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# Microstructural alterations of trabecular and cortical bone in long-term HIV-infected elderly men on successful antiretroviral therapy

Emmanuel Biver[a,*], Alexandra Calmy[b,*], Cécile Delhumeau[b], Claire Durosier[a], Sophie Zawadynski[c] and René Rizzoli[a]

**Objective:** Progress in antiretroviral therapy (ART) has resulted in an almost normal life expectancy for HIV-infected individuals, but an increased risk of fragility fractures has been identified. We investigated the influence of long-term HIV infection on successful ART on bone microstructure in elderly men.

**Design:** A cross-sectional, case–control study.

**Methods:** Dual-energy X-ray absorptiometry (DXA) and high-resolution peripheral quantitative computed tomography (HR-pQCT) were performed in 28 HIV-positive men between 60 and 70 years old on successful ART. Controls were 112 HIV-negative men matched for age (±4 years) and BMI (±4 kg/m$^2$).

**Results:** HIV-positive men (median CD4$^+$ cell count, 589 cells/μl; BMI, 24.8 kg/m$^2$) had a median duration of HIV infection of 18.2 years. Compared with HIV-negative men, they had a lower DXA-measured areal bone mineral density at total hip (−3.2%, $P = 0.050$) and ultra-distal radius (−8.4%, $P = 0.001$). At distal radius and tibia, we observed microstructural alterations with a lower total density (−16%, $P = 0.005$ and −14.3%, $P = 0.039$), trabecular density (−11.6%, $P = 0.012$ and −12.2%, $P = 0.007$) and cortical area (−17.5%, $P = 0.002$ and −12.2%, $P = 0.01$). In addition, they had a lower trabecular number ($P = 0.036$), higher trabecular spacing ($P = 0.027$) and lower cortical thickness (−19.9%; $P = 0.008$) at distal radius. beta-crosslaps (CTX) and vitamin D levels were higher than in controls. By multivariate analyses, HIV status, higher CTX levels, lower physical activity and estradiol levels were determinants of bone density and microstructure alterations.

**Conclusion:** HIV-infected elderly men on successful ART have trabecular and cortical bone microstructure alterations associated with higher bone resorption, despite adequate vitamin D supplementation.



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**Keywords: bone microstructure, bone mineral density, HAARTs, high-resolution peripheral quantitative computed tomography, HIV**

## Introduction

HIV-infected patients are at an increased risk of fractures associated with a decrease of areal bone mineral density (aBMD) occurring upon initiation of antiretroviral therapy (ART) [1–5]. Several factors contribute to the pathophysiology of bone loss in this context, including HIV infection itself, ART, as well as usual risk factors frequently reported in this population, such as low calcium and protein intakes, reduced physical activity and

[a]Division of Bone Diseases, Department of Internal Medicine Specialties, [b]Division of Infectious Diseases, HIV Unit, Department of Internal Medicine Specialties, and [c]Division of Nuclear Medicine, Department of Radiology, Geneva University Hospitals and Faculty of Medicine, Geneva, Switzerland.

Correspondence to Emmanuel Biver, MD, PhD, Division of Bone Diseases, Geneva University Hospitals and Faculty of Medicine, 4 Rue Gabrielle Perret-Gentil, 1211 Geneva 14, Switzerland.

Tel: +41 22 382 99 50; fax: +41 22 382 99 75; e-mail: Emmanuel.Biver@hcuge.ch

* Both Emmanuel Biver and Alexandra Calmy contributed equally to this article.

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high alcohol and tobacco consumption. Age is a main determinant of bone fragility and fracture risk exponentially increases after the age of 60 years in the general population [6].

Major advances in ART over the past 20 years have contributed to a significant increase in the life expectancy of HIV patients. As a result, a substantial number of long-term HIV-positive patients on ART are now approaching 70 years of age and constitute a new population, which has been hitherto poorly investigated. In this emerging group of individuals, it can be expected that the cumulative exposure to the potential adverse effects of HIV infection and/or ART on bone will add to the age-related bone microstructure alterations, thus contributing to lower bone strength and a higher fracture risk than in the general population. To our knowledge, there are no data on the impact of long-term, treated HIV infection on bone mass and microstructure in elderly men. Our objective was to assess the effects of long-term, treated HIV infection on bone density (volumetric bone mineral density, vBMD) and cortical and trabecular bone microstructure in men over 60 years of age using high-resolution peripheral quantitative computed tomography (HR-pQCT). We hypothesized that long-term, HIV-infected men have alterations of vBMD and microstructure not fully appreciated by aBMD when assessed by routine dual-energy X-ray absorptiometry (DXA) evaluation, a particularly important issue to consider in fracture risk assessment in HIV-positive treated patients.

## Materials and methods

### Study design and participants

We conducted a 1 : 4 cross-sectional, case-matched control study in 28 HIV-positive and 112 HIV-negative men matched by age (±4 years) and BMI (±4 kg/m$^2$). Cases were HIV-positive white men, recruited between July and October 2013 without advertisement, during their ambulatory visit with an infectious diseases specialist physician at the Geneva University Hospitals, Geneva, Switzerland. The study was presented as a study on bone to all patients meeting the inclusion criteria, including age between 60 and 70, a minimum duration of HIV infection of 5 years and effective ART (HIV RNA <40 copies/ml) for more than 1 year. Exclusion criteria were a history of neoplasia within the past 5 years, heart, respiratory or renal failure, active opportunistic infections and treatment with antiosteoporotic drugs. Controls were selected from a cohort of healthy retired workers recruited in the Geneva area community [7]. Calcium and protein intakes, as well as physical activity, were assessed by frequency questionnaires as previously described [8]. All individuals provided written informed consent. The study was approved by the ethics committee of Geneva University Hospitals.

### Biochemical determinations

Serum biochemical values were determined by the central chemistry laboratory at Geneva University Hospitals. HIV-related parameters, such as CD4$^+$ cell count, HIV-RNA and CD4$^+$ cell count nadir, were extracted from the Swiss HIV Cohort Study at the closest time point to the measurement visit (3 months' window). Blood samples were collected between 0700 and 1000 h after overnight fast for batchwise determination of serum levels of creatinine, 25-hydroxyvitamin D (25-OH-D), parathyroid hormone (PTH), type 1 collagen amino-terminal telopeptide (P1NP), beta-crosslaps (CTX), total testosterone and estradiol. Estradiol levels less than 20 ng/l were assumed to be 10 ng/l for continuous variables analyses.

### Bone density and body composition measurements

aBMD (g/cm$^2$) of the lumbar spine (L1–L4), non-dominant femoral neck, total hip, 1/3 radius, ultradistal and total radius, as well as body composition, were measured by DXA on a Discovery bone densitometer (Hologic Inc., Bedford, Massachusetts, USA) with a coefficient of variation of repeated measurements varying between 1.0 and 1.5%.

### High-resolution peripheral quantitative computed tomography measurements

Bone density (vBMD) and microstructure variables were determined at the distal radius and distal tibia by HR-pQCT using an Xtreme CT instrument (Scanco Medical, Bassersdorf, Switzerland). A stack of 110 CT slices were acquired over a 9 mm length with an isotropic voxel size of 82 μm, starting proximally at 9.5 and 22.5 mm from a joint margin reference line for distal radius and distal tibia, respectively. The effective dose was 3 μSv and the measurement time 2.8 min. Short-term reproducibility assessed with repositioning was 0.6–1.0 and 2.8–4.9% for density variables and trabecular microstructure, respectively [9]. Determinations were performed on the nondominant limb, unless a fracture was reported in the region of interest. Recorded variables were as follows: total, cortical and trabecular vBMD, expressed as milligrams per cubic centimetre of calcium hydroxyapatite (mg/cm$^3$); total cross-sectional area, and cortical and trabecular areas (mm$^2$); relative trabecular bone volume (BV/TV) (percentage); trabecular number (mm$^{-1}$), thickness and spacing (μm); trabecular spacing standard deviation (SD), estimate of the heterogeneity of the trabecular structure (μm) and mean cortical thickness (μm). Cortical porosity (percentage) was calculated as the number of void voxels in each binary cortex image divided by the total number of voxels [10].

### Fractures

Fracture history was recorded during an interview, in which all patients were asked whether they had ever suffered a fracture, on details on fracture site, age at time of fracture and type and intensity of trauma associated

with the fracture. Fractures of the skull, feet and hands were excluded and fragility fractures were distinguished from all clinical fractures. For vertebral fracture assessment, a lateral scan of the spine (T6–L4) was acquired using DXA on the same day as BMD measurement. According to the Genant visual semi-quantitative method, morphometric vertebral deformations were classified as wedge, biconcave and compression, and graded as mild (20–25% height reduction), moderate (25–40%) and severe (>40%) [11]. We defined all moderate and severe deformations as prevalent vertebral fractures.

### Statistical analysis

The power calculation of this study was computed using our data previously published in premenopausal women [12]. By including at least 21 HIV-positive and 84 HIV-negative men, we estimated that we would be able to detect a difference of 14% in trabecular density to achieve a power level of 90% with an alpha threshold of 5%. The primary endpoint was trabecular density at distal radius and tibia between HIV-positive and HIV-negative men.

Data are expressed as the median ± interquartile range (IQR) by HIV status. Differences between the two groups in aBMD and vBMD and bone microstructure variables were assessed by univariate conditional logistic regression with an alpha threshold of 5%. Determinants of vBMD and bone microstructure in the whole study population ($n = 140$) were analysed for total volumetric density, trabecular density and cortical area, representing the parameters of the three bone compartments (total, trabecular and cortical) that displayed higher alterations in HIV-positive men. They were first analysed using linear mixed models grouped by pair with an alpha threshold of 5%. Linear mixed models did not provide additional information compared with linear regression (likelihood ratio test $P$ values were not statistically significant). The Shapiro–Francia W test and skewness/kurtosis tests were used to verify the normality of the distributions; non-Gaussian variables were normalized using simple mathematical transformations. HIV status, age, inverse BMI, tobacco use (current vs. previous or never), alcohol consumption (>7 units weekly vs. ≤ 7), logarithm calcium intake, protein intake, physical activity, vitamin D level, PTH (all square root transformed), logarithm CTX, inverse square root P1NP, total testosterone and estradiol levels were included in univariate linear regression models adjusted for age and BMI to comply with our baseline age and BMI-matched design.

To identify potential confounding risk factors for osteoporosis, we used a multivariate linear regression model (M1, Table 3) including the clinical risk factors classically associated with BMD alteration, that is HIV status, age, inverse BMI, tobacco use, alcohol consumption and the square root of vitamin D and testosterone levels. We performed additional models to assess the specific contribution of lower physical activity (M2), estradiol (M3), CTX (M4) and P1NP levels (M5) in HIV-associated bone microstructure alterations. Using univariate linear regression, we also looked at the effect of the $CD4^+$ cell count nadir, HIV duration and current or past duration use of each class of ART, including tenofovir, on radius volumetric density and CTX levels. Statistical analyses were performed using STATA software, version 12.1 (StataCorp LP, College Station, Texas, USA).

## Results

### Characteristics of the study population

Ninety-nine HIV-positive men aged between 60 and 70 years were identified in the Geneva HIV ambulatory care unit. Of 49 patients screened, 28 were included in the study; 12 did not meet the inclusion criteria (five under bisphosphonate therapy, three with neoplasia, one with HIV duration <5 years, one with heart failure, one opportunistic infection, one over 70 years old at time of screening), and nine declined to participate.

Characteristics of the cases and matched controls are described in Table 1. HIV-positive men had lower physical activity (−75%), calcium intake (−13%), than HIV-negative controls. Protein intake was slightly, although not significantly, lower in the HIV-positive group. The rate of men with vitamin D supplements was higher in HIV-positive men (61 vs. 8%, respectively) and 25-OH-D levels were consequently higher, reaching the target recommendations (>75 nmol/l) in 18 (64%) HIV-positive patients. HIV-positive men had higher bone resorption marker levels (CTX, +23%; $P = 0.014$). Total testosterone levels were similar, but estradiol levels were lower in HIV-positive men, with more frequently undetectable levels (71 vs. 44%, respectively; $P = 0.009$).

The median duration of HIV infection was 18.2 (IQR, 11–33.2) years and the rate of coinfections with hepatitis B or C was very low [3 (11%) and 2 (7%), respectively]. All HIV-positive men were on successful ART with undetectable HIV RNA, except for one man with HIV RNA more than 40 at the time of assessment (but undetectable at screening) and a median $CD4^+$ cell count of 589 cells/μl (IQR, 430–1216). Current ART included eight patients (29%) on boosted protease inhibitor (bPI)-based therapy and 14 (50%) on non-nucleoside reverse transcriptase inhibitors (NNRTI)-based regimen; 18 (64%) patients had a regimen including tenofovir (Table 1).

### Areal bone mineral density by dual-energy X-ray absorptiometry

HIV-positive men tended to have lower lumbar spine BMD (−6.9%; $P = 0.051$), total hip BMD (−3.2%; $P = 0.050$), total distal radius (−3.6%; $P = 0.064$) and

**Table 1. Characteristics of HIV-positive men and HIV-negative controls (median ± interquartile range or %).**

| | HIV-positive ($n=28$) median [IQR] or % | HIV-negative ($n=112$) median [IQR] or % | $P$ |
|---|---|---|---|
| Sociodemographic characteristics | | | |
| Age (year) | 64 [62.0–67.0] | 64.6 [63.7–66.3] | 0.200 |
| Tobacco consumption:current | 18% | 8% | 0.121 |
| Alcohol consumption: ≥7 units weekly | 29% | 29% | 0.926 |
| Vitamin D supplements | 61% | 8% | <0.001 |
| Nutrition and physical activity | | | |
| Calcium intake (mg/day) | 1074 [664–1343] | 1233 [867–1441] | 0.036 |
| Protein intake (g/day) | 77 [55–99] | 82 [69–100] | 0.111 |
| Protein intake (g/kg BW × day) | 1[0.7–1.3] | 1.1 [0.9–1.3] | 0.379 |
| Physical activity (kcal/day) | 175 [93–248] | 713 [402–961] | <0.001 |
| Fractures | | | |
| All fractures | 64% | 63% | 0.930 |
| Clinical fractures | 57% | 56% | 0.932 |
| Clinical fragility fractures | 14% | 10% | 0.495 |
| Vertebral fracture grades 2 or 3 | 18% | 12% | 0.377 |
| Serum parameters | | | |
| Creatinine (mmol/l) | 0.089 [0.083–0.102] | 0.070 [0.070–0.080] | <0.001 |
| 25 OH vit. D (nmol/l) | 85.4 [70.2–99.9] | 65 [49.9–84.1] | <0.001 |
| PTH (pmol/l) [ref. 1.1–6.8 pmol/l] | 4.6 [3.4–5.8] | 4.2 [3.2–5.1] | 0.291 |
| CTX crosslaps [ref. 104–504 ng/l] | 336 [242–607] | 274 [199–384] | 0.014 |
| P1NP [ref. 15–59 μg/l] | 38.3 [26.1–55.5] | 31.9 [25.1–40.6] | 0.129 |
| Total testosterone [ref. 2.89–8.49 μg/l] | 4.2 [3.3–5.4] | 4 [3–5] | 0.513 |
| Estradiol [ref. <20–41 ng/l] | 10 [10–23.1] | 21.8 [10.0–28.9] | 0.013 |
| Body composition parameters | | | |
| Weight (kg) | 74 [67–85.5] | 78.2 [71.7–86.65] | 0.383 |
| Height (cm) | 173 [168–180] | 173.7 [170.2–179.4] | 0.332 |
| BMI (kg/m$^2$) | 24.8 [23.2–28.4] | 25.5 [23.7–27.8] | 0.880 |
| Subtotal fat mass (g) | 15.0 [12.3–20.2] | 17.8 [15.1–21.7] | 0.031 |
| Percentage subtotal fat (%) | 20.4 [18.7–23.7] | 25.2 [21.8–27.7] | 0.002 |
| HIV characteristics | | | |
| HIV time since diagnosis (year) | 18.2 [11.0–33.2] | | |
| HIV C stage (%) | 18% | | |
| HIV-RNA below 40 copies | 96% | | |
| ART duration (year) | 15.2 [9.1–25.4] | | |
| ART | 100% | | |
| Current NRTI | 82% | | |
| Current integrase inhibitor | 36% | | |
| Current bPI | 29% | | |
| Current non bPI | 4% | | |
| Current NNRTI | 50% | | |
| Current TDF | 64% | | |
| CD4$^+$ cell count nadir (cells/μl) | 192 [128–404] | | |
| CD4$^+$ cell count (cells/μl) | 589 [430–1216] | | |
| Lipodystrophy | 43% | | |
| Coinfection status | | | |
| HBV | 11% | | |
| HCV | 7% | | |

Subtotal fat mass, total fat mass excluding the head. bPI, boosted protease inhibitor; CTX, beta-crosslaps; HBV, hepatitis B virus; HCV, hepatitis C virus; NNRTI, nonnucleoside reverse-transcriptase inhibitor; NRTI, nucleoside reverse transcriptase inhibitor; P1NP, type 1 collagen amino-terminal telopeptide; PTH, parathyroid hormone; TDF, Tenofovir disoproxil fumarate.

ultra-distal radius BMD (−8.4%; $P=0.001$), but had similar femoral neck and radius 1/3 distal BMD. The prevalence of osteoporosis and osteopenia did not differ between the HIV-positive and HIV-negative groups (18 vs. 10% and 61 vs. 57%, respectively; $P=0.317$) (Table 2).

## Volumetric bone mineral density, cortical and trabecular microstructure assessed by high-resolution peripheral quantitative computed tomography

Total volumetric densities at distal radius and tibia were lower [−16% ($P=0.005$) and −14.3% ($P=0.039$), respectively] in HIV-positive men (Fig. 1). These results were associated with alterations of both the trabecular and cortical bone compartments, with lower trabecular density [−11.6% ($P=0.012$) and −12.2% ($P=0.007$), respectively] and lower cortical density [−3.3% ($P=0.011$) and −3.4% ($P=0.056$), respectively]. At the distal radius, trabecular number was lower ($P=0.036$), and trabecular spacing was higher ($P=0.027$) and more heterogeneously distributed ($P=0.041$). At the distal tibia, trabecular thickness was lower ($P=0.011$). The cortical area was lower at both sites [−17.5% ($P=0.002$) and −12.2% ($P=0.01$), respectively] and associated with lower cortical thickness [−19.9% ($P=0.008$) and −7% ($P=0.088$), respectively].

**Table 2. Dual X-ray absorptiometry and high-resolution peripheral quantitative computed tomography measures in HIV-positive men and HIV-negative controls (median ± interquartile range).**

| | HIV-positive ($n = 28$) median [IQR] or % | HIV-negative ($n = 112$) median [IQR] or % | *P* |
|---|---|---|---|
| **DXA scan** | | | |
| Lumbar spine BMD (g/cm$^2$) | 0.933 [0.843–1.053] | 0.995 [0.912–1.132] | 0.051 |
| Lumbar spine T-score | −1.43 [−2.26 to 0.34] | −0.88 [−1.62 to 0.38] | 0.051 |
| Femoral neck BMD (g/cm$^2$) | 0.753 [0.693–0.812] | 0.769 [0.704–0.849] | 0.464 |
| Femoral neck T-score | −1.3 [−1.74 to 0.87] | −1.18 [−1.66 to 0.6] | 0.464 |
| Total hip BMD (g/cm$^2$) | 0.935 [0.851–0.981] | 0.966 [0.89–1.058] | 0.050 |
| Total hip T-score | −0.65 [−1.2 to 0.34] | −0.45 [−0.95 to 0.17] | 0.050 |
| Radius 1/3 distal BMD (g/cm$^2$) | 0.772 [0.737–0.794] | 0.783 [0.727–0.823] | 0.564 |
| Radius 1/3 distal T-score | −0.85 [−1.51 to 0.43] | −0.63 [−1.69 to 0.11] | 0.564 |
| Radius ultra-distal BMD (g/cm$^2$) | 0.45 [0.41–0.488] | 0.491 [0.457–0.552] | 0.001 |
| Radius total BMD (g/cm$^2$) | 0.63 [0.599–0.656] | 0.653 [0.615–0.698] | 0.064 |
| Osteoporotic status | | | |
| Normal | 21% | 33% | 0.317 |
| Osteopenia | 61% | 57% | |
| Osteoporosis | 18% | 10% | |
| **HR-pQCT radius** | | | |
| Total bone parameters | | | |
| Total density (mg HA/cm$^3$) | 277 [252–331] | 329 [284–378] | 0.005 |
| Cross sectional area (mm$^2$) | 363 [317–404] | 357 [316–400] | 0.874 |
| Trabecular parameters | | | |
| Trabecular density (mg HA/cm$^3$) | 152 [141–176] | 172 [150–197] | 0.012 |
| Trabecular bone volume fraction (%) | 0.127 [0.117–0.147] | 0.144 [0.125–0.164] | 0.012 |
| Trabecular area (mm$^2$) | 287 [253–322] | 277 [235–329] | 0.683 |
| Trabecular number (mm$^{-1}$) | 1.96 [1.67–2.16] | 2.02 [1.86–2.24] | 0.036 |
| Trabecular thickness (μm) | 0.069 [0.059–0.075] | 0.071 [0.064–0.08] | 0.184 |
| Trabecular spacing (μm) | 0.446 [0.396–0.529] | 0.419 [0.372–0.475] | 0.027 |
| Distribution of trabecular spacing (μm) | 0.189 [0.161–0.24] | 0.176 [0.151–0.213] | 0.041 |
| Cortical parameters | | | |
| Cortical density (mg HA/cm$^3$) | 846 [780–888] | 875 [843–900] | 0.011 |
| Cortical area (mm$^2$) | 58 [52–72] | 71 [62–81] | 0.002 |
| Cortical thickness (mm) | 0.705 [0.59–0.87] | 0.88 [0.73–1] | 0.008 |
| Cortical perimeter (mm) | 82.3 [76.5–87.9] | 82.4 [77.9–88.5] | 0.923 |
| Cortical porosity (%) | 0.029 [0.021–0.038] | 0.029 [0.022–0.034] | 0.927 |
| **HR-pQCT tibia** | | | |
| Total bone parameters | | | |
| Total density (mg HA/cm$^3$) | 256 [239–317] | 299 [260–340] | 0.039 |
| Cross sectional area (mm$^2$) | 796 [733–935] | 866 [780–973] | 0.238 |
| Trabecular parameters | | | |
| Trabecular density (mg HA/cm$^3$) | 156 [129–194] | 177 [158–204] | 0.007 |
| Trabecular bone volume fraction (%) | 0.13 [0.108–0.162] | 0.148 [0.131–0.17] | 0.007 |
| Trabecular area (mm$^2$) | 662 [583–792] | 720 [617–845] | 0.451 |
| Trabecular number (mm$^{-1}$) | 1.8 [1.56–2.13] | 2 [1.67–2.14] | 0.424 |
| Trabecular thickness (μm) | 0.071 [0.064–0.081] | 0.079 [0.07–0.087] | 0.011 |
| Trabecular spacing (μm) | 0.465 [0.388–0.571] | 0.428 [0.381–0.509] | 0.214 |
| Distribution of trabecular spacing (μm) | 0.218 [0.162–0.287] | 0.191 [0.163–0.247] | 0.280 |
| Cortical parameters | | | |
| Cortical density (mg HA/cm$^3$) | 827 [787–874] | 856 [826–888] | 0.056 |
| Cortical area (mm$^2$) | 126 [113–147] | 143 [120–165] | 0.010 |
| Cortical thickness (mm) | 1.125 [0.985–1.315] | 1.21 [1.02–1.43] | 0.088 |
| Cortical perimeter (mm) | 111.6 [106.4–120.3] | 116.9 [109.8–123.6] | 0.130 |
| Cortical porosity (%) | 0.077 [0.059–0.104] | 0.073 [0.059–0.095] | 0.698 |

BMD, bone mineral density; HR-pQCT, high-resolution peripheral quantitative computed tomography.

No difference was identified in cortical porosity (Table 2; Fig. 2). Bone size assessed by cortical cross-sectional area and cortical perimeter was similar among HIV-positive and HIV-negative men at the distal radius and tibia.

## Determinants of bone mineral density and bone microstructure

Regression analyses of total volumetric density, trabecular density and cortical area were performed to explore the determinants of BMD and bone microstructure alterations in HIV-positive men (Supplemental Table 1, http://links.lww.com/QAD/A573 and Table 3). Nadir $CD4^+$ cell count and HIV-infection duration were not associated with the variability of these bone parameters in HIV-positive men. By univariate analyses adjusted for age and BMI of the 140 study participants, HIV was negatively associated with all bone parameters at the distal radius and tibia (Supplemental Table 1, http://links.lww.com/QAD/A573). Lower physical activity, higher CTX and P1NP levels, and lower estradiol levels

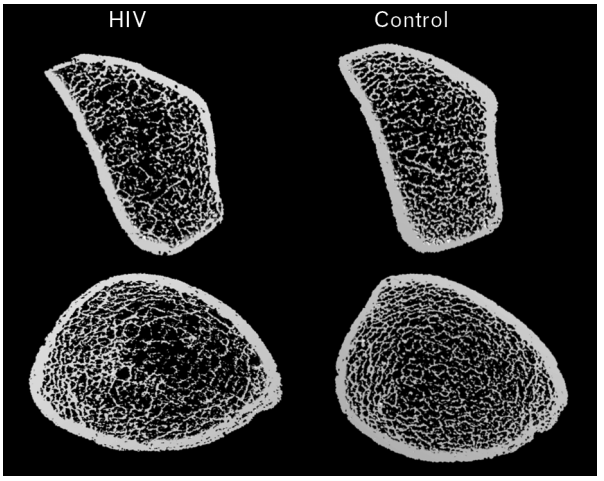


**Fig. 1. High-resolution peripheral quantitative computed tomography images of the radius (top) and tibia (bottom) in representative HIV-positive (left) and HIV-negative (right) men.** Total density at distal radius and tibia are 274 and 279 mg HA/cm$^3$, respectively, for these HIV-positive men, and 336 and 323 mg HA/cm$^3$, respectively, for HIV-negative men (difference 18 and 13%, respectively).

were associated with lower bone microstructural parameters. The negative association with CTX levels was significant for all bone parameters at both distal radius and tibia.

When integrating the classic risk factors of osteoporosis (age, BMI, tobacco use, alcohol consumption, low vitamin D or testosterone levels) in multivariate models, the negative association of HIV status with bone parameters remained significant (M1), thus suggesting that HIV status is an independent clinical determinant of bone microstructure alteration (Table 3). HIV status remained a significant negative determinant of the variability of total density and cortical area at distal radius in a model integrating physical activity (M2). Lower physical activity contributed, but not significantly, to the HIV-associated alterations of distal tibia microstructure and radius trabecular density (M2). Lower estradiol levels contributed to the HIV-associated alteration of distal tibia total density (M3), but there was no association between estradiol and fat mass parameters (data not shown). The only model in which the negative association of HIV status disappeared for all bone parameters was those integrating CTX levels, indicating that HIV-associated bone loss may be mediated by a higher bone resorption associated with HIV status. CTX levels were independent of physical activity ($P = 0.998$) and estradiol levels ($P = 0.232$). When adjusting for P1NP levels, a bone formation marker, HIV status remained significantly associated with bone parameters, except for tibia total density (M4).

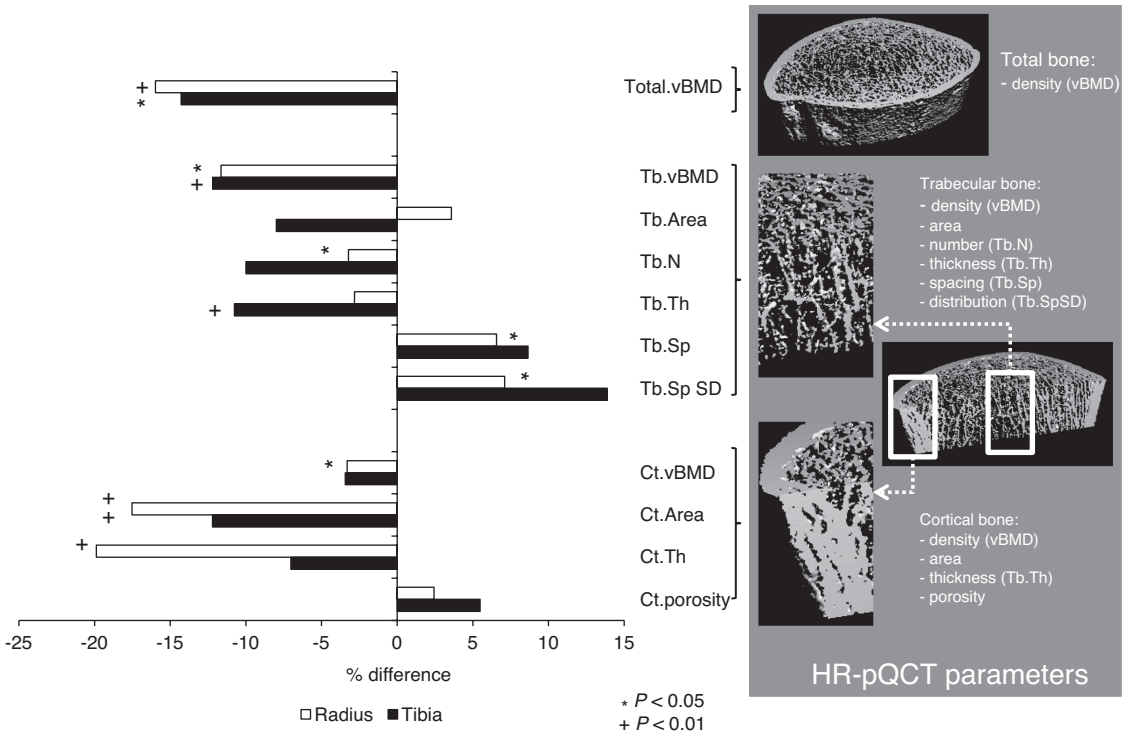


**Fig. 2. Percentage difference in medians in high-resolution peripheral quantitative computed tomography measurements between HIV-positive and HIV-negative men at the radius and tibia.** Ct, cortical; Ct.Th, cortical thickness; Tb, trabecular; Tb.N, trabecular number; Tb.Sp SD, trabecular spacing standard deviation; Tb.Sp, trabecular spacing; vBMD, volumetric bone mineral density. The *P* value of the conditional logistic regression is indicated.

**Table 3. Multivariate models for total density, trabecular density and cortical area at the distal radius and tibia in HIV-positive and HIV-negative men ($n = 140$).**

| | | Total density | | | | | | Trabecular density | | | | | | Cortical area | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Radius | | | Tibia (1/sqrt) | | | Radius | | | Tibia | | | Radius | | | Tibia | | |
| | Models | $R^2$ (p) | $\beta$ | $P$ | $R^2$ (p) | $\beta$ | $P$ | $R^2$ (p) | $\beta$ | $P$ | $R^2$ (p) | $\beta$ | $P$ | $R^2$ (p) | $\beta$ | $P$ | $R^2$ (p) | $\beta$ | $P$ |
| M1 | HIV | 0.037 (0.103) | −36.6 | 0.009 | 0.034 (0.115) | 0.002 | 0.052 | 0.058 (0.038) | −20.23 | 0.019 | 0.082 (0.010) | −24.03 | 0.003 | 0.065 (0.026) | −10.64 | 0.002 | 0.062 (0.030) | −17.81 | 0.009 |
| | Age | | 0.819 | 0.768 | | −0.000 | 0.951 | | 1.846 | 0.286 | | 0.601 | 0.710 | | −0.580 | 0.403 | | −0.724 | 0.592 |
| | BMI (inverse) | | −1596 | 0.176 | | 0.224 | 0.038 | | −1325 | 0.071 | | −1671 | 0.018 | | −646 | 0.029 | | −1606 | 0.007 |
| | Tobacco use | | 6.698 | 0.711 | | −0.001 | 0.586 | | 12.11 | 0.281 | | 13.21 | 0.198 | | −1.139 | 0.800 | | 6.159 | 0.471 |
| | Alcohol consumption | | −12.40 | 0.277 | | 0.001 | 0.159 | | −8.266 | 0.243 | | −11.60 | 0.083 | | −2.014 | 0.478 | | −2.558 | 0.645 |
| | 25 OH vit. D3 (sqrt) | | −1.917 | 0.614 | | 0.000 | 0.687 | | −1.740 | 0.460 | | 1.123 | 0.607 | | 0.219 | 0.817 | | −0.179 | 0.922 |
| | Total testosterone | | 1.490 | 0.691 | | −0.000 | 0.782 | | 3.207 | 0.170 | | −0.379 | 0.864 | | −0.249 | 0.790 | | 1.998 | 0.279 |
| M2 | HIV | 0.045 (0.037) | −38.26 | 0.015 | 0.041 (0.047) | 0.002 | 0.161 | 0.048 (0.031) | −15.33 | 0.116 | 0.094 (0.002) | −13.10 | 0.140 | 0.082 (0.004) | −11.03 | 0.005 | 0.076 (0.006) | −13.46 | 0.070 |
| | Age | | 0.297 | 0.914 | | −0.000 | 0.973 | | 1.39 | 0.419 | | 0.948 | 0.550 | | −0.581 | 0.393 | | −0.873 | 0.510 |
| | BMI (inverse) | | −1498 | 0.174 | | 0.206 | 0.041 | | −960 | 0.165 | | −1459 | 0.026 | | −660 | 0.017 | | −1331 | 0.015 |
| | Physical activity (sqrt) | | −0.053 | 0.934 | | −0.000 | 0.452 | | 0.401 | 0.319 | | 0.673 | 0.078 | | −0.041 | 0.798 | | 0.309 | 0.330 |
| M3 | HIV | 0.056 (0.020) | −34.37 | 0.010 | 0.050 (0.028) | 0.002 | 0.073 | 0.063 (0.013) | −17.66 | 0.035 | 0.076 (0.006) | −20.35 | 0.010 | 0.088 (0.003) | −9.822 | 0.003 | 0.083 (0.003) | −15.29 | 0.018 |
| | Age | | 0.359 | 0.894 | | 0.000 | 0.961 | | 1.156 | 0.493 | | 0.614 | 0.700 | | −0.549 | 0.413 | | −1.016 | 0.437 |
| | BMI (inverse) | | −1553 | 0.154 | | 0.220 | 0.028 | | −1104 | 0.106 | | −1600 | 0.015 | | −665 | 0.015 | | −1425 | 0.009 |
| | Estradiol | | 0.518 | 0.228 | | −0.000 | 0.184 | | 0.468 | 0.083 | | 0.167 | 0.516 | | 0.110 | 0.302 | | 0.306 | 0.149 |
| M4 | HIV | 0.141 (<0.001) | −23.09 | 0.076 | 0.199 (<0.001) | 0.001 | 0.312 | 0.086 (0.003) | −14.37 | 0.088 | 0.167 (<0.001) | −14.51 | 0.052 | 0.181 (<0.001) | −6.763 | 0.036 | 0.181 (<0.001) | −11.03 | 0.070 |
| | Age | | 0.262 | 0.919 | | 0.000 | 0.974 | | 1.101 | 0.509 | | 0.642 | 0.671 | | −0.573 | 0.368 | | −0.998 | 0.420 |
| | BMI (inverse) | | −540 | 0.612 | | 0.108 | 0.250 | | −640 | 0.353 | | −1040 | 0.103 | | −406 | 0.123 | | −900 | 0.084 |
| | CTX (log) | | −36.62 | <0.001 | | 0.004 | <0.001 | | −15.63 | 0.012 | | −21.37 | <0.001 | | −9.456 | <0.001 | | −19.29 | <0.001 |
| M5 | HIV | 0.066 (0.011) | −33.48 | 0.012 | 0.074 (0.006) | 0.002 | 0.069 | 0.056 (0.020) | −18.40 | 0.028 | 0.088 (0.003) | −19.87 | 0.010 | 0.12 (<0.001) | −9.089 | 0.005 | 0.105 (<0.001) | −15.28 | 0.015 |
| | Age | | −0.046 | 0.986 | | 0.000 | 0.792 | | 0.933 | 0.583 | | 0.398 | 0.802 | | −0.685 | 0.300 | | −1.296 | 0.319 |
| | BMI (inverse) | | −1440 | 0.184 | | 0.212 | 0.032 | | −1020 | 0.136 | | −1569 | 0.016 | | −634 | 0.018 | | −1375 | 0.01 |
| | P1NP (1/sqrt) | | 250 | 0.089 | | −0.030 | 0.021 | | 131 | 0.156 | | 129 | 0.075 | | 87 | 0.017 | | 165 | 0.021 |

M1: including HIV status and clinical risk factors classically associated with BMD alteration; M2–5: including HIV status and variables with a $P$ value lower than 0.2 in the univariate models, adjusted for age and BMI, respectively: physical activity (M2), estradiol (M3), CTX (M4) and P1NP (M5). log, logarithm; $R^2$ (p), adjusted $R^2$ and $P$ value of the model; sqrt, square root.

### Subgroup analyses by fracture history

The prevalence of all clinical prevalent fractures was similar in HIV-positive and HIV-negative men (57 vs. 56%, respectively), as well as fragility fractures (14 vs. 10%, respectively; $P = 0.495$) and prevalent vertebral fractures of grades 2 and 3 (18 vs. 12%, respectively; $P = 0.377$) (Table 1). Lateral scan of the spine (morphometric vertebral deformations) identified two additional prevalent nonclinical vertebral fractures in HIV-positive men and eight in HIV-negative men (7% of the population for both). To examine whether aBMD and vBMD were associated with fracture history, we grouped together all 140 study participants. Compared with men without a history of fracture, those with a history of clinical or nonclinical vertebral fractures ($n = 89$) had lower aBMD only at the total hip ($P = 0.007$), and lower total vBMD at the distal radius and tibia (both $P = 0.008$). When considering only the HIV-positive group, differences in aBMD and vBMD between men with and without a history of clinical or vertebral fractures were no longer significant due to the low number of patients (18 with fracture and 10 without fracture) (data not shown).

### Effect of specific antiretroviral therapy use

Assessment of the relationship between specific ART and radius total density, including the level of bone resorption assessed by CTX, did not show any significant effect of current use or duration of ART, in particular tenofovir or bPI (data not shown).

## Discussion

Using HR-pQCT, we found that HIV-infected elderly men on successful ART have alterations of trabecular and cortical bone microstructure associated with higher bone resorption markers, despite adequate vitamin D supplementation. To our knowledge, this cross-sectional case–control study is the first to assess bone microstructure alterations in this emerging population. We carefully matched HIV-positive elderly men for age (±4 years) and BMI (±4 kg/m$^2$) to limit the effect of potential confounding factors on bone parameters. Fracture prevalence was in accordance with that expected in elderly men [13]. During screening, we excluded patients on bisphosphonate therapy who were more likely to have osteoporosis. Consequently, the prevalence of osteoporotic patients in our HIV-positive group was relatively low (18 vs. 10%, respectively). Moreover, the prevalence of vitamin D supplements was high, reflecting the application of guidelines recommending to check vitamin D status and to provide supplements to patients with vitamin D deficiency or insufficiency as standard care. Low sun exposure and treatment with efavirenz and protease inhibitors, two relatively highly prevalent conditions in the Swiss HIV cohort, may have contributed to vitamin D levels requiring supplements. Coinfection with hepatitis B and C was low. Two recent reviews have shown that the prevalence of low BMD and the risk of fracture are higher in hepatitis C-HIV coinfected patients than in HIV mono-infected controls [14,15], reflecting probably additional risk factors of osteoporosis associated with hepatitis C infection. Despite the exclusion of men treated for osteoporosis, optimization of vitamin D levels and low rate of coinfection with hepatitis B and C, we detected lower total vBMD at distal radius and tibia (16 and 14.3%, respectively) in HIV-positive men. Both trabecular and cortical bone were altered. The trabecular network was disorganized with a lower trabecular number or thickness and higher heterogeneity in trabecular spacing; cortical area was decreased, especially because of thinner cortex. These alterations might be worse in nonselected-HIV men, not screened for osteoporosis, with higher rates of vitamin D deficiency and coinfections. Similar data were obtained in younger men with HIV infection [16], indicating that HIV exerts deleterious effects on adult bone, in addition to growing individuals. We previously reported that premenopausal HIV-positive women have trabecular and cortical bone alterations [12]. Such bone microstructure alterations were however not found in postmenopausal HIV-positive women in another study, despite lower aBMD at the spine, total hip and ultradistal radius assessed by DXA [17].

These microstructure alterations were only partially captured by aBMD assessed by DXA. One explanation is that the sensitivity of DXA is not sufficient to detect alterations of bone microstructure captured by HR-pQCT. A second one is that alterations of bone microstructure, especially at the cortical compartment, are not totally detected by DXA aBMD. Microstructure alteration is an aBMD-independent determinant of fracture risk that contributes to bone mechanical properties, as it has been demonstrated in women [18] and men [19]. HR-pQCT can discriminate osteopenic women with and without prevalent fragility fracture, suggesting that it captures additional bone fragility factors not captured by DXA [20]. Previous studies indicate that biomechanical properties assessed by finite element analysis based on HR-pQCT images remain significantly associated with fractures after adjustment for aBMD [21,22].

In our population, the negative association of HIV status with distal radius and tibia total density lost significance after adjustment for aBMD at total hip and ultra-distal radius. This suggests that HIV-related deterioration in trabecular and cortical bone microstructure is captured by aBMD, but not sufficiently to be detected at the individual level. Moreover, the contribution of these bone microstructure alterations to bone fragility and fracture risk remains to be investigated in prospective studies in HIV-positive patients, in particular in this emerging elderly population. We identified that increased bone resorption, as assessed by higher CTX levels in HIV-positive men, was the main determinant of the negative association of HIV status on aBMD and bone microstructure (M1 and M3).

Interestingly, higher bone resorption was previously highlighted in HIV-positive women [17], as well as the increase in bone turnover markers after initiation of ART [23]. The SMART study ('Strategies for Management of Anti-Retroviral Therapy') reported that continuous ART increases bone turnover as assessed with bone turnover markers [24]. All our HIV-positive men were on long-term treatment with ART and it is likely that the long-term effect of HIV status on bone microstructure may be associated with the increase of bone resorption induced by ART.

The mechanism by which ART affects bone turnover remains unclear. It may not be mediated by the RANKL/OPG pathway [24] and appears to be independent of any specific ART class. Tenofovir has been shown to interfere with bone metabolism, and higher bone turnover markers were described in HIV-positive patients treated with this drug [25,26]. However, we did not find that high bone turnover was associated with the use of a specific ART class, in particular tenofovir. By including 64% of HIV-positive treated by tenofovir, our study was not designed to answer this issue. In addition, previous data showed that proximal tubular renal dysfunction in HIV-infected patients was not associated with higher levels of bone turnover markers [26]. Bone changes have also been described with other drug classes, such as bPI [27] or zidovudine [28], but with inconsistent results in studies with bone parameters as secondary outcomes [29]. Larger studies are necessary to better explore this issue.

Lower physical activity also contributed to the negative effect of HIV status on trabecular microstructure, in particular at the distal tibia, more exposed to physical loading than the radius. Mechanical loading is a well known factor that contributes to bone health [30,31]. However, CTX levels were not associated with physical activity in our population, suggesting that higher bone resorption and lower physical activity are two independent factors associated with the negative effect of HIV status on bone.

The negative association of HIV status with distal tibia total density disappeared after adjustment for estradiol levels, suggesting that lower levels in HIV-positive men may contribute to the effect of HIV status on bone. It was also independent of testosterone levels. Large epidemiological studies in community-dwelling men have indicated that bioavailable and total testosterone are not associated with bone mass, density and remodelling. In contrast, low estradiol levels are associated with cortical and trabecular BMD in men, and are an independent predictor of bone remodelling. In addition, low estradiol levels are associated with accelerated hip aBMD decrease, and with a 50% increase in incident fractures in the cohort MrOs, a prospective study of community-dwelling men older than 65 years [32–35]. The role of lower estradiol levels in HIV-positive men remains to be investigated in larger cohorts of patients with other steroid markers, such as bio-available 17-beta-estradiol or sex hormone binding globulin. Fat mass is a source of estradiol and was lower in HIV-infected men than in controls. Lipo-atrophy may explain part of the lower estradiol levels observed in HIV-infected patients. However, estradiol levels were not associated with fat mass parameters in univariate regression models, suggesting that additional factors contribute to lower estradiol levels in HIV-infected men.

Our study has limitations, in particular the small sample size. The design allowed us to investigate an 'HIV effect' on bone microstructure, but not whether this effect was linked to the virus itself, the host, ART or all HIV-associated factors. By matching HIV-positive men and controls by age and BMI, we aimed to detect an 'HIV effect' on bone less dependent of fat mass. HIV infection is known to affect metabolism and fat mass distribution that might be confounding factors influencing bone metabolism in addition to the 'HIV effect'. Long-term exposure to ART of this population of elderly men probably contributes to these alterations in addition to age. We did not find any association between the duration of ART or tenofovir use, and bone microstructure. However, the study was not designed to address this issue and additional studies would be necessary to draw specific conclusion regarding the respective role of HIV and ART in bone microstructure alterations.

It is likely that the effect of HIV together with its metabolic-related complications, as well as lifestyle habits and comorbidities, affect bone to a higher extent than in this highly selected HIV-positive population.

In conclusion, long-term infected HIV-positive men, at the age when fracture risk markedly increases in the general population, have microstructural bone alterations at the trabecular and cortical compartments, only partially captured by aBMD assessed by DXA, and associated with higher bone resorption. These alterations were observed despite adequate HIV replication control by efficient ART and despite vitamin D supplementation. These alterations may contribute to higher bone fragility in the emerging population of long-term HIV-infected men older than 60. Apart from the recommendation of BMD testing and vitamin D in HIV population, a specific emphasis should be placed on fracture risk prevention in HIV-infected men after the age of 60, even though their BMD T-score does not reach the −2.5DS threshold for osteoporosis diagnosis. Further longitudinal research is needed in this population to determine the lifelong kinetics of bone alterations, the contribution of various ART regimens and the efficacy of preventive measures to limit fracture risk and consequences on quality of life in later years.

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E.B., A.C. and R.R. conceived the study and designed the protocol. E.B., A.C., C.Du. and R.R. conducted the study. E.B. and C.De. collected the data. E.B. and C.De. analysed the data. E.B., A.C. and R.R. interpreted the results. E.B. takes responsibility for the integrity of the data analysis. E.B., A.C. and R.R. drafted the article and revised the manuscript content. All authors edited the article and approved the final article.

## Conflicts of interest

The authors report no conflicts of interest pertaining to this manuscript.

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