Stein serves on a Data Safety Monitoring Board for Abbott, Lilly, and Takeda. Dr McComsey has served as a consultant or received research grants from BMS, Pfizer, and GSK. Dr Ribaudo and Ms Chen and Rothenberg have no Duality of Interest disclosures.

Conclusions—Among ART-naïve HIV-infected individuals, lower BMD was associated with lower lean mass, higher adiponectin, and lower OPG, but not HIV disease variables or any of the inflammatory markers. These findings may have implications for bone metabolism in untreated HIV, in which hypoadiponectinemia and higher OPG may mitigate bone loss.

Keywords

Bone mineral density; Body composition; Human Immunodeficiency Virus; Inflammation

Osteoporotic fractures are a major source of morbidity and mortality in aging populations[1]. Among HIV-infected populations, the prevalence of osteoporosis is several fold higher than HIV-uninfected control populations and likely accounts for the emerging data suggesting a higher than expected risk of fragility fracture in HIV-infected patients[2–6]. The etiology of osteoporosis is multifactorial. While traditional risk factors, such as hypogonadism, smoking, heavy alcohol use, and certain components of antiretroviral therapy (ART) are important contributors, chronic infection with HIV and the resulting inflammation and immune activation have been hypothesized to lead to decreased bone mineral density (BMD). In pre-clinical models, both high levels of HIV-viral proteins and inflammatory cytokines, such as TNF-α and IL-6, have been associated with decreased osteoblast function and increased osteoclast formation and activity[7–10], potentially leading to an uncoupling of bone formation and bone resorption and net bone loss. In untreated HIV-infected patients, a similar pattern is observed with higher markers of bone resorption and relatively lower concentrations of markers of bone formation[11]. However, the extent to which systemic inflammation is related to lower BMD in untreated HIV-infected persons has not been clearly established.

Other factors may also influence BMD in untreated HIV-infected individuals. In the general population, lower lean body mass and lower fat mass have been independently associated with lower BMD [12;13]. In HIV-infected populations, lower body mass index is a major contributor to the increased prevalence of osteoporosis [14]. In addition, as in the general population[15], relative higher levels of abdominal visceral fat have also been associated with lower BMD in HIV-infected populations[16;17], although this has not been investigated to date in ART-naïve, HIV-infected patients. Part of the effect of adipose tissue on bone may be mediated through the adipose derived hormones, adiponectin and leptin, which may be altered in HIV-infected populations and have been associated with BMD independently of fat mass in the general population[18].

Other biomarkers may also be associated with abnormal bone metabolism in untreated HIV-infected persons. Osteoprotegerin (OPG) and receptor activator of NFκB Ligand (RANKL) are osteoblast-secreted factors which have a major role in the coupling of bone formation and resorption. Secreted RANKL binds to RANK on the cell surface of osteoclast precursors, leading to osteoclast activation and bone resorption. As a control mechanism, OPG is also secreted by osteoblast precursors to bind to RANKL, thereby preventing the interaction of RANK and RANKL and slowing bone resorption[19]. Although these proteins act locally in the bone microenvironment, circulating concentrations of these markers and their ratio have been associated with osteoporosis in the general population [19]. Interestingly, both of these cytokines are produced by activated immune cells[20;21] and decrease with ART-initiation [11].

To date most studies evaluating factors associated with BMD in HIV-infected populations have focused on patients receiving ART. In the current report, we assessed the prevalence of low BMD among ART-naïve patients and determined the associations between soluble

markers of inflammation, body composition, adipocytokines, and OPG/RANKL and site-specific BMD.

Methods

This was a cross-sectional, baseline evaluation of ART-naïve, HIV-infected individuals who enrolled in a randomized ART treatment trial AIDS Clinical Trials Group Study (ACTG) A5257 and agreed to undergo testing for subclinical cardiovascular disease (CVD), BMD, body composition, and specialized serum biomarkers as part of a cardiovascular/metabolic substudy ACTG A5260s. The parent study and substudy (clinicalTrials. gov Identifier NCT00851799) were approved by the Institutional Review Boards of all participating institutions and all subjects provided written informed consent. Study entry criteria included: 1) 18 years of age or older, 2) documented HIV-1 infection, 3) ART-naïve (defined as ≤10 days of ART at any time prior to entry), and 4) screening HIV-1 ribonucleic acid (RNA) ≥1000 copies/mL within 90 days prior to study entry. Because the primary endpoint of A5260s was subclinical CVD, the major exclusion criteria were known CVD, diabetes mellitus, uncontrolled thyroid disease, or use of lipid-lowering medications.

For all subjects, information regarding demographics, health-related behaviors, medical conditions and prescribed medications were obtained. Physical activity was assessed using the International Physical Activity Questionnaire (IPAQ) –Short Form and activity was stratified into low, medium, and high activity based on IPAQ definitions[22]. BMD was assessed by dual energy x-ray absorptiometry (DXA) at the lumbar spine, total hip, and femoral neck using Hologic or Lunar scanners. All scans were read centrally using a standardized protocol at the Body Composition Analysis Center, Tufts University (Boston, MA, USA). Z-scores were calculated from the site-specific BMD measures using normative data matched for age, gender and race. The Z-score is the number of standard deviations a participant's BMD falls from the mean BMD of a gender-, age-, and race-matched population. Low BMD was defined as Z-score ≤ −2 at any of the three sites. Given the relatively young age of the population, Z- scores were used as the primary outcome measurement in accordance with National Osteoporosis Foundation guidelines [23]. Single slice abdominal quantitative computed tomography (CT) at the L4–L5 level was used to assess visceral adipose tissue (VAT) and scans were read centrally at the Los Angeles Biomed (Harbor-UCLA, Torrance, CA). Total body fat, limb fat, and lean mass were measured by whole body DXA and analyzed centrally (Tufts University, Boston, MA, USA).

Laboratory Assessment

Fasting (at least 8 hours) blood samples were obtained by phlebotomists and sent to core laboratories for analysis. Inflammatory biomarkers and adipocytokines were measured on plasma stored at −70 degrees at the University of Vermont Laboratory for Clinical Biochemistry Research lab (Burlington, VT, USA), and included high-sensitivity C-reactive protein (hsCRP) by nephelometry (Inter-Assay CV range 2.96–6.24%) and interleukin-6, total adiponectin, leptin, OPG, and RANKL by enzyme-linked immunosorbent assay (Inter-Assay CV range 5.26–12.45%).

Statistical Analysis

Continuous variables are described as medians (1st-3rd quartile, Q1–Q3); categorical variables are presented as percentages. The prevalence of low BMD in our sample was compared to the expected prevalence of low BMD in a simulated reference population including 100,000 variants from a standard (zero mean and variance of one) trivariate normal distribution with correlation as observed in the study sample (Figure 1). Univariate

associations between demographic variables, HIV-related measures, body composition measurements, biomarkers and BMD Z-scores (continuous) at the lumbar spine, total hip, and femoral neck were assessed by non-parametric k-sample tests and tests of non-zero Spearman correlation. Associations that were nominally significant ($p<0.05$) were included in adjusted analyses. Adjusted analyses used multivariable linear regression modeling with candidate variable selection based on the Akaiki Information Criterion. Final model selection was done manually with clinical input and consideration of collinearity and final model R^2. Parameters estimates (95% confidence intervals) represent average difference in BMD Z-scores per unit changes or population subgroups as described. All models included age, sex, and race. In order to determine the associations between body composition compartments and BMD Z-scores, body mass index was replaced in the models with total lean mass and total fat mass.

Because strong associations between lean body mass and BMD Z-score in the above analyses, a post-hoc, exploratory, multivariable regression analysis was conducted to determine whether lean body mass was associated with inflammatory (hsCRP and IL-6) and HIV disease related variables (HIV-1 RNA level, CD4, and known HIV duration). To a model which included age, race, and sex, each of the above covariates was added to evaluate their association with total lean body mass.

Results

Subject Characteristics (Table 1)

Of the 331 participants, 89% were male, 44% were non-Hispanic white, 32% were black, and 20% were of Hispanic race/ethnicity. Twenty-three percent had a prior clinical AIDS diagnosis. The median (Q1, Q3) HIV-1 RNA level was 4.5 (4.0, 5.1) $\log_{10}$copies/mL and CD4 cell counts were 349 (207, 455)/mm^3. There were 38% current smokers and 20% former smokers; 29% reported alcohol intake ≥ 3 drinks/day and 6% reported current proton pump inhibitor use. The median BMI was 25 (22, 28) kg/m^2. None of the subjects had a history of osteoporosis treatment.

Bone Mineral Density

The median of total hip Z-score of was −0.1 (−0.6, 0.6); of femoral neck Z-score was −0.1(−0.7, 0.5) and the lumbar spine Z-score was −0.4 (−1.2, 0.4). These three endpoints were correlated with each other ($r\geq0.59$, $p<0.001$). In particular, the estimated correlation (Spearman) between hip and femoral neck Z-score was 0.89 (Figure 1). The prevalence of low BMD at the lumbar spine, total hip, and femoral neck were 9%, 1%, and 2%, respectively. With low BMD defined as Z-score ≤ −2.0 at any of the 3 sites, the observed prevalence of 10% (33 of 331) [95% CI: 6.7%–13.2%] was higher than expected under the standard trivariate normal assumption (16 cases expected, $P<0.001$).

Univariate Associations with BMD Z-scores

In univariate analyses (Table 2), CD4 cell count, HIV-1 RNA levels, hsCRP, or IL-6, were not associated with BMD Z-score at any site. Associations with smoking, heavy alcohol use, physical activity, hepatitis C, or current proton pump inhibitor use were also not detected (data not shown). All body composition parameters (limb fat, VAT, total body lean mass, total body fat mass) were associated with higher BMD Z-scores at all sites. Lower adiponectin and higher leptin concentrations were associated with higher Z-score BMD. Higher OPG was associated with higher BMD Z-scores, but no association was observed with RANKL or the OPG/RANKL (data not shown). Of the covariates with statistically significant associations with BMD Z-score, the magnitude of these associations was relatively small.

Multivariable Associations with BMD Z-scores (Figure 2)

In multivariable analyses, females had higher BMD than males, compared to their respective reference populations. Lean body mass was more strongly associated with BMD Z-score, than total fat mass. For the latter, although associations of similar magnitude were estimated across all sites, only the association at the femoral neck achieved formal statistical significance (P=0.13 for lumbar; P=0.08 for hip; P=0.019 for femoral neck). The association of VAT with BMD Z-score at any of the 3 sites was no longer statistically significant after adjustment for total fat mass (data not shown, all p>0.15).

After adjustment for fat mass, an inverse association of adiponectin with BMD Z-score was still detected although the magnitude of the effect size was reduced. In contrast, associations with leptin were no longer statistically significant in multivariable analysis (p=0.17 at the lumbar spine; p=0.51 at the total hip; p=0.30 at the femoral neck). Higher levels of OPG were associated with higher BMD Z-score at all sites, whereas the associations with RANKL or the OPG/RANKL ratio (data not shown) were not detected.

Exploratory Analysis Investigating the Associations between Lean Body Mass and Inflammatory and HIV Disease-Related Variables

Because of the consistent association between lean body mass and BMD Z-score at all 3 sites and lack of association between inflammatory and HIV disease-related variables and BMD Z-score, associations between lean body mass and hsCRP, IL-6, HIV-1 RNA level, or CD4 cell count were examined. In separate multivariable linear regression models which included age, race, and sex, residual associations lean body mass with IL-6 (p=0.006), CD4 cell count (p=0.03), and HIV-1 RNA level (p=0.002) and were observed, but association with hsCRP (p=0.24) was not apparent.

Discussion

In this cohort of ART-naïve HIV-infected persons ready to start HIV therapy, we found a higher than expected prevalence of low BMD, particularly at the lumbar spine. Lower BMD was related to lower lean body mass and two soluble biomarkers, adiponectin and OPG, which have been associated with BMD and fracture in the general population. However, associations between BMD and HIV disease parameters, such as HIV-1 RNA or CD4 cell count, or the inflammatory markers, hsCRP or IL-6 were not detected. However, some of these HIV disease parameters and IL-6 were associated with lower lean mass. Our findings provide insight into the balance of bone metabolism in the setting of untreated HIV infection.

Our finding regarding the higher than expected prevalence of low BMD in a population of ART-naïve HIV-infected patients was also observed in ACTG 5224s, in which 31% of subjects prior to ART-initiation had osteopenia (median age 38 years)[24]. It has been speculated that systemic inflammation and immune activation associated with untreated HIV is an important contributor to low BMD among HIV-infected patients, as in other inflammatory conditions, such as rheumatoid arthritis or inflammatory bowel disease[29;30]. In the absence of longitudinal data in untreated HIV-infected persons, however, it is impossible to determine the extent to which chronic untreated HIV contributes to bone loss. Low BMD in ART-naïve populations could also have pre-dated HIV-infection, as was suggested in studies examining high risk, HIV-uninfected men in the CDC pre-exposure prophylaxis (PrEP) trial and men with primary HIV infection[29;30]. In the PreP trial, for example, the prevalence of low BMD (Z-score ≤ −2) was almost identical to our study[25]. Taken together, these findings may suggest that the impact of untreated HIV-infection on BMD may be less than anticipated.

Consistent with the hypothesis that untreated HIV infection does not have a markedly negative effect on BMD, we did not detect any association between low BMD and inflammatory markers (hsCRP and IL-6) or HIV disease related variables (CD4 cell count or HIV-RNA). This is somewhat surprising in that IL-6, for example, has been shown to increase osteoclast activity in pre-clinical models [21] and in epidemiologic studies in post-menopausal women, higher concentrations of IL-6 have been associated with BMD loss[26;27]. However, these results extend the findings of other studies which similarly have shown no relation between inflammatory markers and BMD or bone turnover in untreated HIV-infected patients[11;28]. One limitation of the current analysis was that we measured only two inflammatory markers. In future studies, other soluble and cellular inflammatory markers should be examined for their relationship with BMD in untreated HIV-infected patients.

We also investigated the relative contribution of lean and fat mass to BMD in this population and found strongest association with lean mass, which is an independent predictor of fracture in the general population[29]. Possible explanations include mechanical effects of muscle contraction of bone mass and strength, secreted factors that may allow metabolic communication between muscle and bone, and genetic, hormonal, and behavioral factors that may affect both muscle and bone. In general, HIV-infected populations have lower BMI and lean mass than HIV-uninfected control populations[38;30]. One possible explanation for differences in lean mass by HIV serostatus is the effect of chronic HIV-infection on muscle metabolism. Inflammatory cytokines, such as TNF-alpha and IL-6 have direct effects on muscle breakdown and these markers have been associated with sarcopenia in the general elderly population[31;32]. Indeed, in our exploratory analysis, higher IL-6 was associated with lower lean body mass independent of age, sex, and race in these HIV-infected, ART-naïve individuals, although the direction of this association cannot be determined in this cross-sectional study. Longitudinal studies are required to understand the contributors to lower lean mass in HIV-infected populations, including chronic inflammation, and the relationship between lean mass and bone mineral density and fracture.

Total fat mass also was associated with femoral neck BMD even after adjustment for lean mass, with similar trends at the other sites. In the general population, there is conflicting data in the general population regarding the relative effect of fat mass on BMD[12;13]. It is hypothesized that increased adipose tissue contributes to the mechanical effect on bone mass. In addition, adipose tissue may secrete hormonal factors which affect bone metabolism. We investigated whether two fat-derived hormones, adiponectin and leptin, were associated with BMD in untreated HIV-infected persons. In the general population, lower adiponectin and higher leptin have been associated with increased BMD, although the associations appear to be stronger for adiponectin compared to leptin and are independent of fat-mass[18]. Similarly, we found that both adiponectin and leptin were associated with BMD in the expected directions in univariate analyses, but after adjustment for fat mass and the other covariates, only adiponectin showed independent associations, with a statistically significant association at the total hip and a similar trend at the other sites.

Adiponectin is a protein secreted by adipocytes in high concentration and adiponectin receptors have also been found in osteoblasts and osteoclasts[33]. Activation of these receptors is thought lead to increased bone turnover. Perhaps thorough this mechanism, higher concentrations of adiponectin have been associated with lower BMD[18] and have been associated with an increased risk of fracture, even independent of BMD, in some[34;35], but not all studies[36]. Among HIV-infected populations, relative hypoadiponectinemia has been described [37]. We speculate that lower circulating levels of adiponectin may also serve to protect BMD in HIV-infected populations.

Other secreted factors may influence the balance of bone formation and resorption in our population, including osteoprotegerin and RANKL. In some epidemiologic studies in the general population, however, higher levels of OPG have been associated with lower BMD, which is thought to be a compensatory mechanism for abnormal bone metabolism[38;39], but these findings have not been consistent[40].

We found that higher concentrations of OPG were associated with increased BMD at all three sites. In addition to osteoblasts, other cell types including activated T-cells and B-cells produce OPG[41]. In a small previous study of OPG and other markers of inflammation and bone turnover in various disease states, the median OPG concentration in under-treated HIV-infected persons was 50% greater than the concentration observed in age- and sex-matched healthy controls, and was related to TNF-α levels, but not to bone turnover markers[42]. These findings suggest that OPG, which is a member of the TNF superfamily, is upregulated in untreated HIV-infection. Indeed, the mean OPG concentration among men in our study, adjusted for age, race, and BMI, was 31% higher than the adjusted mean OPG concentration in 141 HIV-uninfected men participating in the Multicenter AIDS Cohort Study cardiovascular substudy, which used an identical assay from the same laboratory (Wendy Post, personal communication). We speculate that higher concentrations of OPG, derived from activated immune cells in the untreated HIV-infected persons may help protect from increased bone resorption, as initially proposed by Seminari[43].

Osteoporosis and fracture risk in HIV-infected persons results from a complicated interaction between HIV-disease related factors, antiretroviral therapy, and traditional risk factors for bone loss found in high prevalence in HIV-infected persons. In order to begin to disentangle these multiple etiologies, it is useful to examine an untreated HIV-infected population for factors that may affect bone metabolism. To our knowledge, this is the largest study of BMD in ART-naïve persons with concomitant measures of body composition, adipocytokines, and OPG/RANKL.

Our study had several limitations. Similar to other ART-naïve treatment studies with specialized procedures[24], our study comprised mostly men. Further studies should focus on the bone health in HIV-infected women given the high burden of osteoporosis in women with aging. Second, we did not measure parathyroid hormone, 25 hydroxyvitamin D, free testosterone, bone turnover markers, or other markers of immune activation, systemic inflammation, or immune senescence as this was the baseline analysis of a clinical trial. We plan to examine these factors in further analyses. Next, the prevalence of some traditional osteoporosis risk factors was low in our population, such as hepatitis C and proton pump inhibitor use, limiting our ability to detect associations with BMD. Finally, our study was cross-sectional in nature and it is impossible to determine causality. Longitudinal studies in ART-naive persons will be critical to establish temporality.

In conclusion, we found a high prevalence of low BMD in this ART-naïve cohort, which was strikingly similar to men with primary HIV-infection or at risk men starting PrEP. Our findings suggest a delicate balance of factors affecting bone formation and bone resorption in untreated HIV-infected persons. While lower lean body mass, perhaps impacted by systemic inflammation, was associated with lower BMD, lower adiponectin and higher OPG appeared to have protective effects. In ongoing follow-up with this cohort, we will be able to carefully monitor whether the factors that we found to be associated with BMD at baseline predict changes in BMD with initiation, since the first 48–96 weeks after ART initiation are consistently associated with BMD losses and compromised bone health.

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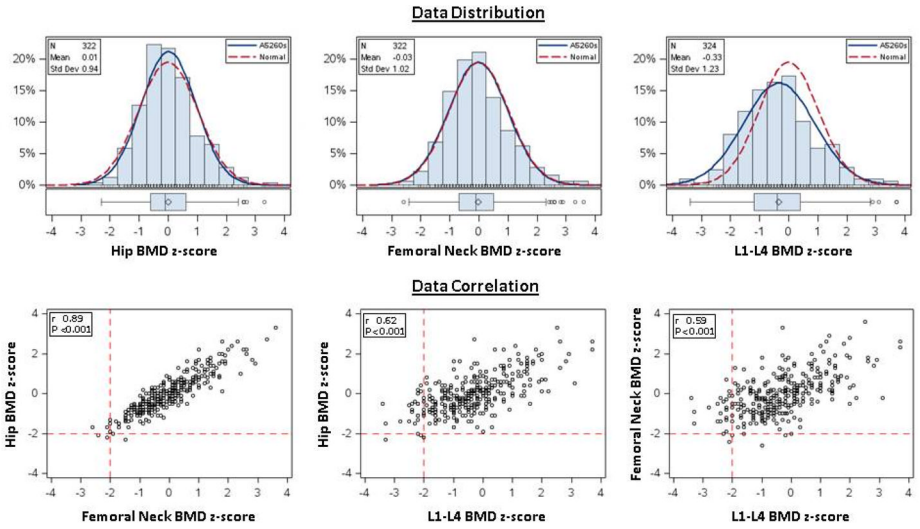

Figure 1.

Distribution of Z-scores at Hip, Femoral Neck, and Lumbar Spine in ART-naïve persons compared to the expected distribution of Z-scores. Pair-wise Correlation of Z-scores at the 3 BMD sites.

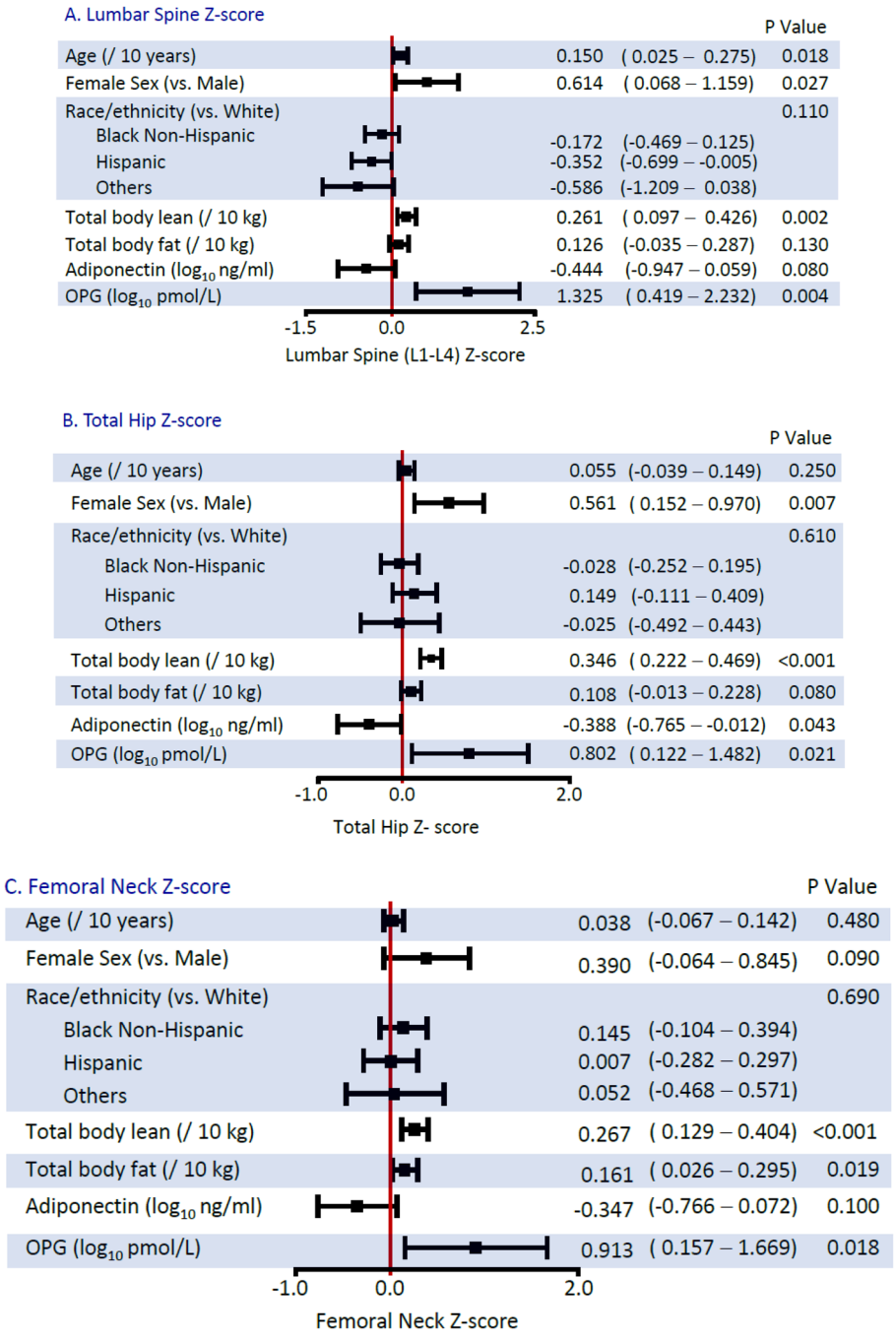

Figure 2.
Multivariable Linear Regression Models for Bone Mineral Density Z-scores

Table 1

Subject Characteristics (n=331)

Parameter	Median (Q1, Q3) or Percent
Age (years)	36 (28, 45)
Sex	89% Male, 11% Female
Race/ethnicity[a]	44% White, 32% Black, 20% Hispanic
Smoking status[a]	38% Current, 40% Never
Alcohol Use (≥ 3/drinks per day)[a]	29%
Current Proton Pump Inhibitor Use	6%
Physical Activity[a]	23% Low, 60% Moderate, 18% High
HIV-1 RNA (log_{10} copies/mL)	4.5 (4.0, 5.1)
CD4+ cell count (/mm^3)	349 (207, 455)
Time since HIV diagnosis (months)	5.7 (2.4 - 31.5)
Prior AIDS	23%
Nadir CD4 cell count (/mm^3)	311 (195, 417)
Hepatitis C	7%
Body Composition	
Body-mass index (kg/m^2)	25 (22, 28)
Visceral Adipose Tissue (cm^2)	73 (39, 107)
Total body fat mass (kg)	17 (11, 24)
Total body lean mass (kg)	56 (50, 63)
Biomarkers	
C-reactive protein(mg/L)	1.4 (0.7, 3.0)
Interleukin-6 (pg/mL)	1.8 (1.2, 3.0)
Adiponectin (ng/mL)	7780 (5355, 11540)
Leptin (pg/mL)	5288 (3017, 9558)
OPG (pmol/L)	4.2 (3.3, 5.1)
RANKL (pg/mL)	34 (16, 57)
Bone Parameters	
Lumbar Spine T-score	−0.5 (−1.2, 0.3)
Lumbar Spine Z-score	−0.4 (−1.2, 0.4)
Lumbar Spine Z-score ≤ −2 N (%)[a]	29 (9%)
Total Hip T-score	−0.3 (−0.8, 0.4)
Total Hip Z-score	−0.1 (−0.6, 0.6)
Total Hip Z-score ≤ −2.0 [a]	4 (1%)
Femoral Neck T-score	−0.5 (−1.1, 0.2)
Femoral Neck Z-score	−0.1 (−0.7, 0.5)
Femoral Neck Z-score ≤ −2 [a]	7 (2%)

[a]Percentages are calculated for participants with data; 1 participant is missing race/ethnicity, 1 participant is missing smoking history; 28 participants are missing alcohol use; 14 participants are missing physical activity; 9 participants are missing hip and femoral neck BMD Z scores; and 7 participants are missing lumbar spine BMD Z scores.

Table 2

Univariate Relationships Demographic Variables, Body Composition Data, and Biomarker Concentrations and Bone Mineral Density Z-scores at the Lumbar Spine, Total Hip, and Femoral Neck

Covariates	Lumbar Spine Z		Total Hip Z		Femoral Neck Z	
	rho	P	rho	P	rho	P
CD4+ cell count (/mm^3)	−0.02	0.68	−0.02	0.66	−0.02	0.67
HIV-1 RNA (log_{10} copies/ml)	0.02	0.77	0.04	0.50	0.02	0.66
Time since 1st HIV+ diagnosis (months)	0.11	0.049	0.05	0.36	0.09	0.13
VAT (cm^2)	0.14	0.014	0.18	0.001	0.14	0.010
Total body lean (kg)	0.24	<0.001	0.31	<0.001	0.28	<0.001
Total body fat (kg)	0.26	<0.001	0.26	<0.001	0.27	<0.001
CRP (log_{10} ug/ml)	0	0.96	0.02	0.69	0.02	0.68
IL-6 (log_{10} pg/ml)	0.01	0.84	0.05	0.38	0.06	0.28
Adiponectin (log_{10} ng/ml)	−0.12	0.037	−0.18	0.001	−0.19	<0.001
Leptin (log_{10} pg/ml)	0.17	0.002	0.22	<0.001	0.21	<0.001
OPG (log_{10} pmol/L)	0.2	<0.001	0.14	0.011	0.15	0.006
RANKL (log_{10} pg/ml)	0.04	0.52	0.04	0.48	0.05	0.40