Low Bone Mineral Density in Patients With Well-Suppressed HIV Infection: Association With Body Weight, Smoking, and Prior Advanced HIV Disease

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Background. Human immunodeficiency virus (HIV) and combination antiretroviral therapy (cART) may both contribute to the higher prevalence of osteoporosis and osteopenia in HIV-infected individuals.

Methods. Using dual-energy X-ray absorptiometry, we compared lumbar spine, total hip, and femoral neck bone mineral density (BMD) in 581 HIV-positive (94.7% receiving cART) and 520 HIV-negative participants of the AGEhIV Cohort Study, aged ≥45 years. We used multivariable linear regression to investigate independent associations between HIV, HIV disease characteristics, ART, and BMD.

Results. The study population largely consisted of men who have sex with men (MSM). Osteoporosis was significantly more prevalent in those with HIV infection (13.3% vs 6.7%; P < .001). After adjustment for body weight and smoking, being HIV-positive was no longer independently associated with BMD. Low body weight was more strongly negatively associated with BMD in HIV-positive persons with a history of a Centers for Disease Control and Prevention class B or C event. Interestingly, regardless of HIV status, younger MSM had significantly lower BMD than older MSM, heterosexual men, and women.

Conclusions. The observed lower BMD in treated HIV-positive individuals was largely explained by both lower body weight and more smoking. Having experienced symptomatic HIV disease, often associated with weight loss, was another risk factor. The low BMD observed in younger MSM remains unexplained and needs further study.

Keywords. HIV; bone mineral density; osteoporosis; osteopenia; body weight; men who have sex with men.
a combination of TDF with emtricitabine [9–11] but not in
those who switch to a different (non–TDF-containing) anti retro-
viral regimen [12,13]. Although the rate of BMD decline observed
after starting cART generally stabilizes with time [14, 15], there
are indications that the prolonged use of TDF may be associated
with a persistent increase in the rate of BMD decline [16].

The pathogenesis of the increased prevalence of reduced
BMD is probably multifactorial. It might be partially explained
by a lower average body weight of HIV-infected individuals [17]
and other traditional risk factors, such as hypogonadism, smok-
ing, alcohol or opiate use, and vitamin D deficiency which are
more prevalent in HIV-positive populations [18]. It remains
unclear whether and how HIV per se may be independently as-
associated with a reduced BMD. The objective of this study was to
further elucidate the relationship between BMD and HIV-1, use
of cART, and traditional risk factors for low BMD in a cohort of
HIV-1–infected individuals, predominantly receiving cART,
and HIV-uninfected controls with a comparable background,
all aged ≥45 years.

METHODS

Patients

The AGEhIV Cohort Study is an ongoing, prospective compar-
ative cohort study. Between 2010 and 2012, a total of 598 HIV-
1–infected individuals were recruited from the HIV outpa
t clinic of the Academic Medical Center in Amsterdam, the
Netherlands. As a control group, 550 HIV-uninfected individu-
als were recruited from the sexual health clinic and the
Amsterdam Cohort Studies on HIV/AIDS at the Amsterdam
Public Health Service, from the same geographic region and
with similar sociodemographic and behavioral (risk) factors
[19]. The inclusion criteria were age ≥45 years and laboratory-
confirmed presence (HIV-infected participants) or absence
(HIV-uninfected controls) of HIV infection. Dual-energy
X-ray absorptiometry (DXA) scanning for BMD measurement
is part of the assessment at baseline and biennial follow-up
study visits. Patients typically have several routine clinic visits
in between study visits; DXA scanning is not part of routine
clinical care. We performed a cross-sectional analysis of base-
line BMD measurements, obtained at study enrollment. All par-
ticipants provided written consent; the study was approved by
the local ethics review board (ClinicalTrials.gov identifier
NCT01466582).

Study Procedures and Definitions

The BMD of the lumbar spine (L1–L4), total hip, and femoral
neck was measured with Hologic QDR 4500 W and Hologic
Discovery A densitometers (software versions 12.4 and 13.3);
measurements were given in grams per square centimeter. Scann-
ers were cross-calibrated with a standard phantom. Scans for
both HIV-infected and –uninfected participants were obtained
centrally at the Academic Medical Center. The reference data-
base of the National Health and Nutrition Examination Survey
was used to calculate T and Z scores. T scores refer to the dis-
tance in standard deviations (SDs) from the mean of a sex–
race–matched reference population, aged 20–29 years; Z scores
refer to the distance in SDs from the mean of an age–, sex–, and
race–matched reference population.

In accordance with the World Health Organization defini-
tions, osteoporosis was defined as a T score of −2.5 SD or lower
and osteopenia as a T score between −1 and −2.5 SD.
In men aged <50 years and premenopausal women, it is advis-
not to diagnose osteoporosis based on BMD measurements
alone, but instead to define a Z score of −2 SD or lower as
“below the expected range for age” [20]. To verify that classify-
ng men aged <50 years and premenopausal women using
T scores did not affect our conclusions, we repeated the analysis,
using a Z score of −2 SD or lower as equivalent to osteoporosis
in these individuals.

Participants were asked to complete a questionnaire evaluat-
ing demographics, (family) medical history, use of medications,
substance use, physical activity, intake of calcium/vitamin D,
and sexual (risk) behavior. Blood and urine samples were col-
ered for extensive laboratory testing. Markers of inflamma-
tion (high-sensitivity C-reactive protein [hsCRP], coagulation
[D-dimer], microbial translocation (soluble CD14 [sCD14])
and monocyte activation (soluble CD163 [sCD163]) were deter-
mined for all study participants. Hepatitis B virus (HBV) and
hepatitis C virus (HCV) serostatus was also determined, as
were plasma HIV-1 RNA levels in the HIV-infected partici-
pants. Detailed information concerning HIV infection and an-
tiretroviral therapy (ART) history was extracted from the Dutch
HIV Monitoring Foundation database [21].

Statistical Analysis

Statistical analysis was performed using Stata software (version
12; StataCorp). All reported P values are 2 sided. Study groups
were compared using χ², Wilcoxon rank sum, or nonparametric
trend tests where appropriate.

Multivariable linear regression models were constructed,
with BMD in the lumbar spine, total hip, and femoral neck as
continuous dependent variables. We used multiple imputation
to handle missing observations of independent covariates. Con-
tinuous variables were transformed or categorized when neces-
sary. All models were adjusted for version of DXA software. To
ensure that no bias resulted from using multiple software ver-
sions, we verified that observed associations were not different
when including only participants scanned with one or the other
scanner in the models.

To assess whether HIV-positive status was independently as-
associated with BMD in the 3 bone locations, all models were ad-
justed for predefined recognized risk factors for osteoporosis,
potentially confounding the association between HIV status
and BMD: age, sex, menopausal status, body weight, race (black or nonblack), and smoking status. We preferred adjusting for body weight rather than for body mass index (BMI) because the constructed model fit the data better with body weight as a covariate as opposed to BMI.

In addition to the above-mentioned traditional risk factors, we explored other potential, biologically plausible determinants for reduced BMD in the multivariable models, and we investigated whether they confounded the association between HIV and BMD. These were physical activity, intake of dairy/fish, prescription drug and substance use, family history of hip fracture, and HBV and HCV infection or coinfec­tion. The 3 final presented models are adjusted for these covariates. A covariate was considered a confounder if addition to the model resulted in a change in the coefficient of HIV of ≥10%. Biologically plausible interactions between independent co­variates were also explored. Levels of hsCRP, D-dimer, sCD163, sCD14, and 25-hydroxy vitamin D2+D3 were explored as potential mediators in the association between HIV and BMD in the models.

Within the HIV-positive group, associations between BMD and known duration of HIV infection, prior Centers for Disease Control and Prevention (CDC) events, historic body weight, current and nadir CD4 cell counts, and HIV-1 plasma viral load were explored as covariates in the above-mentioned multivariable models. Current and prior cART use and its duration were also investigated.

RESULTS

Subject Characteristics

Bone mineral density measurements were available for 581 HIV-infected (97.2%) and 520 HIV-uninfected (94.6%) participants. Study groups were comparable with respect to age, sex, and proportion of men who have sex with men (MSM), but HIV-infected individuals had a lower median body weight, were more likely to smoke, and were more often black. A history of intravenous drug use was more common among HIV-infected participants, as were chronic HBV and HCV infection. The percentage of individuals currently using bisphosphonates was low and did not differ significantly between groups (Table 1). The vast majority of HIV-infected participants were receiving cART, had well-suppressed plasma HIV-1 loads, and had CD4 cell counts within the normal range (Table 2).

HIV Status and Osteoporosis

The prevalence of osteoporosis and osteopenia in each of the 3 skeletal locations was significantly higher in HIV-infected than HIV-uninfected individuals (Figure 1). Results were not significantly different with use of a Z score below −2 SD as equivalent to osteoporosis in men aged <50 years and premenopausal women.

Multivariable Analysis

Adjusted for age, sex, menopausal status, and race, HIV-positive status was significantly associated with lower BMD in the femoral neck (−0.021 g/cm²; P = .01) and the total hip (−0.031 g/cm²; P < .001), but not in the lumbar spine (−0.009 g/cm²; P = .32). With further adjustment for body weight, HIV-positive status was no longer associated with lower BMD in the femoral neck (−0.008 g/cm²; P = .26), and the association between HIV and BMD was attenuated in the total hip (−0.016 g/cm²; P = .04).

A lower current body weight was associated with a lower BMD; this association was significantly stronger in HIV-positive than in HIV-negative individuals. To explore this interaction further, the HIV-positive group was subdivided into those with no history of CDC events, those with a history of a CDC-B event, and those with a history of a CDC-C event [22]. The association between body weight and BMD was stronger in individuals who had experienced a CDC-B event, and even more so in those who had experienced a CDC-C event, compared with HIV-positive individuals without a history of CDC-B or CDC-C events and HIV-negative individuals (Table 3). The interaction between body weight and CDC class was statistically significant at the femoral neck for CDC-B (P = .04) and CDC-C (P = .005) and at the total hip for CDC-C (P = .001) and marginally significant at the total hip for CDC-B (P = .09) and at the lumbar spine for CDC-C (P = .06). A history of CDC-B or CDC-C events by itself was not independently associated with a lower BMD.

Inclusion of the other explored covariates in the models resulted in only modest (<10%) changes in the association between HIV status and BMD, and the interaction between CDC classification, body weight, and BMD. Statistically significant positive associations were found between the frequency of practicing heavy physical activity, use of proton pump inhibitors, and consumption of dairy or fish and BMD in ≥1 bone location. There was a statistically significant negative association between BMD and chronic HCV infection but not between BMD and chronic HBV infection. Furthermore, no statistically significant associations were observed between BMD and the frequency of moderate physical activity, heavy alcohol intake, recreational drug use at least once a month, current use of systemic corticosteroids or statins, or a family history of hip fracture.

Although MSM status was not independently associated with BMD, a statistically significant interaction was observed between age and MSM status for BMD in the lumbar spine (P = .03), femoral neck (P = .01), and total hip (P = .002) (Table 3). Whereas age was negatively associated with BMD
Table 1. Characteristics of HIV-Infected and HIV-Uninfected Study Participants

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>HIV-Positive Participants</th>
<th>HIV-Negative Participants</th>
<th>P Valuea</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, median (IQR), y</td>
<td>52.7 (48.3–59.4)</td>
<td>52.0 (47.9–58.0)</td>
<td>.23</td>
</tr>
<tr>
<td>Male sex, No. (%)</td>
<td>514 (88.5)</td>
<td>441 (84.8)</td>
<td>.07</td>
</tr>
<tr>
<td>MSM, No. (%)</td>
<td>398 (74.0)</td>
<td>357 (70.4)</td>
<td>.20</td>
</tr>
<tr>
<td>Black race, No. (%)</td>
<td>84 (14.5)</td>
<td>42 (8.1)</td>
<td>.001</td>
</tr>
<tr>
<td>Postmenopausal status (in women), No. (%)</td>
<td>30 (51.7)</td>
<td>40 (54.8)</td>
<td>.73</td>
</tr>
<tr>
<td>Body weight, median (IQR), kg</td>
<td>77.7 (69.1–86.5)</td>
<td>79.5 (72.1–86.7)</td>
<td>.01</td>
</tr>
<tr>
<td>Smoking</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Current, No. (%)</td>
<td>172 (24.3)</td>
<td>122 (24.3)</td>
<td></td>
</tr>
<tr>
<td>Past, No. (%)</td>
<td>186 (35.0)</td>
<td>198 (39.4)</td>
<td>.02</td>
</tr>
<tr>
<td>Pack-years of smoking for both current and former smokers, median (IQR)</td>
<td>22.5 (7.8–36.8)</td>
<td>13.8 (4.3–28.9)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Heavy alcohol intake in past 6 mo, No. (%)a</td>
<td>26 (4.9)</td>
<td>37 (7.4)</td>
<td>.10</td>
</tr>
<tr>
<td>Intravenous drug use, No. (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Current</td>
<td>0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Past</td>
<td>18 (2.4)</td>
<td>5 (1.0)</td>
<td>.005</td>
</tr>
<tr>
<td>Recreational drug use, at least once monthly in past 6 mo, No. (%)da</td>
<td>119 (22.6)</td>
<td>108 (21.5)</td>
<td>.66</td>
</tr>
<tr>
<td>Moderate physical activity (30 min), No. (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0 d/wk</td>
<td>109 (21.0)</td>
<td>65 (13.0)</td>
<td></td>
</tr>
<tr>
<td>1–4 d/wk</td>
<td>242 (46.5)</td>
<td>242 (48.2)</td>
<td></td>
</tr>
<tr>
<td>5–7 d/wk</td>
<td>169 (32.5)</td>
<td>195 (38.8)</td>
<td>.002</td>
</tr>
<tr>
<td>Heavy physical activity, No. (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;3 sessions/wk or &lt;20 min</td>
<td>377 (71.5)</td>
<td>321 (64.1)</td>
<td></td>
</tr>
<tr>
<td>≥3 sessions/wk or ≥20 min</td>
<td>150 (28.5)</td>
<td>180 (35.9)</td>
<td>.01</td>
</tr>
<tr>
<td>Hepatitis coinfection, No. (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hepatitis B (HBsAg and/or HBV DNA positive)</td>
<td>24 (4.1)</td>
<td>3 (0.6)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Hepatitis C (HCV RNA positive)</td>
<td>20 (3.4)</td>
<td>5 (1.0)</td>
<td>.006</td>
</tr>
<tr>
<td>Dairy consumption, median (IQR), times per day</td>
<td>2 (1–3)</td>
<td>2 (1–3)</td>
<td>.06</td>
</tr>
<tr>
<td>Consumption of oily fish, No. (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Never</td>
<td>58 (11.0)</td>
<td>45 (9.0)</td>
<td></td>
</tr>
<tr>
<td>Sometimes</td>
<td>346 (65.4)</td>
<td>320 (63.8)</td>
<td></td>
</tr>
<tr>
<td>Often</td>
<td>125 (23.6)</td>
<td>137 (27.3)</td>
<td>.12</td>
</tr>
<tr>
<td>Family history of hip fracture, No. (%)</td>
<td>64 (12.2)</td>
<td>57 (11.4)</td>
<td>.69</td>
</tr>
<tr>
<td>History of fractures, No. (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Any fracture</td>
<td>162 (30.8)</td>
<td>192 (38.2)</td>
<td>.01</td>
</tr>
<tr>
<td>Fracture in wrist, spine, or hip</td>
<td>16 (3.0)</td>
<td>19 (3.8)</td>
<td>.52</td>
</tr>
<tr>
<td>Prescription drug use at enrollment, No. (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bisphosphonate</td>
<td>8 (1.5)</td>
<td>3 (0.6)</td>
<td>.15</td>
</tr>
<tr>
<td>Proton pump inhibitor</td>
<td>40 (7.7)</td>
<td>42 (8.4)</td>
<td>.69</td>
</tr>
<tr>
<td>Systemic corticosteroid</td>
<td>2 (0.4)</td>
<td>3 (0.6)</td>
<td>.62</td>
</tr>
<tr>
<td>Statin</td>
<td>73 (14.0)</td>
<td>37 (7.4)</td>
<td>.001</td>
</tr>
<tr>
<td>25-OH vitamin D2+D3, median (IQR), nmol/L</td>
<td>46 (29–71)</td>
<td>54 (29–72)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>hsCRP, median (IQR), mg/L</td>
<td>1.5 (0.7–3.5)</td>
<td>1 (0.6–1.9)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>D-dimer, median (IQR), mg/L</td>
<td>0.22 (0.20–0.35)</td>
<td>0.24 (0.20–0.39)</td>
<td>.02</td>
</tr>
<tr>
<td>sCD14, median (IQR), ng/mL</td>
<td>1579 (1308–2010)</td>
<td>1361 (1087–1734)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>sCD163, median (IQR), ng/mL</td>
<td>289 (207–417)</td>
<td>248 (181–343)</td>
<td>&lt;.001</td>
</tr>
</tbody>
</table>

Abbreviations: 25-OH vitamin D2+D3, 25-hydroxy vitamin D2+D3; HBsAg, hepatitis B surface antigen; HBV, hepatitis B virus; HCV, hepatitis C virus; HIV, human immunodeficiency virus; hsCRP, high-sensitivity C-reactive protein; IQR, interquartile range; MSM, men who have sex with men; sCD14, soluble CD14; sCD163, soluble CD163.

a P values obtained with χ², Wilcoxon rank sum, and nonparametric test for trend where applicable.
b Birth country from individual or both parents is Suriname, Netherlands Antilles, or sub-Saharan Africa.
c Defined as alcoholic consumptions ≥3/d for women and ≥5/d for men.
d Cannabinoids, gamma-Hydroxybutyric acid (GHB), XTC, cocaine, (meth)amphetamine, uppers, downers, poppers, hallucinogen, or opiates.
in heterosexual men and women, in MSM such an association was not present or even reversed (Figure 2), independent of the investigated behavioral covariates (smoking, alcohol intake, and recreational drug use), race, or body weight. This statistically significant interaction was similar when analyzed separately in HIV-positive and HIV-negative cohorts (data not shown).

Factors Possibly Involved in the Causal Pathway
The level of 25-OH vitamin D2+D3 was lower in HIV-infected individuals, while levels of hsCRP, sCD163, and sCD14 were higher. Introduction of any of these markers into the models did not alter the association between HIV and BMD or the interaction between CDC classification, body weight, and BMD, nor were these markers independently associated with BMD in any of the 3 bone locations. Excluding patients currently taking bisphosphonates from the models did not significantly affect the results.

HIV-Associated Covariates

To explore possible associations of HIV-related variables (other than CDC classification) with BMD, we added these to the multivariable models from Table 3, while including only HIV-positive individuals. The HIV-related covariates that were independently associated with BMD in ≥1 bone location are included in the multivariable models (Table 4). The duration of severe immunodeficiency in this population (defined as a CD4 cell count <200 cells/mm³), although short (median duration, 0.94 months) showed a statistically significant negative association with BMD only in the total hip (−0.045 g/cm² per 5 years, P = .02), but this association became nonsignificant once covariates regarding ART and historic body weight were included in the model. Immunodeficiency defined by other cutoff values (50, 100, 350, 500, and 700 cells/mm³) was not associated with BMD.

Table 2. HIV-Related Characteristics

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>HIV-Positive Participants (n = 581)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Time since diagnosis of HIV infection, median (IQR), y</td>
<td>12.2 (6.5–17.2)</td>
</tr>
<tr>
<td>CD4 cell count, median (IQR), cells/mm³</td>
<td></td>
</tr>
<tr>
<td>In year before enrollment</td>
<td>565 (433–740)</td>
</tr>
<tr>
<td>Nadir</td>
<td>170 (70–260)</td>
</tr>
<tr>
<td>Cumulative duration of CD4 cell count &lt;200 cells/mm³, median (IQR), mo</td>
<td>0.94 (0–9.4)</td>
</tr>
<tr>
<td>CDC [22] classification, No. (%)</td>
<td></td>
</tr>
<tr>
<td>History of CDC-B event</td>
<td>138 (23.8)</td>
</tr>
<tr>
<td>History of CDC-C event</td>
<td>180 (31.0)</td>
</tr>
<tr>
<td>HIV-1 load &lt;200 copies/mL in year before enrollment, No. (%)</td>
<td>510 (88.5)</td>
</tr>
<tr>
<td>Duration of suppressed HIV-1 viremia, median (IQR), y</td>
<td>7.1 (3.4–10.5)</td>
</tr>
<tr>
<td>ART exposure</td>
<td></td>
</tr>
<tr>
<td>Current use, No. (%)</td>
<td>550 (94.7)</td>
</tr>
<tr>
<td>Cumulative ART exposure (excludes ART-naive participants), median (IQR), y</td>
<td>9.9 (4.3–14.2)</td>
</tr>
<tr>
<td>Pretreatment with mono/dual NRTI therapy before cART initiation, No. (%)</td>
<td>119 (21.4)</td>
</tr>
<tr>
<td>ART regimen, No. (%)</td>
<td></td>
</tr>
<tr>
<td>Containing NRTI/TDF</td>
<td>530 (96.4)/424 (77.1)</td>
</tr>
<tr>
<td>Containing protease inhibitor</td>
<td>240 (42.6)</td>
</tr>
<tr>
<td>Containing NNRTI/nevirapine</td>
<td>332 (60.4)/166 (30.2)</td>
</tr>
</tbody>
</table>

Abbreviations: ART, antiretroviral therapy; cART, combination antiretroviral therapy; CDC, Centers for Disease Control and Prevention; HIV, human immunodeficiency virus; IQR, interquartile range; NNRTI, nonnucleoside reverse-transcriptase inhibitor; NRTI, nucleoside reverse-transcriptase inhibitor; TDF, tenofovir disoproxil fumarate.

* Excludes participants with current HIV-1 viremia; transient viremia <200 copies/mL was ignored.

Figure 1. Prevalence of osteoporosis and osteopenia in human immunodeficiency virus (HIV)-positive and HIV-negative participants. P values obtained by nonparametric test for trend.
The known duration of HIV infection, current or nadir CD4 cell count, and CD4/CD8 cell count ratio were not associated with BMD, nor were markers of HIV replication (current HIV-1 viral load >200 copies/mL, cumulative duration of HIV-1 viremia, or peak HIV-1 viral load) or pretreatment with mono/dual nonnucleoside reverse-transcriptase inhibitors before initiation of cART. The lowest recorded historic body weight was associated significantly with BMD in the lumbar

### Table 3. Covariates Independently Associated With BMD in Multivariable Linear Regression Analysis

<table>
<thead>
<tr>
<th>Covariate</th>
<th>Lumbar Spine BMD&lt;sup&gt;a&lt;/sup&gt;,&lt;sup&gt;b&lt;/sup&gt;,&lt;sup&gt;c&lt;/sup&gt; (95% CI, g/cm²)</th>
<th>P Value</th>
<th>Femoral Neck, BMD&lt;sup&gt;a&lt;/sup&gt;,&lt;sup&gt;b&lt;/sup&gt;,&lt;sup&gt;d&lt;/sup&gt; (95% CI, g/cm²)</th>
<th>P Value</th>
<th>Total Hip, BMD&lt;sup&gt;a&lt;/sup&gt;,&lt;sup&gt;b&lt;/sup&gt;,&lt;sup&gt;e&lt;/sup&gt; (95% CI, g/cm²)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>HIV-status/CDC-class</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HIV negative (reference)</td>
<td></td>
<td>. .</td>
<td></td>
<td>. .</td>
<td></td>
<td>. .</td>
</tr>
<tr>
<td>HIV positive, CDC-A</td>
<td>0.007 (&lt;−15 to 13)</td>
<td>.93</td>
<td>−0.055 (&lt;−17 to 06)</td>
<td>.35</td>
<td>−0.043 (&lt;−16 to 08)</td>
<td>.47</td>
</tr>
<tr>
<td>HIV positive, CDC-B</td>
<td>−0.047 (&lt;−21 to 12)</td>
<td>.58</td>
<td>−0.150 (&lt;−29 to 01)</td>
<td>.03</td>
<td>−0.161 (&lt;−30 to 02)</td>
<td>.03</td>
</tr>
<tr>
<td>HIV positive, CDC-C</td>
<td>−0.127 (&lt;−29 to 03)</td>
<td>.12</td>
<td>−0.211 (&lt;−34 to 08)</td>
<td>.001</td>
<td>−0.266 (&lt;−40 to 13)</td>
<td>.001</td>
</tr>
<tr>
<td><strong>Body weight, per 10 kg&lt;sup&gt;f&lt;/sup&gt;</strong></td>
<td></td>
<td>. .</td>
<td></td>
<td>. .</td>
<td></td>
<td>. .</td>
</tr>
<tr>
<td>HIV negative</td>
<td>0.035 (&lt;0.03 to 0.05)</td>
<td>.&lt;.001</td>
<td>0.035 (&lt;0.03 to 0.04)</td>
<td>.&lt;.001</td>
<td>0.043 (&lt;0.03 to 0.05)</td>
<td>.&lt;.001</td>
</tr>
<tr>
<td>HIV positive, CDC-A</td>
<td>0.038 (&lt;0.02 to 0.05)</td>
<td>.&lt;.001</td>
<td>0.042 (&lt;0.03 to 0.05)</td>
<td>.&lt;.001</td>
<td>0.048 (&lt;0.04 to 0.06)</td>
<td>.&lt;.001</td>
</tr>
<tr>
<td>HIV positive, CDC-B</td>
<td>0.042 (&lt;0.02 to 0.06)</td>
<td>.&lt;.001</td>
<td>0.054 (&lt;0.04 to 0.07)</td>
<td>.&lt;.001</td>
<td>0.062 (&lt;0.05 to 0.09)</td>
<td>.&lt;.001</td>
</tr>
<tr>
<td>HIV positive, CDC-C</td>
<td>0.054 (&lt;0.04 to 0.07)</td>
<td>.&lt;.001</td>
<td>0.061 (&lt;0.05 to 0.08)</td>
<td>.&lt;.001</td>
<td>0.075 (&lt;0.06 to 0.09)</td>
<td>.&lt;.001</td>
</tr>
<tr>
<td><strong>Male sex</strong></td>
<td></td>
<td>. .</td>
<td></td>
<td>. .</td>
<td></td>
<td>. .</td>
</tr>
<tr>
<td>Non-MSM</td>
<td>−0.006 (&lt;−0 to 0.02)</td>
<td>.68</td>
<td>−0.039 (&lt;−0.06 to −0.02)</td>
<td>.001</td>
<td>−0.033 (&lt;−0.06 to −0.01)</td>
<td>.007</td>
</tr>
<tr>
<td>MSM</td>
<td>0.030 (&lt;0.02 to 0.04)</td>
<td>.&lt;.001</td>
<td>−0.007 (&lt;−0.02 to −0.01)</td>
<td>.28</td>
<td>0.008 (&lt;−0.00 to 0.02)</td>
<td>.21</td>
</tr>
<tr>
<td><strong>Pack-years of smoking, per 10 y</strong></td>
<td>−0.005 (&lt;−0.01 to −0.00)</td>
<td>.04</td>
<td>−0.004 (&lt;−0.01 to −0.00)</td>
<td>.04</td>
<td>−0.005 (&lt;−0.01 to −0.00)</td>
<td>.01</td>
</tr>
</tbody>
</table>

Abbreviations: BMD, bone mineral density; CDC, Centers for Disease Control and Prevention; CI, confidence interval; HIV, human immunodeficiency virus; MSM, men who have sex with men.

<sup>a</sup> For univariable associations between reported coefficients and BMD, see Supplementary Table 1.

<sup>b</sup> Adjusted for dual-energy X-ray absorptiometry software version used, regular heavy and moderate physical activity, use of proton pump inhibitors/corticosteroids/statins, consumption of dairy or oily fish, regular (≥ monthly) recreational and (current/historic) intravenous drug use, family history of hip fracture, alcohol abuse and presence of hepatitis B surface antigen or hepatitis C virus RNA.

<sup>c</sup> $R^2 = 0.191$ (estimated, based on Fisher $z$ transformation); $P < .001$.

<sup>d</sup> $R^2 = 0.266$; $P < .001$.

<sup>e</sup> $R^2 = 0.311$; $P < .001$.

<sup>f</sup> Coefficients for interaction between body weight and CDC classification: lumbar spine, $P = .79$, $P = .53$, and $P = .07$ for CDC-A, CDC-B, and CDC-C, respectively; femoral neck, $P = .36$, $P = .03$, and $P = .002$, respectively; and total hip, $P = .52$, $P = .04$, and $P < .001$, respectively.

<sup>g</sup> Coefficients for interaction between age and MSM status: lumbar spine, $P = .03$; femoral neck, $P = .01$; and total hip, $P = .003$.

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Figure 2. Bone mineral density (BMD) in relation to age in men who have sex with men (MSM) compared with non-MSM male and female subjects in the lumbar spine, femoral neck, and total hip.
Table 4. HIV-Related Characteristics Associated With BMD in Multivariable Linear Regression Analysis (HIV-Positive Individuals Only)a

<table>
<thead>
<tr>
<th>Covariate</th>
<th>Lumbar Spine, BMDb,c (95% CI, g/cm²)</th>
<th>P Value</th>
<th>Femoral Neck, BMDb,d (95% CI, g/cm²)</th>
<th>P Value</th>
<th>Total Hip, BMDb,e (95% CI, g/cm²)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cumulative duration of CD4 cell count &lt;200 cells/mm³, per 5 y</td>
<td>0.009 (−0.04 to .05)</td>
<td>.74</td>
<td>−0.000 (−0.04 to .04)</td>
<td>1.00</td>
<td>−0.034 (−0.07 to .00)</td>
<td>.08</td>
</tr>
<tr>
<td>Lowest registered body weight after HIV infection, per 10 kg</td>
<td>0.028 (.01 to .05)</td>
<td>.01</td>
<td>0.023 (.00 to .04)</td>
<td>.01</td>
<td>0.018 (−0.00 to .04)</td>
<td>.07</td>
</tr>
<tr>
<td>Cumulative exposure to high-dose ritonavir, per 1 y</td>
<td>−0.003 (−0.01 to .00)</td>
<td>.40</td>
<td>−0.007 (−0.01 to −0.00)</td>
<td>.02</td>
<td>−0.007 (−0.01 to −0.00)</td>
<td>.02</td>
</tr>
<tr>
<td>Current nevirapine use</td>
<td>0.028 (.00 to .06)</td>
<td>.05</td>
<td>0.027 (.00 to .05)</td>
<td>.02</td>
<td>0.028 (.00 to .05)</td>
<td>.02</td>
</tr>
</tbody>
</table>

Abbreviations: BMD, bone mineral density; CI, confidence interval; HIV, human immunodeficiency virus.

a For univariable associations between reported coefficients and BMD: see Supplementary Table 2.

b Adjusted for dual-energy X-ray absorptiometry software version used, Centers for Disease Control and Prevention class, current body weight, race, age, men who have sex with men (MSM), the interaction between age and MSM, pack-years of smoking, regular heavy and moderate physical activity, use of proton pump inhibitor, corticosteroid, or statin, consumption of dairy or oily fish, regular (at least monthly) recreational and (current/historic) intravenous drug use, family history of hip fracture, alcohol abuse, and presence of hepatitis B surface antigen or hepatitis C virus RNA.

c $R^2 = 0.223$ (estimated, based on Fisher z transformation); $P < .001$.

d $R^2 = 0.357; P < .001$.

e $R^2 = 0.394; P < .001$.

spine and femoral neck, and borderline significantly with total hip BMD. A significant positive association was observed between current use of nevirapine and BMD in all 3 locations, and a significant negative association between the duration of exposure to high-dose (≥400 mg/d) ritonavir and BMD in the femoral neck and total hip. Other types of ART, including current or prior TDF use or its duration, were not associated with BMD.

**DISCUSSION**

In this cross-sectional analysis of BMD measurements within a cohort of largely well-suppressed HIV-positive and HIV-negative subjects aged ≥45 years, we observed a higher prevalence of osteoporosis and osteopenia in HIV-positive subjects. This is consistent with previous reports of a higher prevalence of osteoporosis in HIV-infected individuals [1, 17]. However, after adjustment for possible confounding by traditional risk factors, HIV did not remain independently associated with lower BMD. We found a lower median body weight in the HIV-positive population to be the strongest confounder of the association between HIV status and BMD. A history of symptomatic HIV disease seems to aggravate the negative effect of low body weight on BMD.

Our findings regarding the confounding effect of body weight in the association between HIV status and BMD are consistent with a meta-analysis by Bolland et al [17], which pointed out that lower BMD in HIV-positive subjects is largely explained by differences in body weight. The association between body weight, HIV status, and BMD is complex, because HIV and certain ART regimens affect body weight, body composition, and the distribution of fat and lean mass. Bone mineral density is strongly associated with fat mass and, even more strongly, lean mass in the general population [23] as well as in HIV-infected individuals [24, 25].

We do have indications that those with a history of advanced HIV disease have a lower BMD. We observed a weak association between immunodeficiency, the duration of a CD4 cell count <200, and lower BMD in the total hip. In addition, the lowest body weight of HIV-infected individuals ever recorded was strongly associated with lower BMD. A subgroup of HIV-positive individuals with low current body weight and a CDC-B or CDC-C classification had lower BMD than both HIV-positive subjects with higher body weight or a CDC-A classification and HIV-negative subjects. These individuals may have irreversibly lost lean body mass in the past owing to more advanced HIV disease.

The observed associations between body weight and BMD may be direct consequences of changed body weight and composition, or alternatively, may result from severe illness at the time this weight loss occurred. Treatment with corticosteroids as part of the management of common prior opportunistic infections, such as *Pneumocystis jiroveci*, pneumonia, may also have contributed to loss of BMD. The relative contributions of changes in body composition and prior severe illness to BMD loss cannot be distinguished with certainty in this cross-sectional study with very limited data on body composition. Longitudinal BMD measurements and planned additional body composition measurements within this cohort may provide further insight into the pathophysiological mechanisms.

Drawing definitive conclusions on causation between specific antiretroviral drugs and BMD is impossible owing to the cross-sectional and observational nature of our study; we merely report associations. Our statistical power to detect associations between (specific) ART and BMD is very limited, given that
only a small proportion of the HIV-positive study group has never been exposed to ART, and those who started treatment have often used many sequential cART regimens. Furthermore, any observed association may be biased as a result of nonrandom allocation of particular antiretroviral agents. The observed negative association between the duration of exposure to high-dose ritonavir and BMD may be indicative of historic severe HIV-related disease, considering the greater prevalence of patients with more severe HIV-related disease at the time high-dose ritonavir was commonly prescribed. However, a direct negative effect of ritonavir on BMD cannot be ruled out. Several potential mechanisms for the negative effect of PIs on bone have been suggested by findings of ex vivo studies, involving effect of PI on bone formation as well as on bone resorption [26, 27]. In cross-sectional studies, exposure to high-dose ritonavir has been associated with reduced BMD among children [28], and use of any PI with reduced BMD in adults [1, 29–31]. Furthermore, the duration of treatment with lopinavir combined with ritonavir has been associated with an increased risk of fractures [32].

We did not observe a negative association between duration of TDF use and BMD, in contrast with previous reports of lower BMD in individuals exposed to TDF [16, 33]. However, our power to detect such an association was very low. For many years, all preferred first-line cART regimens in our hospital contained TDF. Most patients who used alternative nucleoside reverse-transcriptase inhibitors did so because of a particular contraindication for the use of TDF or because of side effects of TDF.

We observed a statistically significant positive association between the current use of nevirapine and BMD, consistently in all 3 bone locations. It is uncertain whether nevirapine has an actual effect on bone metabolism. Similar positive associations between nevirapine use and BMD have been reported elsewhere [34], but to our knowledge the effect of nevirapine on bone cells has not been studied ex vivo.

The BMD of the younger MSM subjects was surprisingly low, in both HIV-positive and HIV-negative participants, as demonstrated by the observed unexpected relationship between age and BMD in this group. Whereas, as expected, older age in heterosexual men and women was negatively associated with BMD, a different pattern was observed in MSM. No statistically significant negative association between BMD and age was observed in the femoral neck and total hip, while in the lumbar spine we even observed a significant positive association. The relatively lower BMD in younger MSM is probably confounded by current or historic lifestyle differences between the younger and the older MSM population. We could not explain the observation by including in our models body weight or any self-reported information on behavior, such as smoking or use of recreational drugs. The questions regarding behavior, however, largely covered only the past 6 months, whereas BMD changes in this population may have happened years ago. We speculate that the younger MSM within this cohort may have had a different historic pattern of engagement in active sports, diet, use of anabolic steroids, or recreational drugs than the older MSM, possibly at an age before reaching peak bone mass.

Previous studies have demonstrated reduced BMD in MSM, compared with reference values, in HIV-infected as well as HIV-uninfected men, including those with primary HIV infection [35–37], corroborating our findings. In addition, several studies, involving predominantly MSM populations, showed no differences between ART-naive HIV-infected (including those with primary infection) and HIV-uninfected individuals [37–39], suggesting that the low BMD observed in HIV-positive MSM at least partly predates HIV infection. Previous studies may therefore have overestimated the effect of HIV on bone by inclusion of insufficiently comparable control groups.

In summary, in this predominantly male and MSM cohort, aged ≥45 years, we observed lower BMD in predominantly cART-treated HIV-positive individuals than in those who were HIV negative. This finding could largely be explained by a lower median body weight and more smoking in the HIV-positive group. Prior symptomatic and more severe HIV disease in particular and associated weight loss due to wasting may have persistently negatively affected BMD in a subgroup of HIV-positive individuals. Finally, we observed a strikingly low BMD in HIV-positive as well as HIV-negative younger MSM in this population, suggesting that low BMD in these men may to a considerable extent predate their acquisition of HIV.

The observed lower BMD in individuals who had experienced loss of body weight associated with advanced HIV disease supports the need for earlier identification and treatment of individuals with HIV infection. Furthermore, clinicians should be aware of the high prevalence of low BMD, particularly in the (relatively) young MSM population. Such individuals may be particularly prone to develop osteoporosis/osteopenia because of the BMD decline generally observed after cART initiation [5]. Avoidance of regimens associated with greater BMD loss, supportive treatment with vitamin D and calcium [40], and BMD monitoring, especially in the presence of additional risk factors for low BMD, may each be worth considering in such men.

**Supplementary Data**

Supplementary materials are available at The Journal of Infectious Diseases online (http://jid.oxfordjournals.org). Supplementary materials consist of data provided by the author that are published to benefit the reader. The posted materials are not copyedited. The contents of all supplementary data are the sole responsibility of the authors. Questions or messages regarding errors should be addressed to the author.

**Notes**

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Potential conflicts of interest. K. W. K. has received travel grants from
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grants from Gilead Sciences, Bristol-Myers Squibb, Boehringer Ingelheim, ViVi Healthcare, and AbbVie. J. S. has received travel grants from Gilead Sciences, ViVi Healthcare, and Boehringer Ingelheim. M. v. d. V. has
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Bristol-Myers Squibb. P. R. has received research support from Gilead Sci-
cences, Janssen Pharmaceutica, Merck, Bristol-Myers Squibb, and ViVi
Healthcare, and travel grants from Gilead Sciences; he serves as a scientific
advisor to Gilead Sciences and on a data safety monitoring committee for
Janssen Pharmaceutica. All other authors report no potential conflicts.

All authors have submitted the ICMJE Form for Disclosure of Potential
Conflicts of Interest. Conflicts that the editors consider relevant to the con-
tent of the manuscript have been disclosed.

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