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Predictors of longitudinal change in bone mineral density in a cohort of HIV-positive and negative subjects

Authors: Willard TINAGO[1, 2], Aoife G COTTER[1, 3], Caroline SABIN[4], Alan MACKEN[1], Eoin KAVANAGH[5], Jennifer BRADY[6], Geraldine McCARTHY[7], Juliet COMPSTON[8], Patrick WG MALLON[1,3], on behalf of the HIV UPBEAT Study Group

[1]HIV Molecular Research Group, School of Medicine, University College Dublin, Dublin, Ireland

[2] Department of Community Medicine, College of Health Sciences, University of Zimbabwe, Harare, Zimbabwe

[3] Department of Infectious Diseases, Mater Misericordiae University Hospital, Dublin, Ireland

[4]Research Department of Infection and Population Health, University College London, London, United Kingdom

[5]Department of Radiology, Mater Misericordiae University Hospital, Dublin, Ireland,

[6]Department of Clinical Chemistry and Diagnostic Endocrinology, Mater Misericordiae University Hospital, Dublin, Ireland

[7]Department of Rheumatology, Mater Misericordiae University Hospital, Dublin, Ireland,

[8]Department of Medicine, Cambridge Biomedical Campus, United Kingdom

Correspondence and requests for reprints:

Willard Tinago, HIV Molecular Research Group, Catherine McAuley Education and Research Centre, 21 Nelson Street, Dublin 7, Ireland.

Tel: + 353 1 7166548, Fax: + 353 1 7166335,

Email: wtinago@gmail.com

Abstract

Objective: Although low bone mineral density (BMD) is prevalent in HIV, changes in BMD over time remain unclear. We aimed to compare rates of, and factors associated with, BMD change between HIV-positive and HIV-negative subjects.

Methods: In a prospective, 3-year cohort, HIV-positive and HIV-negative subjects provided annual demographic and clinical data, fasting bloods and dual x-ray absorptiometry (DXA). Using longitudinal mixed models we compared and determined predictors of rate of change in BMD.

Results: Of 384 subjects (45.8% HIV-positive), 120 contributed two and 264 contributed three BMD measurements. Those with HIV were younger (median (IQR) 39 (34-46) vs 43 (35-50) years; p=0.04), more often male (61% vs 46%; p=0.003) and less likely Caucasian (61% vs 82%; p<0.001). Although BMD was lower in those with HIV, BMD declined in both groups, with non-significant between-group difference in rate of BMD change over time.

Within the HIV group, starting ART within 3 months of enrolment was associated with greater BMD decline at all anatomical sites (all p<0.001). Age >30 years, Caucasian ethnicity, and not being on ART during follow-up were associated with greater decline and higher parathyroid hormone associated with a smaller decline in BMD at the femoral neck. We found no association between BMD change and exposure to tenofovir disoproxil fumarate or protease inhibitors.

Conclusions: We observed no difference in rate of BMD decline regardless of HIV status and in HIV positive subject, having started ART within the previous three months was the only factor associated with greater BMD decline at all 3 sites.

Keywords: bone mineral density, HIV, longitudinal change, antiretroviral therapy

Introduction

Treatment of HIV-infection with antiretroviral therapy (ART) has significantly reduced morbidity and mortality [1, 2]. However several disorders associated with chronic HIV-infection and ART exposure, such as osteoporosis are increasingly emerging with long-term survival of treated patients [3].

Low bone mineral density (BMD) and osteoporosis are commonly reported across numerous cohorts of both men and women with HIV-infection [4-6], with a greater decline in BMD with antiretroviral therapy initiation. In several studies, including a number of randomised clinical trials, initiation of ART, regardless of the selected regimen, has been found to reduce BMD by 2%-6% over the first 2 years of therapy [7-10], with subsequent stabilization [11-13]. Several factors likely contribute to reduced BMD in HIV-infected patients, including the pro-inflammatory effects of chronic HIV-infection, effects of ageing on BMD, genetic predisposition, endocrine dysfunction such as menopause, low calcium and vitamin D intake, smoking and low body mass index (BMI) [14, 15]. Exposure to the nucleotide reverse transcriptase inhibitor tenofovir disoproxil fumarate (TDF), some protease inhibitors (PIs) [7, 9, 16-18] and pre-ART immunodeficiency [19] have also been implicated in greater BMD loss upon ART initiation.

Bone undergoes continual remodelling mediated by tightly integrated contrasting activity of bone resorbing osteoclasts and bone forming osteoblasts. The functional balance and cross-talk between osteoblasts and osteoclasts is pivotal in regulating bone mass [20]. Circulating levels of bone turnover markers (BTM) reflect these underlying processes and can be used to indicate changes in either bone resorption or formation. Markers of bone resorption include C-terminal cross-linking telopeptide of type 1 collagen (CTX-1) and N-terminal cross-linking telopeptide of type 1 collagen (NTX-1) and markers for bone formation include bone-specific

alkaline phosphatase (Bone ALP), procollagen type 1 N-propeptide (P1NP) and osteocalcin (OC) [21]. When bone resorption exceeds bone formation, a net reduction of bone mass results. With initiation of ART in HIV-infection, this balance is impaired, resulting in preferential bone resorption and subsequent loss of bone mass [20].

In cross-sectional analyses, HIV infection and high bone turnover as measured by BTM have both been associated with low BMD [1, 22-25], a relationship which was further defined in a baseline cross-sectional analysis of the *Understanding the Pathology of Bone Disease in HIV infected Subjects* (HIV UPBEAT) cohort, in which both HIV positive and negative groups were compared [22]. Whether such an association is maintained in longitudinal studies remains unclear. This study aimed to compare rates of, and factors associated with, change in BMD over time between HIV-positive and HIV-negative subjects and to determine HIV-related predictors of change in BMD.

Methods

Population and study design

The HIV UPBEAT study is a 3 year, single site, prospective observational cohort study that enrolled HIV-positive and HIV-negative subjects from similar demographic backgrounds. HIV-positive subjects were recruited from the Infectious Disease clinic at the Mater Misericordiae University Hospital, Dublin, Ireland, whereas the HIV-negative subjects were recruited from the local Dublin area using a range of recruitment strategies. Of note, these subjects were recruited from the local community rather than from other hospital-based chronic disease clinics.

Between February 2011 and July 2012, subjects enrolled into the study had their baseline visit and dual x-ray absorptiometry (DXA) scans at femoral neck (FN), total hip (TH) and lumbar spine (LS) performed and annually thereafter. All subjects were scanned on a Lunar

Prodigy-energy X-ray absorptiometry scanner (GE Medical Systems, Madison, Wisconsin, USA) utilising Encore software version 12.10 for acquisition and analysis. Dual femur (femoral neck, total hip) and lumbar spine scans were obtained using a standardized protocol. Short term in vivo variability was 1.12% at femoral neck of hip.

Demographic and treatment history information were collected at study entry. Plasma and serum samples were collected at study entry and annually until end of study (following an 8-hour overnight fast, water permitted). Fasting blood tests including full blood count, urea and electrolytes, liver function tests (bilirubin, alkaline phosphatase (ALP), alanine aminotransferase (ALT), gamma-glutamyl transferase (GGT), albumin), bone profile (corrected calcium, phosphate), lipid profile, glucose, thyroid function tests, were analysed by routine methodologies. HIV and hepatitis B and C serology were assessed in the HIV-negative group. Intact parathyroid hormone (PTH) was measured using sandwich immunoassay with chemi-luminescent detection (Siemans, Centaur, Erlangen, Germany); Serum and plasma samples were stored at -80 degrees Celsius. Serum 25(OH)D was measured by radio-immunoassay (Diasorin, Saluggia, Italy). After baseline visit completion, stored plasma samples were utilised for bone biomarker analyses. CTX-1, OC (N-mid OC[1-43]) and P1NP were measured using electrochemi-luminescence immunoassays (Roche Diagnostics, Basel, Switzerland). Coefficients of variation for all assays were within acceptable limits. CD4+ T-cell count and HIV-1 RNA levels were measured as part of routine clinical care concurrently with other measures.

BMD at femoral neck, total hip and lumbar spine were measured in grams per centimetre squared (g/cm^2). A detailed description of the HIV UPBEAT study, BMD assessments and laboratory methods have been described previously [22]. The present analysis was restricted only to subjects with at least two longitudinal BMD measurements. All participants gave written, informed consent and approval was granted by the local ethics committee.

Statistical Analysis

Between-group differences in demographic and clinical variables at baseline were assessed using the Mann-Whitney U test for continuous variables and the Chi-squared test for categorical variables. The study primary-endpoint was the difference in rate of change in BMD between the HIV-positive and HIV-negative groups at FN, TH and LS over 3 years. To address this primary-endpoint, we compared BMD values (log transformed to stabilize the variance and linearize changes over time) at baseline and over time between the HIV-positive and HIV-negative group's at all three anatomical sites using mixed effects regression models. The mixed models included patient-specific random intercepts and random slopes over time to allow for heterogeneity in patients' initial BMD levels and subsequent changes. The initial model included time (calculated from the date of the first assessment and measured in years), HIV status and the interaction between time and HIV status as independent variables. This allowed us to investigate the overall rate of change of BMD in the full sample, and to formally assess whether this change differed significantly between the HIV-positive and HIV-negative groups.

We next investigated whether changes in BMD over follow-up were associated with traditional risk factors for low BMD, including age (≤30; >30 years), sex (male, female), ethnicity (Caucasian, non-Caucasian), smoking status (current smoker, noncurrent smoker) and BMI, through the inclusion of additional interaction terms between each covariate and time. Factors that were not significantly associated with the absolute BMD or its change over time ($p>0.05$) were then removed from this model.

We next performed a subgroup analysis on the HIV-positive group only, to identify HIV-related predictors of absolute change in BMD at FN, TH and LS. Due to the very large number of potential covariates, we firstly performed an exploratory analysis using baseline BMD values and a naïve linear regression (with the estimated BMD slope as the outcome

variable) to select the appropriate form for each covariate. In the exploratory analysis, variables that exhibited inconsistent directions of associations with BMD across the three anatomical sites were not considered for further analysis (assuming a lack of biological plausibility and likelihood of type I errors). In addition to the demographic variables and BMI, these exploratory analyses also considered the following variables: laboratory variables (alkaline phosphatase, phosphate, parathyroid hormone (PTH), 25-hydroxy-vitamin D (25(OH)D, bone turnover marker variables (OC, CTX-1 and P1NP) and HIV-specific variables (HIV acquisition risk (injection drug use (IDU); non-IDU), current TDF use, current PI use, current non-nucleoside reverse transcriptase inhibitor (NNRTI) use, ART status (on continuous ART during follow-up and having started ART >3 months prior to study enrolment; having started ART at sometime between <3 months prior to or after study enrolment; not on ART). Mixed effects models were then used to select variables to be included in the multivariable analyses based on their potential effect on baseline BMD levels or absolute decline in BMD.

Multivariable mixed effects models were then constructed to include relevant variables from the exploratory analysis, with covariates considered for inclusion in the following order: demographics, BMI, laboratory variables, bone turnover markers and HIV-specific variables. On adding each set of variables into the model, a backward selection approach was used to remove any variables that did not remain independently associated with baseline BMD level and/or the absolute change in BMD. Once a parsimonious model had been achieved, we then proceeded to add the next set of variables. Using a similar approach, we examined the additional effects of current and nadir CD4+ T-cell counts and baseline HIV RNA (≤40 copies/ml vs >40 copies/ml) on absolute change in BMD in a sub-group of subjects either on continuous ART or having started ART >3 or <3 months prior to or after study enrolment.

For interpretation, all model estimates were back transformed. All analyses were conducted using STATA 13.1 (College Station, Texas).

Results

474 enrolled subjects (of whom 210 were HIV-positive) completed the baseline study visit [22]. The longitudinal analyses included 384 subjects (176 HIV-positive) with at least two BMD measurements. There were no significant differences in demographic characteristics, laboratory variables and bone-turnover markers between the 474 enrolled subjects and the 384 subjects with at least two follow-up BMD measurements (data not shown).

Of the 384 subjects, 120 (57 HIV-positive) contributed two and 264 (119 HIV-positive) contributed three BMD annual measurements. Subjects were followed up for median (interquartile range (IQR)) duration of 103 (88-177) weeks.

Baseline characteristics of the HIV-positive and HIV-negative groups included in the analysis are presented in Table 1. Compared to the HIV-negative group, those with HIV were younger and more likely to be male, of African ethnicity, to currently smoke and to be hepatitis C antibody positive. There were no significant differences in median BMI at baseline between the HIV-positive and HIV-negative groups.

Laboratory variables and bone turnover markers

Baseline alkaline phosphatase, corrected calcium, parathyroid hormone and bone biomarkers; OC, P1NP, CTX-1 were significantly higher in the HIV-positive group than in the HIV negative group. (Table 1) as previously reported [22]. There were no significant differences in 25(OH) D between the HIV-positive and HIV-negative groups.

Baseline differences and differences in absolute change in bone mineral density

Compared to the HIV-negative group, the HIV-positive group had significantly lower baseline BMD at the FN (median (IQR) 1.024 (0.927-1.135) g/cm^2 vs 1.055 (0.964-1.159) g/cm^2; p=0.003), TH (1.061 (0.942-1.157) g/cm^2 vs 1.107 (1.000-1.196) g/cm^2; p=0.003) and LS (1.164 (1.061-1.304) g/cm^2 vs 1.238 (1.135-1.348) g/cm^2; p=0.001).

In general BMD declined at all three anatomical sites within the overall cohort and in both sub-groups. From the mixed effects regression models, the decline in BMD in the overall cohort was significant at all three anatomical sites (-0.0046 g/cm^2/yr at FN,-0.0041 g/cm^2/yr at TH and -0.0033 g/cm^2/yr at LS, all p<0.001). In the HIV-positive group, BMD significantly declined at FN (-0.0063 g/cm^2/yr; p=0.001), TH (-0.0044 g/cm^2/yr; p<0.001) but not at LS (-0.0024 g/cm^2/yr; p=0.25). Similarly, in the HIV-negative group, BMD significantly declined at FN (-0.0028 g/cm^2/yr; p=0.023) and TH (-0.0036 g/cm^2/yr; p<0.001) but not at LS (-0.0041 g/cm^2/yr; p=0.08). Despite the within-group changes in BMD, there were no significant between-group differences in the rate of absolute change in BMD at FN, TH or LS (Figure 1). Further adjustment for age, gender, ethnicity, smoking status and BMI in a multivariable mixed effects regression analysis minimally affected the estimated rates of BMD decline (Table 2).

HIV-specific analysis

Predictors of absolute change in BMD in HIV-infected subjects

In the unadjusted mixed effects regression models (Table 3), having started ART<3 months prior to or after study enrolment was significantly associated with greater subsequent BMD decline at FN, TH and LS, while not being on ART during study follow-up was also significantly associated with greater subsequent BMD decline but only at FN. Interestingly, current PI use was significantly associated with a lesser decline in BMD but only at FN.

There was no association between change in BMD and current or cumulative exposure to tenofovir disoproxil fumarate (TDF).

Analysis of circulating factors associated with bone function revealed inconsistent results. Higher baseline corrected calcium was significantly associated with reduced subsequent BMD decline but only at LS, while, surprisingly, a higher baseline PTH was significantly associated with reduced subsequent decline in BMD at FN and TH but not LS. Higher baseline ALP was significantly associated with reduced subsequent BMD decline at FN and LS but not TH, while a higher baseline P1NP was significantly associated with reduced subsequent decline in BMD at FN, TH and LS, and higher baseline OC and CTX-1 were significantly associated with a reduced decline in BMD but only at FN.

In multivariable mixed effects models within the HIV-positive group (Table 4), age over 30 years and Caucasian ethnicity were independently associated with a greater subsequent decline in BMD at FN of -0.013 g/cm^2/year (p=0.02) and -0.011 g/cm^2/year (p=0.006), respectively. Compared to being on continuous ART or having started ART >3 months prior to study enrolment, having started ART<3 months prior to or after study enrolment and not being on ART during study follow-up were independently associated with a greater subsequent BMD decline at FN of -0.016 g/cm^2/year (p=0.002) and of -0.019 g/cm^2/year (p=0.012), respectively, while the effect of recent ART initiation was also seen at TH and LS of -0.014 g/cm^2/year and -0.025 g/cm^2/year, respectively (all p<0.001). In addition, surprisingly, a one pmol/L increase in baseline PTH was independently associated with a reduced subsequent BMD decline at FN of 0.0017 g/cm^2/year (p=0.007). In an analysis restricted to subjects on stable ART (>12 months), to exclude the possibility that the effects of PTH on BMD decline might be affected by measurement of individuals who started ART after or just prior to baseline, PTH remained independently associated with a smaller decline in BMD at femoral neck (data not shown).

In a further sub-group analysis of subjects either on continuous ART or having started ART >3 months prior to or after study enrolment and those having started ART <3 months prior to or after study enrolment, to assess the independent effects of baseline CD4+ T-cell count and HIV RNA<40 copies/ml on absolute change in BMD, higher baseline CD4+ T-cell count remained independently associated with higher baseline BMD at FN (+1.00 g/cm^2, p=0.006) and TH (+1.00 g/cm^2, p=0.005) but not with absolute change in BMD.

Discussion

In this 3 year, prospective study of subjects with and without HIV, although BMD declined in the whole cohort at all 3 anatomical sites, there was no evidence of between-group differences in the rate of BMD decline over time between the HIV-positive and HIV-negative groups in analyses adjusted for traditional risk factors for low BMD including age, gender, ethnicity, smoking and BMI. In an HIV-specific sub-analysis, having started ART within the previous three months was the only factor associated with greater BMD decline at all 3 sites, with no evidence to support an effect of ART itself, or specific components such as TDF or PI, in accelerating bone loss.

That the rate of BMD decline was similar in both those with and without HIV infection is consistent with similar observations reported in other longitudinal studies with a comparative HIV-negative group, with some specific study population composition differences. Dolan et al [5] and Sharma et al [4] reported that rates of change in BMD were not different between HIV-infected women and healthy controls at the LS, FN, or TH. Yin et al. found no difference in the rate of bone loss at LS and FN between premenopausal HIV-infected and uninfected women [26]. Bolland et al [12] and Sharma et al [3] found no evidence of between-group differences in the rate of BMD loss at LS, TH or FN between highly active

antiretroviral therapy (HAART) treated HIV-infected men and HIV-negative controls. In these BMD longitudinal studies among men or women with a comparative HIV-negative group, the duration of follow-up ranged from 1.5 [3, 4] to 6 years [27], with sample size ranging between 81 [27] and 464 [4]. This present study of medium duration of follow-up and relatively large sample size further reaffirms this observation of lack of a between-group difference in the rate of BMD decline, with the advantage of including a more heterogeneous, European-based population with a control group derived from a similar demographic as the HIV positive cohort.

The effect of ART on BMD in people living with HIV has been well defined in prospective, randomized clinical trials with much of the change in BMD with ART observed within the first year after ART initiation [9, 10, 28]. Our finding that subjects recently started on ART had greater decline in BMD is consistent with these data. Furthermore, no evidence for greater BMD decline once on established ART for >3 months was observed. This is similar to observations from a US cohort in younger men [11] and cohorts of men or premenopausal women in which the majority of subjects treated with effective ART at baseline had stable or increasing BMD during follow-up [6].

Similar to other analyses, such as that from the SMART study, an international randomized trial comparing continuous with intermittent antiretroviral treatment [29], we did not find evidence for greater BMD decline in those with exposure to specific antiretrovirals such as TDF or PI. This is also in agreement with Nolan et al [13], who did not find evidence of greater BMD decline. While the association between initiation of ART containing TDF and larger reductions in BMD has been demonstrated in several randomized trials [30-32], the role of PIs has been contradictory, ranging from an increase [11, 13] to a decrease [17, 33] in BMD in ART-experienced or ART-naïve subjects receiving ART regimens that included PI. The lack of association in our study could potentially be explained by the fact that the effects

of individual drugs may have been too weak to be identified in the presence of other factors more strongly influencing BMD change, or more likely that once ART is initiated for > 3 months, there is no additional, clinically relevant impact from specific antiretroviral drugs on progressive BMD loss.

The observation of greater BMD decline in those aged above 30 years and in those of Caucasian ethnicity is not surprising, as these are well-established risk factors for low BMD in many studies. However, this analysis also observed associations between higher PTH levels and a slower rate of BMD decline that runs contrary to a previous randomised trial comparing an NNRTI versus NRTI-containing ART regimen, both used in combination with PI, in ART-naïve men, which demonstrated a correlation between increasing PTH levels and a greater reduction in BMD at LS [28]. However in that study, in the NNRTI study group that experienced the greatest PTH increase, BMD loss was less compared to the NRTI group, suggesting that PTH may not explain this greater BMD loss. Although increased PTH levels have been associated with low BMD in HIV, our study suggests little evidence for a significant role.

Within the baseline analysis of this cohort, significantly higher bone turnover markers were observed in those with HIV and were associated with lower BMD [22]. Higher bone turnover in HIV is well described, with temporary uncoupling of bone turnover that coincides with initiation of antiretroviral therapy resulting in a catabolic window during which BMD loss occurs [18]. In this study, bone turnover markers were not associated with BMD decline in adjusted analyses, bringing into question their role in driving ongoing change in BMD beyond the period of ART initiation. Although there are few prospective data other than the present study describing sustained high bone turnover states and the consequence on BMD in those with HIV, based on our data, we would speculate that if bone turnover is coupled, even

at an overall higher level in those with HIV, that progressive loss of bone may not be observed.

There are several limitations to our study. At study enrolment, a majority of the HIV-positive subjects had already been on ART for extended periods of time, limiting our ability to determine the impact of ART initiation on BMD loss. The majority of the HIV-positive subjects were on TDF-including regimens (83%), limiting our ability to determine whether associations between TDF and BMD loss were TDF-specific or reflecting a class-wide association. Although we were able to detect statistically relevant declines in BMD over time, the absolute changes were relatively small and the limited follow-up in our cohort prevents us from determining if these declines will continue and whether they will result in clinically relevant BMD loss over a longer period of follow-up. Our cohort was also of a relatively young age (average age 42 years) and hence at low overall risk of BMD loss, resulting in small absolute BMD changes, with potentially larger changes expected in an older population. Despite these limitations, that our study is relatively large in its makeup and includes an appropriate control group enables important and clinically relevant observations to be made that significantly contribute to our understanding of long-term changes in BMD in people living with HIV.

In conclusion, our findings suggest that although those with HIV have low BMD, there is no difference in the rate of BMD decline between HIV-positive and HIV-negative subjects over time, with recent ART initiation a major factor determining a greater decline in BMD and a relatively stability of BMD over time once on established ART.

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Conflict of interest

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Table 1: Characteristics of HIV-positive and HIV-negative subjects included in analysis

	HIV-positive (n=176)	HIV-negative (n=208)	P-value
Age (median (IQR) years)	39 (34-46)	43 (35-50)	0.04
Male gender, n (%)	107 (61%)	95 (46%)	0.003
African ethnicity, n (%)	68 (39%)	37 (18%)	<0.001
Hepatitis C Ab positive, n (%)	23 (13%)	2 (1%)	<0.001
Hepatitis B sAg positive, n (%)	4 (2%)	1 (0.5%)	0.18
Current smoker, n (%)	60 (34%)	31 (15%)	<0.001
BMI (median (IQR) kg/m^2)	26 (23-30)	27 (24-30)	0.07
Education, n (%)			
Third level	88 (50%)	138 (66%)	0.002
Primary/Secondary level	77 (44%)	66 (32%)	
Undisclosed	11 (6%)	4 (2%)	
Any history of fractures, n (%)	53 (30%)	50 (24%)	0.18
HIV transmission risk group, n (%)		-	
Heterosexual	90 (51%)	-	

Homosexual	55 (31%)	-
Injection drug user	29 (17%)	-
Other	2(1%)	-
Current ART, n (%)	155 (88%)	-
Total ART exposure (median (IQR) years)	2.9 (0.7-5.4)	-
TDF, n (%)	128 (83%)	-
exposure (median (IQR) years)	1.3 (0.1-2.9)	-
PI, n (%)	73 (47%)	-
exposure (median (IQR) years)	0.3 (0.0-2.6)	-
NNRTI, n (%)	77 (50%)	-
exposure (median (IQR) years)	0.2 (0.0-2.6)	-
HIV RNA ≤40 cp/ml, n (%)	121 (78%)	-
Nadir CD4+ T-cell (median (IQR) cell/mm^3)	218 (134-309)	-
Current CD4+ T-cell (median (IQR) cell/mm^3)	508 (370-650)	-

Current CD8+ T-cell (median (IQR) cell/mm^3)	787 (596-1085)	-	
Time since HIV diagnosis (median (IQR) years)	4.0 (2.0-9.0)	-	
Laboratory variables			
Albumin (median (IQR) g/L)	39 (37-42)	41 (40-43)	<0.001
ALT (median (IQR) IU/L)	28 (20-36)	26 (19-36)	0.04
ALP (median (IQR) IU/L)	79 (65-101)	63 (53-74)	<0.001
Corrected calcium (median (IQR) mmol/L)	2.31 (2.25-2.36)	2.27 (2.22-2.31)	<0.001
Phosphate (median (IQR) mmol/L)	1.06 (0.95-1.17)	1.03 (0.93-1.13)	0.13
25 (OH) D (median (IQR) nmol/L)	50.5 (31.0-70.5)	53.0 (37.0-75.0)	0.33
PTH (median (IQR) pmol/L)	5.9 (4.6-8.4)	5.3 (4.2-6.8)	0.001
Free T4 (median (IQR) pmol/L)	13.0 (11.9-14.1)	13.2 (12.2-14.4)	0.13
TSH (median (IQR) mIU/l)	1.71 (1.14-2.42)	1.53 (1.11-2.20)	0.12
Haemoglobin (median (IQR) g/dL)	13.9 (12.6-15.0)	13.9 (12.7-14.7)	0.69

Platelets (median (IQR) x10^9/L)	226 (195-279)	256 (215-288)	0.005
Bone turnover markers			
OC (median (IQR) ug/L)	21.5 (15.8-28.4)	17.2 (16.7-29.9)	<0.001
CTX-1 (median (IQR) ug/L)	0.489 (0.306-0.616)	0.344 (0.261-0.465)	<0.001
P1NP (median (IQR) ug/L)	49.2 (35.7-65.7)	37.0 (28.5-46.1)	<0.001

BMI-body mass index, Ab-antibody, sAg-surface antigen, TDF-tenofovir disoproxil fumarate, PI-protease inhibitor, NRTI-nucleoside reverse transcriptase, NNRTI-non-nucleoside reverse transcriptase inhibitor, ALT-alanine aminotransferase, ALP-alkaline phosphatase, 25 (OH)D-25-hydroxy-vitamin D, PTH-parathyroid hormone, TSH-thyroid-stimulating hormone, OC-osteocalcin, CTX-1-C-terminal cross-linking telopeptide of type-1 collagen, P1NP-procollagen type-1 amino-propeptide

Table 2 Results from unadjusted and adjusted mixed effects regression models for the between-group difference in absolute change in BMD between HIV-positive and HIV-negative subjects

	Between group difference in BMD at FN (g/cm^2/yr)	95% CI	P-value	Between group difference in BMD at TH (g/cm^2/yr)	95% CI	P-value	Between group difference in BMD at LS (g/cm^2/yr)	95% CI	P-value
Unadjusted mixed effects regression model									
HIV-positive vs HIV-negative	-0.0035	-0.0078; 0.00078	0.11	-0.0008	-0.0038; 0.0026	0.71	0.0017	-0.0036; 0.0073	0.51
***Adjusted mixed effects regression model**									
HIV-positive vs HIV-negative	-0.0038	-0.0081; 0.00046	0.08	-0.001	-0.0038; 0.0025	0.69	0.0018	-0.0036; 0.0073	0.51

*adjusted for age, gender, ethnicity, smoking status, educational level and body mass index (BMI)

FN-femoral neck, TH-total hip, LS-lumbar spine, CI-confidence interval

Table 3: Results from unadjusted mixed effects regression models results for factors associated with BMD slope at FN, TH and LS in HIV-positive subject

	[a]Effect on absolute change in FN BMD	95% CI	P-value	[a]Effect on absolute change in TH BMD	95% CI	P-value	[a]Effect on absolute change in LS BMD	95% CI	P-value
Demographic variables									
Female gender vs Male gender	0.0016	-0.0058; 0.0091	0.67	0.0013	-0.0039; .0066	0.62	-0.0041	-0.013; 0.0044	0.35
Age>30 vs Age<30	-0.0082	-0.019; 0.0028	0.15	-0.0051	-0.013; 0.0027	0.20	-0.0025	-0.015; 0.010	0.70
Caucasian vs non-Caucasian	-0.0033	-0.011; 0.0041	0.39	0.0018	-0.0034; 0.0070	0.50	-0.0035	-0.012; 0.0050	0.42
Unknown vs 1st/2nd level education	0.0013	-0.014; 0.017	0.87	-0.0017	-0.013; 0.0096	0.77	-0.0053	-0.024; 0.013	0.58
3rd vs 1st/2nd level education	-0.0053	-0.013; 0.0021	0.16	-0.0021	-0.0073; 0.0031	0.44	-0.00064	-0.0092; 0.0079	0.88
IDU vs non-IDU HIV acquisition	0.0026	-0.0072; 0.012	0.60	-0.0033	-0.010; 0.0036	0.35	-0.0026	-0.014; 0.0087	0.66
BMI (per 5kg/m^2 higher)	0.00086	-0.0023; 0.0040	0.59	0.00090	-0.0013; 0.0031	0.43	0.00030	-0.0034; 0.0040	0.87
Baseline laboratory variables									
ALP (per 5IU/L increment)	**0.00075**	**0.000096; 0.0014**	**0.03**	0.00038	-0.000089; 0.00084	0.11	**0.00078**	**0.000045; 0.0015**	**0.04**

Calcium (per mmol/L increment)	-0.012	-0.051; 0.026	0.53	-0.011	-0.038; 0.016	0.44	**-0.047**	**-0.091; -0.0040**	**0.03**
Phosphate (per mmol/L increment)	-0.0061	-0.026; 0.014	0.55	-0.0031	-0.017; 0.011	0.67	-0.013	-0.036; 0.0097	0.26
25 (OH) D (per 5 nmol/L increment)	-0.00019	-0.00075; 0.00036	0.50	-0.00016	-0.00054; 0.00022	0.40	-0.00012	-0.00075; 0.0005	0.69
PTH (per pmol/L increment)	**0.0014**	**0.00028; 0.0025**	**0.01**	**0.0011**	**0.00035; 0.0019**	**0.004**	0.0012	-0.00004; 0.0025	0.06
Baseline bone turnover markers									
OC (per ug/L higher)	**0.0019**	**0.00022; 0.0037**	**0.03**	0.00073	-0.00049; 0.0019	0.24	0.0012	-0.00085; 0.0032	0.26
CTX-1 (per 0.100 ug/L increment)	**0.0017**	**0.000048; 0.0034**	**0.04**	0.00075	-0.00044; 0.0019	0.22	0.00064	-0.0013; 0.0026	0.52
P1NP (per 10ug/L increase)	**0.0019**	**0.00036; 0.0034**	**0.02**	**0.0012**	**0.00016; 0.0023**	**0.024**	**0.0021**	**0.00035; 0.0038**	**0.02**
HIV-specific variables									
Started ART<3 months pre/after enrolment vs continuous follow-up ART	**-0.019**	**-0.029; -0.0097**	**<0.001**	**-0.016**	**-0.023; -0.0094**	**<0.001**	**-0.026**	**-0.037; -0.015**	**<0.001**

Not on ART vs continuous follow-up ART	**-0.021**	**-0.035; -0.0059**	**0.006**	-0.0029	-0.013; 0.0075	0.58	-0.0070	-0.024; 0.010	0.43
Current TDF vs no current TDF use	0.0069	-0.0013; 0.015	0.10	0.0028	-0.003; 0.0087	0.34	-0.0049	-0.015; 0.0046	0.31
Current PI vs no current PI use	0.0074	0.000088; 0.015	**0.05**	0.00067	-0.0045; 0.0058	0.80	0.0039	-0.0045; 0.012	0.37
Current NNRTI vs no current NNRTI use	-0.00058	-0.0079; 0.0067	0.88	0.0015	-0.0036; 0.0066	0.57	-0.0020	-0.010; 0.0064	0.64

[a]Effects on change represents the interaction between the individual variable with time in the mixed effects model

IDU-injection drug use, BMI-body mass index, ALT-alanine aminotransferase, ALP-alkaline phosphatase, 25 (OH) D-25-hydroxy-vitamin D, PTH-parathyroid hormone, OC-osteocalcin, CTX-1-C-terminal cross-linking telopeptide of type-1 collagen, P1NP-procollagen type-1 amino-propeptide, TDF-tenofovir disoproxil fumarate, PI-protease inhibitor, NRTI-nucleoside reverse transcriptase, NNRTI-non-nucleoside reverse transcriptase inhibitor

Table 4: Results from multivariable mixed effects regression models for factors independently associated with absolute change in BMD at FN, TH and LS BMD in HIV-positive subjects

	FN BMD*			TH BMD‡			LS BMD†		
	[a]Effect on FN slope	95% CI	*P*	[a]Effect on TH slope	95% CI	*P*	[a]Effect on LS slope	95% CI	*P*
Age >30 vs <30 years	-0.013	-0.02; -0.002	0.02	n/a			n/a		
Caucasian vs non-Caucasian Ethnicity	-0.011	-0.02; -0.003	0.006	n/a			n/a		
PTH (per 1 pmol/L)	0.0017	0.0005; 0.003	0.007	0.0006	-0.0002; 0.001	0.12	n/a		
ART< 3months pre/after study enrolment vs continuous ART	-0.016	-0.026; -0.006	0.002	-0.014	-0.02; -0.007	<0.001	-0.025	-0.04; -0.01	<0.001

Not on ART vs continuous ART	-0.019	-0.03; -0.004	0.01	-0.0024	-0.01; 0.008	0.65	-0.0062	-0.02; 0.01	0.48

[a]Effects on absolute change represents the interaction between the individual variable with time in the mixed effects model

,PTH-parathyroid hormone

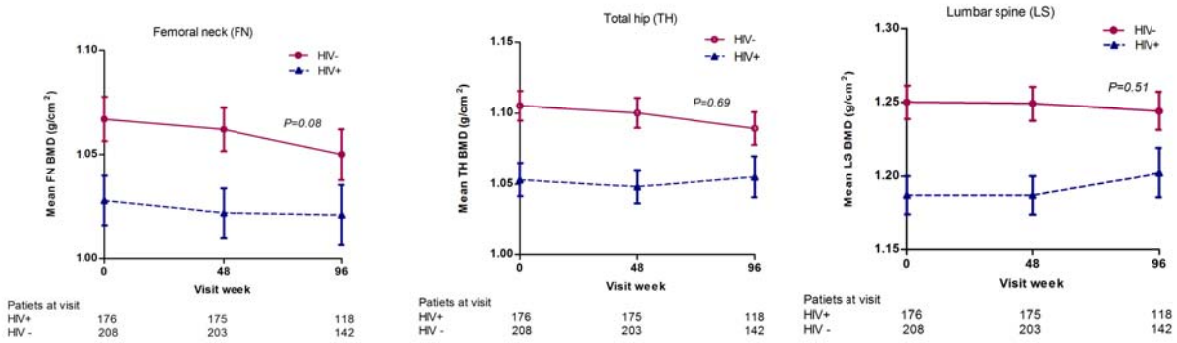
*Model adjusted for baseline variables: gender, education level, BMI, ALP, 25(OH) D and CTX-1

[‡]Model adjusted for gender, ethnicity, BMI, baseline ALP, PTH and CTX-1

[†]Model adjusted for ethnicity, BMI, baseline ALP, and CTX-1

Captions

Figure 1: Change in BMD from study enrolment at FN, TH and LS in HIV-positive and HIV-negative subjects



Footnote Figure 1

Between-group difference p-values is obtained from the time*HIV status interaction in the mixed effects regression model