Prevalence of low bone mineral density among people living with HIV

Terese L. Katzenstein1*, Maria Wessman2, Ellen Moseholm3, Haakon Sandholdt3, Ann-Brit E Hansen1, Anne-Mette Lebech5, Niklas R Jørgensen1 and Nina Weis1,4

Abstract: Increased prevalence of low bone mineral density (BMD) and fractures among people living with HIV (PLWH) have been reported. The aim of the DANHIV-OSTEO study is to longitudinally monitor BMD among successfully treated PLWH. Here we report the baseline Dual-energy X-ray Absorptiometry (DXA) data. Furthermore, we analyze the influence of mode of analysis on BMD results. Well-treated PLWH aged 40–70 (women) and 50–70 years (men) were included. Using T-scores and a newly described Z-score grading we investigated the frequencies of low BMD. Logistic regression models were used to delineate the influence of age, sex, BMI, smoking, exercise, tenofovir (TDF) and protease inhibitor (PI) usage on low BMD (Z/T scores < -1). 226 PLWH had baseline DXA scans. The frequency of low BMD was 57% (osteopenia and osteoporosis: 44 and 13%). Higher age, current smoking and male sex were associated with higher risk of low BMD. Higher BMI and exercise were protective. We found an OR suggesting a negative effect of TDF. PI usage was not associated with low BMD. Mode of analysis influenced the findings. Low BMD was highly prevalent among Danish well-treated PLWH. Neither TDF nor PI usage was significantly associated with low BMD. Greater uniformity in the mode of analysis is recommended.

Subjects: Epidemiology; Health Conditions; Medicine

ABOUT THE AUTHOR

Terese L Katzenstein (TLK) has been involved in HIV research for > 25 years. Dr Katzenstein introduced viral load monitoring in Denmark and the early research activities were on viral load trajectories and the interplay between various clinical and virological parameters and viral load. Currently, Dr Katzenstein is also involved in studies in Tanzania on viral load monitoring. TLK is part of a Danish and a Nordic group with special interest in women living with HIV (WLWH). The activities have included Nordic Conferences on WLWH, patient meetings and information material as well as several scientific publications. The current study on bone health among individuals living with HIV is a collaboration between two University Hospitals in Copenhagen. The aim of the study is to monitor study participants every 2 years for 10 years to get a better understanding of the interplay between HIV, HIV drugs and bone health.

PUBLIC INTEREST STATEMENT

The huge improvements in HIV treatment has led to the life expectancy of individuals living with HIV approaching that of the general population. Along with many individuals being diagnosed at age > 50 years, the population of individuals living with HIV is ageing. People living with HIV has been reported to have an increased risk of bone health issues including osteoporosis. The aim of the current study is to follow a cohort of well-treated individuals living with HIV with bone scans every second year for 10 years to improve our understanding of the possible interplay between HIV, HIV drugs and bone health. The data from the initial bone scans confirm that many have low bone mineral density and that the risk is increasing with age. We do not find an influence on HIV drugs on bone health. The study is ongoing.
Keywords: People living with HIV (PLWH); bone mineral density (BMD); DXA scan; tenofovir; protease inhibitor

1. Introduction

With the introduction of potent antiretroviral therapy (ART), the life expectancy of individuals living with Human Immunodeficiency Virus (HIV) (PLWH) infection (and access to treatment) is approaching that of the general population (Lohse & Obel, 2016; Teeravanichai et al., 2017; van Sighem et al., 2010). Furthermore, diagnosis of new HIV infections among individuals older than 50 years is not unusual (Ripa et al., 2017), combined leading to an aging HIV population. In Denmark the proportion of PLWH aged >50 years increased from 13 % in 1995 to 43 % in 2014 (Rasmussen et al., 2015). This shift towards an older PLWH population is likely to continue (Compston, 2016; Hood et al., 2017). A recent projection of the demographic composition of PLWH in the US estimated that the proportion of PLWH aged ≥ 55 years would double between 2013 and 2045, with a sharp increase between 2013 and 2025 (from 25 to 38 % of PLWH) (Hood et al., 2017). While combination ART (cART) has significantly reduced morbidity and mortality due to a decrease in immunodeficiency inflicted infections (Palella et al., 1998) and virally associated malignancies (Shiels & Engels, 2017), other causes of morbidity has not decreased or not decreased to the same extent. Among the co-morbidities gaining increasing importance among PLWH, in lieu of the ageing HIV population, is low bone mineral density (BMD) (Compston, 2016). Several studies have found increased frequencies of low BMD (Goh et al., 2018) and found increased occurrences of fractures among PLWH (Borges et al., 2017; Hansen et al., 2012; Starup-Linde et al., 2020; Triant et al., 2008; Womack et al., 2011). The mechanisms behind the decreased BMD among PLWH is incompletely understood. Traditional risk factors for osteoporosis i.e. low body weight, smoking, vitamin D deficiency and substance abuse is high among some HIV population and are likely to contribute (Borges et al., 2017; Compston, 2016; Goh et al., 2018). In some pre-exposure prophylaxis (PrEP) studies higher rates of low BMD than expected have been found, suggesting that lifestyle factors contribute to low BMD among some men who have sex with men, even prior to HIV acquisition (Liu et al., 2011). However, it is also thought that the HIV infection by itself, as well as the HIV induced inflammation, contribute both among untreated and successfully treated PLWH. Studies have uniformly found declines in BMD within the first 6–12 months after cART initiation with a reversion towards baseline values hereafter (R.J. Bedimo et al., 2014; Hansen et al., 2011). This early cART induced decline in BMD is most likely caused by cART induced viral suppression and ensuing immune reconstitution leading to increased bone turnover with involvement of the immune-skeletal interface (Compston, 2016; Ofotokun & Weitzmann, 2011). Patients with lower CD4 cell counts at baseline have a larger initial decline in BMD (Grant et al., 2013). Tenofovir disoproxil fumarate (TDF) has been shown to induce a larger decline than comparator drugs (R. J. Bedimo et al., 2014; Martin et al., 2009) and substitution has been shown to revert this effect (McComsey et al., 2018). The effect on BMD has also been found among individuals receiving TDF as PrEP (Liu et al., 2011). Protease inhibitors (PI) have also been incriminated (R. Bedimo et al., 2012; Cervero et al., 2018; Hirakawa et al., 2017; Womack et al., 2011), though the findings for PI’s are not as consistent as the findings for TDF (Arnsten et al., 2007).

Classically decreased BMD is evaluated by Z scores for pre-menopausal women and men < 50 years, and T scores for those post-menopausal and > 50 years (International Society for Clinical Densitometry, 2015). However, the interpretation of the BMD data generated by Dual-energy X-ray absorptiometry (DXA) scans varies. We used the BMD data to investigate the influence of different modes of analyzing the data on the findings.

Only few longitudinal studies of BMD in PLWH including both sexes and a control group have been performed (Tinago et al., 2017). We have established the DANHIV-OSTEO cohort with PLWH from the two largest HIV clinics in Denmark, jointly caring for more than 2/3 of PLWH in Denmark. It is planned that the participants will have DXA-scans performed biennial for a period of 10 years, aiming at contributing to a better understanding of the interplay between HIV, HIV drugs and bone
health. Currently, there is no established treatment to counteract the HIV/cART induced reduction in BMD, though the search is ongoing (Ofotokun et al., 2016). A better delineation of the longitudinal BMD changes and the influence of the contributing factors will hopefully help pave the way for such strategies. In a biomarker analysis we will relate the levels to those found in a matched cohort of HIV-uninfected individuals (not included in this manuscript).

Here we report the findings from the baseline visit. First, we evaluate the prevalence of low BMD, secondly the influence of various HIV related and unrelated factors on low BMD. Finally we describe the influence of mode of analysis on BMD results.

2. Methods

2.1. Study population
Study participants were recruited among PLWH monitored at two clinics of Infectious Diseases at Copenhagen University Hospitals, Hvidovre Hospital and Rigshospitalet, Copenhagen. The two clinics jointly care for the majority of PLWH in Denmark Recruitment was done in conjunction with planned clinical visits.

We aimed to include 150 females and 150 male PLWH, with equal contribution from the two clinics.

2.2. Inclusion criteria
Viral suppression (HIV RNA < 50 copies/mL) for ≥1 year. Age for men 50–70 and for women 40–70 years. Due to the known influence of ethnicity on BMD (Zengin et al., 2015), we aimed at including only Caucasians; however, due to the ethnic composition of women living with HIV in Denmark (Wessman et al., 2017), we were only able to almost fully comply with this for the men.

Study participants had to have the ability and willingness to have the supplementary tests performed (DXA scans and extra blood tests). We aimed at combining these with the timing of the patients scheduled visit/blood sampling.

2.3. Exclusion criteria
Patients suffering from the following diseases possibly affecting bone health were excluded: Diabetes, heart disease, hypogonadism, diseases of the thyroid/parathyroid glands, malignancies, chronic kidney disease, Morbus Bechterew and psychiatric diseases. PLWH with substance use and/or co-infected with viral hepatitis B and C were also excluded.

Likewise, PLWH receiving chemotherapy, systemic steroids, heparin, pioglitazone or anti-epileptic drugs were excluded. For women, pregnancy was an exclusion criterion.

Ethical clearance was obtained from the Local Ethical Committee (Den Videnskabsetiske Komite D for Region Hovedstaden, H-15006943 approval date 24.06.2015). All participant provided written informed consent.

2.4. HIV related data
Data on mode of HIV transmission, time since HIV diagnosis, nadir CD4 cell counts and treatment history were obtained from the Danish HIV Cohort (Omland et al., 2014). Regarding treatment history, we extracted cumulative time on TDF and whether the participants were receiving TDF and/or PI at the time of study participation.

2.5. Questionnaire
All study participants filled in a questionnaire at enrollment collecting demographic data including age, sex, self-perceived health, smoking and alcohol usage, body mass index (BMI), previous
fractures, physical activity, for women also date of menopause where applicable, and medications for illnesses unrelated to HIV.

2.6. Samples obtained
All patients were scheduled for a DXA-scan and within 6 months hereof, in conjunction with routine blood sampling, blood was drawn for either immediate analysis (including Ca**, 25-hydroxy vitamin D, phosphate, magnesium, thyroid stimulating hormone (TSH), T3, T4, parathyroid hormone (PTH) and for the women estradiol, follicle-stimulating hormone (FSH) and luteinizing hormone (LH) or for storage at—80°C for later analyses (for bone-turnover and inflammation markers).

2.7. DXA-scans
At Copenhagen University Hospital, Rigshospitalet DXA scans were performed by Lunar Prodigy (GE Healthcare, Madison, WI, USA). At Copenhagen University Hospital, Hvidovre Hospital Hologic DXA scanner (Hologic Inc., MA, USA) was used. From DXA scans raw BMD values from L2-L4 (columna), left and right femur were extracted. These were converted into T-scores (standard deviations (SD) from the mean of a young, healthy gender matched population) and Z-scores (standard deviations (SD) from the mean of a healthy age and gender matched population).

2.8. Definitions of low BMD, osteoporosis and menopause
For patients > 50 years of age the WHO classification of osteoporosis was applied, defining osteopenia as a T-score < −1 but > −2.5 and osteoporosis as T-score ≤ −2.5 (International Society for Clinical Densitometry, 2015). For women younger than 50 years (estimated to be pre-postmenopausal) Z scores were used in accordance with the International Society of Clinical Densitometry i.e. a Z score > −2 denoting osteoporosis. Further, for this group, we defined low BMD as a Z score > −1 as recently proposed by Shaiykova et al. (2018).

At the two Danish hospitals the standard procedure is to measure BMD at columna, left and right femur. The bone health status is categorized according to the lowest T/Z scores using all three sites i.e. values from columna and both left and right femur. This is in contrast to the 2015 recommendations from the International Society for Clinical Densitometry to measure BMD at the columna and either femur (International Society for Clinical Densitometry, 2015), but in line with other studies both within and outside of the HIV field (Afzelius et al., 2017; Cervero et al., 2018; Hwang et al., 2012).

2.9. Statistical analyses
We estimated the prevalence of low BMD and osteoporosis according to the definitions described above. We presented the data as frequencies. To test for differences between patients with normal and low BMD, we performed chi-square tests.

Univariate and multiple logistic regression analysis was used to assess the impact of explanatory variables including age, sex, BMI, smoking, exercise and a number of HIV-related risk factors including nadir CD4 cell count, exposure to TDF and/or PI. Effect on the dichotomous independent variable low BMD defined as a Z or T score < −1, using Z or T scores as appropriate, and using the minimum value of the three BMD measurements.

Univariate and multiple mixed logistic regression analysis was used in almost the same setting as mentioned for the logistic regression, differing in that we used all BMD measurements for all patients and a random term of patient effect, accounting for the possible correlation of observations from the same patient. In the multivariate analysis we adjusted for all variables in the model (listed above). The validity of the multivariate models were tested using the Hosmer and Lemeshow goodness-of-fit test.

Furthermore, we performed logistic regression analyses using data either from columna and left femur or columna and right femur (information on dominant site not available) Table 1 and 2 (Table 3).
Furthermore, we compared the results using the standard method at the clinical sites i.e. low BMD (osteopenia/osteoporosis) evaluation based on the lowest value at the three monitoring sites with including only values from columna and the left femur. Analyses were carried out using STATA 15 (STAT Corporation, College Station, Texas, USA) and p-values of <0.05 were considered significant.

2.10. Power calculation
We aimed to include 300 patients, with equal contribution from the two clinics and with a 50–50 male to female participation. This seemed reasonable relative to other studies within the field i.e. the HIV UPBEAT study which included 384 study participants, hereof 176 PLWH (Tinago et al., 2017).

3. Results
As planned, we enrolled >150 men (in total 164), of whom 141 had a DXA-scan performed. Due to the composition of individuals living with HIV in Denmark, with the majority being male and a large proportion of the women being born outside Denmark (Thorsteinsson et al., 2012), and with a number of these being unable to fill in the questionnaire in Danish, we only managed to enroll 106 women (out of the planned 150) and of these 85 had a DXA-scan performed (Table 1).

We estimated women < 50 years to be pre-menopausal, which was supported by our questionnaire data (data not shown). For women aged 40–50 years we therefore used Z scores.

We evaluated frequencies of osteopenia and osteoporosis per the method used at the participating sites and the Z-score grading as per the method described by Shaiykova et al (Shaiykova et al., 2018) (i.e. defining Z-score <-1 and >-2 as osteopenia and < -2 as osteoporosis). Table 1 shows that 57 % of the 226 participants had either osteopenia or osteoporosis. One-hundred-and-one participants (44 %) had osteopenia and 29 (13 %) had osteoporosis. When reanalyzing the data including only T and Z-scores from the columna and left femur the frequency of low BMD was 53 % (120/226).

We next analyzed, in a logistic regression model, the influence of various parameters (age, sex, BMI, DXA site, smoking status, physical activity, nadir CD4 cell count and TDF and PI usage) on low BMD (< -1 using T-scores for individuals > 50 years and Z-scores for women < 50 years). For this analysis we used the lowest values from the three BMD measured sites; columna, left and right femur (Table 2). The odds ratio (OR) for low BMD was lower among the participating women relative to their male counterparts (Table 2). However, in the adjusted analyses there was no significant association between sex and low BMD.

Increasing age was associated with low BMD values (minimum osteopenia). Higher BMI was significantly associated with lower risk of having BMD values denoting osteopenia or osteoporosis (Table 2). These results did not change significantly in the multivariate analysis.

We found the extent of physical activity to be associated with BMD, with higher activity levels associated with lower risks of low BMD (Tables 2 and 3). In the logistic regression model (Table 3) the finding was of borderline significant when including DXA data (T and Z scores) from columna and left femur, but significant when using DXA values from columna and right femur. Moderate physical activity was associated with reduced risk of low BMD in all the adjusted analyses.

The grading of risk of low BMD relative to smoking was with highest risks among those currently smoking relative to never-smokers with the risk for previous smokers lying in between. The difference between current and never smokers was statistically significant, while this was not the case when comparing current smokers with ex-smokers (Tables 2 and 3). Smoking was also not significantly associated with low BMD in any of the adjusted analyses.

We found no influence of nadir CD4 cell counts (</>/ 200 x 10⁶/L) on risk of low BMD (Table 2).
|                         | Total  | Normal BMD | Low BMD | p-value* |
|-------------------------|--------|------------|---------|----------|
|                         | (n = 226) | (n = 96) | (n = 130) |          |
| n (%)                   | n (%)  | n (%)      |         |          |
| Male                    | 141 (62)  | 51 (33)  | 90 (69) |          |
| Female                  | 85 (38)   | 45 (47)  | 40 (31) |          |
| Missing                 | 0       |           |         |          |
| Male                    | 141 (62)  | 51 (53)  | 90 (69) |          |
| Age, years              |         | <0.001    |         |          |
| <50                     | 45 (20)   | 31 (32)  | 14 (11) |          |
| 50–60                   | 113 (50)  | 45 (47)  | 68 (52) |          |
| >60                     | 68 (30)   | 20 (21)  | 48 (37) |          |
| Missing                 | 0       |           |         |          |
| BMI                     | <0.01    |           |         |          |
| <24                     | 105 (47)  | 39 (41)  | 66 (51) |          |
| 24–30                   | 93 (41)   | 38 (39)  | 55 (42) |          |
| >30                     | 28 (12)   | 20 (20)  | 9 (7)   |          |
| Missing                 | 0       |           |         |          |
| Smoking                 | 0.03     |           |         |          |
| Never                   | 85 (38)   | 42 (44)  | 43 (33) |          |
| Current smoker          | 57 (25)   | 16 (17)  | 41 (32) |          |
| Former smoker           | 84 (37)   | 38 (39)  | 46 (35) |          |
| Missing                 | 0       |           |         |          |
| Physical activity pr week | 0.10    |           |         |          |
| 0–120 minutes           | 74 (33)   | 24 (25)  | 50 (38) |          |
| 2–4 hours               | 68 (30)   | 33 (34)  | 35 (27) |          |
| More than 4 hours       | 84 (37)   | 39 (40)  | 45 (35) |          |
| Missing                 | 0       |           |         |          |
| Nadir CD4 cell count    | 0.47     |           |         |          |
| <200                    | 108 (48)  | 44 (46)  | 64 (49) |          |
| >200                    | 112 (50)  | 51 (53)  | 61 (47) |          |
| Missing                 | 6 (2)    | 1 (1)    | 5 (4)   |          |
| Treatment w/ Tenofovir  | 0.14     |           |         |          |
| No                      | 50 (22)   | 26 (27)  | 24 (18) |          |
| Yes                     | 171 (76)  | 69 (72)  | 102 (78)|          |
| Missing                 | 5 (2)    | 1 (1)    | 4 (4)   |          |
| Treatment w/ Protease inhibitors | 0.32   |           |         |          |
| No                      | 78 (35)   | 30 (31)  | 48 (37) |          |
| Yes                     | 143 (63)  | 65 (68)  | 78 (60) |          |
| Missing                 | 5 (2)    | 1 (1)    | 4 (3)   |          |

* P values are based on the χ2-test
Table 2. Logistic regression analysis of low BMD

|                      | Logistic regression with lowest BMD value | Mixed model with all bone measurements |
|----------------------|------------------------------------------|---------------------------------------|
|                      | OR (95% CI)                              | OR (95% CI)                           |
|                      | Unadjusted p-value                       | Unadjusted p-value                    |
|                      | Adjusted* p-value                       | Adjusted* p-value                    |
| Sex                  |                                          |                                      |
| Male                 | ref                                      | ref                                  |
| Female               | 0.50 (0.29; 0.87)                        | 1.01 (0.47; 2.19)                    |
|                      | 0.01                                     | 0.98                                 |
|                      |                                          | 0.46 (0.26; 0.81)                    |
|                      |                                          | <0.01                                |
|                      |                                          | 0.96 (0.43; 2.12)                    |
|                      |                                          | 0.91                                 |
| Age, years           |                                          |                                      |
| <50                  | ref                                      | ref                                  |
| 50–60                | 3.35 (1.60; 6.97)                        | 3.20 (1.18; 8.70)                    |
|                      | <0.01                                    | 0.03                                 |
|                      |                                          | 3.54 (1.66; 7.55)                    |
|                      |                                          | <0.01                                |
|                      |                                          | 3.22 (1.16; 8.98)                    |
|                      |                                          | 0.03                                 |
| >60                  | 5.31 (2.34; 12.05)                       | 4.60 (1.54; 13.68)                   |
|                      | <0.001                                   | <0.01                                |
|                      |                                          | 6.72 (2.84; 15.91)                   |
|                      |                                          | <0.001                               |
|                      |                                          | 5.94 (1.90; 18.54)                   |
|                      |                                          | <0.01                                |
| BMI                  |                                          |                                      |
| <24                  | ref                                      | ref                                  |
| 24–30                | 0.86 (0.48; 1.51)                        | 0.84 (0.44; 1.60)                    |
|                      | 0.59                                     | 0.59                                 |
|                      |                                          | 0.88 (0.37; 2.10)                    |
|                      |                                          | 0.77                                 |
|                      |                                          | 0.86 (0.44; 1.67)                    |
|                      |                                          | 0.65                                 |
| >30                  | 0.28 (0.11; 0.68)                        | 0.23 (0.11; 0.79)                    |
|                      | <0.01                                    | 0.02                                 |
|                      |                                          | 0.17 (0.01; 216.5)                   |
|                      |                                          | 0.63                                 |
|                      |                                          | 0.27 (0.10; 0.74)                    |
|                      |                                          | 0.01                                 |
| Measure site         |                                          |                                      |
| col                  | ref                                      | ref                                  |
| Femur sin            | 0.04 (0.01; 0.33)                        | <0.01                                |
|                      |                                          | 0.35 (0.16; 0.76)                    |
|                      |                                          | <0.01                                |
| Femur dxt            | 0.17 (0.03; 1.10)                        | 0.06                                 |
|                      |                                          | 0.34 (0.16; 0.72)                    |
|                      |                                          | <0.01                                |
| Smoking              |                                          |                                      |
| Never                | ref                                      | ref                                  |
| Current              | 2.50 (1.22; 5.12)                        | 1.64 (0.74; 3.64)                    |
|                      | 0.01                                     | 0.22                                 |
|                      |                                          | 3.94 (0.10; 149.1)                   |
|                      |                                          | 0.46                                 |
|                      |                                          | 1.85 (0.81; 4.20)                    |
| Former               | 1.18 (0.65; 2.17)                        | 1.13 (0.58; 2.23)                    |
|                      | 0.59                                     | 0.73                                 |
|                      |                                          | 1.54 (0.38; 6.30)                    |
|                      |                                          | 0.54                                 |
|                      |                                          | 1.35 (0.66; 2.76)                    |
| Physical activity    |                                          |                                      |
| 0–120 minutes        | ref                                      | ref                                  |
| 2–4 hours            | 0.51 (0.26; 1.01)                        | 0.45 (0.21; 0.96)                    |
|                      | 0.05                                     | 0.04                                 |
|                      |                                          | 0.49 (0.11; 2.13)                    |
|                      |                                          | 0.34                                 |
|                      |                                          | 0.44 (0.20; 0.97)                    |
|                      |                                          | 0.04                                 |
| More than 4 hours    | 0.55 (0.29; 1.06)                        | 0.57 (0.27; 1.19)                    |
|                      | 0.09                                     | 0.14                                 |
|                      |                                          | 1.04 (0.23; 4.71)                    |
|                      |                                          | 0.96                                 |
|                      |                                          | 0.52 (0.24; 1.11)                    |
|                      |                                          | 0.09                                 |
Table 2. (Continued)

|                | Logistic regression with lowest BMD value | Mixed model with all bone measurements |
|----------------|-------------------------------------------|----------------------------------------|
| Nadir CD4 cell count |                                            |                                        |
| <200           | ref                                       | ref                                    | ref          |
| ≥200           | 0.82 (0.48; 1.40)                          | 0.47                                   | 0.76 (0.41; 1.40) |
|                |                                            |                                        | 0.37         | 0.83 (0.31; 2.23) |
|                |                                            |                                        | 0.71         | 0.84 (0.45; 1.59) |
|                |                                            |                                        | 0.60         |              |
| Tx w/Tenofovir |                                            |                                        |              |              |
| No             | ref                                       | ref                                    | ref          |
| Yes            | 1.60 (0.85; 3.02)                          | 0.15                                   | 1.26 (0.62; 2.55) |
|                |                                            |                                        | 0.53         | 1.51 (0.79; 2.87) |
|                |                                            |                                        | 0.21         | 1.10 (0.53; 2.28) |
|                |                                            |                                        | 0.80         |              |
| Tx w/Protease Inhibitors |                                    |                                        |              |              |
| No             | ref                                       | ref                                    | ref          |
| Yes            | 0.75 (0.43; 1.32)                          | 0.32                                   | 0.82 (0.43; 1.55) |
|                |                                            |                                        | 0.54         | 0.75 (0.42; 1.32) |
|                |                                            |                                        | 0.32         | 0.83 (0.43; 1.59) |
|                |                                            |                                        | 0.57         |              |

*Adjusted for all variables in table. Significant results are in bold.
Table 3. Logistic regression analysis of low BMD (lowest value of columna and fem.sin OR columna fem.dxt)

|                          | Model w/col & left fem | Model w/col & right fem |
|--------------------------|------------------------|-------------------------|
|                          | OR (95% CI)            | p-value                 | OR (95% CI) | p-value |
|                          | Unadjusted             | Adjusted*               | Unadjusted  | Adjusted*               |
| Sex                      |                        |                         |            |                     |
| Male                     | ref                    | ref                     | ref        | ref                  |
| Female                   | 0.63 (0.37; 1.08)      | 0.09                    | 1.33 (0.62; 2.85) | 0.47 | 0.48 (0.27; 0.82) | <0.01 |
| Age, years               |                        |                         |            |                     |
| <50                      | ref                    | ref                     | ref        | ref                  |
| 50–60                    | 3.58 (1.70; 7.56)      | 0.001                   | 3.77 (1.39; 10.22) | <0.01 | 4.00 (1.87; 8.52) | <0.001 |
| >60                      | 3.52 (1.57; 7.87)      | <0.01                   | 3.16 (1.08; 9.22) | 0.04 | 6.60 (2.84; 15.31) | <0.001 |
| BMI                      |                        |                         |            |                     |
| <24                      | ref                    | ref                     | ref        | ref                  |
| 24–30                    | 0.91 (0.52; 1.60)      | 0.74                    | 0.92 (0.49; 1.72) | 0.80 | 0.82 (0.46; 1.44) | 0.48 |
| >30                      | 0.36 (0.15; 0.86)      | 0.02                    | 0.36 (0.49; 0.95) | 0.04 | 0.29 (0.12; 0.71) | <0.01 |
| Smoking                  |                        |                         |            |                     |
| Never                    | ref                    | ref                     | ref        | ref                  |
| Current                  | 2.15 (1.07; 4.30)      | 0.03                    | 1.65 (0.76; 3.58) | 0.21 | 2.62 (1.28; 5.38) | 0.01 |
| Former                   | 1.02 (0.56;1.87)       | 0.94                    | 0.96 (0.49; 1.86) | 0.90 | 1.13 (0.62; 2.06) | 0.7 |
| Physical activity        |                        |                         |            |                     |
| 0-120 minutes            | ref                    | ref                     | ref        | ref                  |
| 2-4 hours                | 0.51 (0.26; 1.00)      | 0.05                    | 0.48 (0.23; 0.99) | 0.05 | 0.48 (0.24; 0.95) | 0.03 |
| More than 4 hours        | 0.55 (0.28; 1.04)      | 0.06                    | 0.59 (0.29; 1.21) | 0.15 | 0.50 (0.26; 0.96) | 0.04 |
| Nadir CD4 cell count     |                        |                         |            |                     |
| <200                     | ref                    | ref                     | ref        | ref                  |
| ≥200                     | 0.76 (0.44; 1.26)      | 0.27                    | 0.68 (0.37; 1.24) | 0.20 | 0.80 (0.47; 1.35) | 0.40 |

(Continued)
Table 3. (Continued)

|                  | Model w/col & left fem |                        | Model w/col & right fem |                        |
|------------------|------------------------|------------------------|-------------------------|------------------------|
|                  | ref ref ref ref        | ref ref ref ref        | ref ref ref ref        | ref ref ref ref        |
| No               |                        |                        |                         |                        |
| Yes              | 1.59 (0.84; 3.00)      | 0.15                   | 1.31 (0.65; 2.64)      | 0.45                   |
|                  |                        |                        |                         |                        |
| Tx w/Protease    |                        |                        |                         |                        |
| Inhibitors       | ref ref ref ref        | ref ref ref ref        | ref ref ref ref        | ref ref ref ref        |
| No               |                        |                        |                         |                        |
| Yes              | 0.87 (0.50; 1.52)      | 0.63                   | 0.95 (0.51; 1.77)      | 0.87                   |
|                  |                        |                        |                         |                        |

*Adjusted for all variables in table. Significant results are in bold.
Likewise, we found no significant influence of TDF or PI usage on the risk of low BMD. There was, however, a trend towards lower BMD with TDF usage (Tables 2 and 3).

When using either columna and left femur versus columna and right femur in the logistic regression analysis of low BMS results differed somewhat (Table 3).

4. Discussion
In this prospective HIV-OSTEO study of bone health among successfully treated PLWH in Copenhagen, Denmark, baseline DXA-scans results from 226 (141 men and 85 women) PLWH showed that 42, 44 and 13 % had normal BMD values, osteopenia and osteoporosis, respectively. In a recently published Spanish study the proportion of individuals with low BMD was also very high, at 59 %, hereof 10 % with osteoporosis (Cervero et al., 2018).

A meta-analysis from 2020 found that PLWH were at increased risk of both low BMD and fragility fractures compared to controls. Interestingly, they report that the reduced BMD did not fully explain the increased fracture risk (Starup-Linde et al., 2020). There is a need to better understand this discrepancy. Obtaining an ex vivo method for evaluating the interplay between the various cell types—including osteoblasts, osteoclasts and cells of the immune system as well as the various cytokines has proven difficult. Hopefully further development within this field will help delineation of the complex interactions (Ehnert et al., 2020). As expected, we found that age was an independent risk factor for low BMD.

We found the OR for low BMD was lower for women relative to men. In the current study we included women from a younger age (>40 yrs) relative to the men (>50 yrs). The different trajectories in BMD decline relative to gender (Goltzman, 2019) might have contributed to the gender difference. This is supported by the adjusted analysis, where the difference was no longer significant after adjustment for various parameters, including age. However, lower BMD levels among young men who have sex with men, irrespective of HIV status, relative to older MSM, heterosexual men and women has previously been reported (Kooij et al., 2015). Likewise, Bonjoch et al. (2010), in a longitudinal study, found male sex to be a risk factor for bone loss. However, in the large study by Erlandson et al. (2018) (n = 2.598) the main finding was a steeper decline in BMD among women relative to men. Differences in the study populations might contribute to the very mixed results regarding the influence of sex on BMD and BMD trajectories among individuals living with HIV.

In line with the literature (Erlandson et al., 2018; Multanen et al., 2014), we found physical exercise to be protective against low BMD.

Higher BMI was associated with protection against low BMD. This is in agreement with the literature (Akkawi & Zmerly, 2018) including bone health studies among PLWH (Erlandson et al., 2018).

We did not find a significant difference between study participants treated with or without PI and low BMD (<1 in either lowest T or Z score). In the meta-analysis by Goh et al. (2018) the proportion with low BMD was higher among those treated with PI's relative to those on non-PI containing cART regimens, but the difference was not statistically significant. Likewise, we did not find a significant effect of TDF containing cART (either investigated as current or cumulative TDF exposure), but a trend towards lower BMD values among those on TDF. In the meta-analysis by Goh et al. (2018) only one of the studies included data on TDF usage and therefore an odd ratio was not calculated, but low BMD was more common among TDF versus non-TDF treated individuals. In the latest meta-analysis by Starup-Linde et al the risk of declines in BMD were significantly higher for patients initiating TDF relative to those initiated on either abacavir (ABC) or tenofovir alafenamide fumarate (TAF) (Starup-Linde et al., 2020). In the study by Erlandson et al. (2018) longer duration of TDF treatment was associated with lower BMD. Cervero et al. (2018) found that both PI and TDF usage were independent risk factors for low BMD. The PLWH included in the Spanish study differed from ours in several ways; the DXA scans were performed over a 30-year period, a large proportion having acquired HIV by intravenous drug use and being chronic hepatitis C virus (HCV) infected (43 and 40 %, respectively), while HCV infection in our study was an exclusion criterion. On
the other hand, the Spanish study population had a median age of only 46.5 years as opposed to 54.4 (interquartile range 50.6;61.7) years in our study (Cervero et al., 2018).

At the study hospitals in Copenhagen, Denmark BMD is routinely measured at L1-L4 and both left and right femur, and the lowest values from any of the three sites are used to categorize the individuals as having either normal BMD values, osteopenia or osteoporosis. According to the International Society for Clinical Densitometry’s 2015 position paper BMD is measured at one femur (no preference for non-dominant site) (International Society for Clinical Densitometry, 2015). BMD studies reporting using data from either the right femur only (Haskelberg et al., 2012) or both left and right femur (Cervero et al., 2018) have been published. Most studies, however, do not specify the femur data used (Escota et al., 2016; Liu et al., 2011; McComsey et al., 2018; Shaiykova et al., 2018). Also within the bone literature outside of the HIV field the optimal data analysis method is debated (Hwang et al., 2012). We found influence on mode of analysis (i.e. using all data, data from columna and either right or left femur) on the results.

5. Strength’s
The cohort is well-defined with exclusion of individuals with other health factors associated with low BMD. We included patients from the two largest HIV Clinics in Denmark, jointly caring for more than 2/3 of all PLWH in the country. Furthermore, through the Danish HIV Cohort Study we had access to complete data on cART usage.

6. Limitations
As we mainly included virologically suppressed Caucasian PLWH (>90 % of the men, but only 55% of the women), these findings cannot be extrapolated to PLWH with a different ethnic composition or untreated/not fully successfully treated PLWH. Furthermore, we did not reach the number of study participants stipulated and used for study design. However, the number of study participants is equal to or higher than the participants included in other studies investigating BMD among cART treated PLWH (Cervero et al., 2018; Tinago et al., 2017).

The two sites included used different DXA scanners. This is, however, not uncommon, i.e. in the study by Graat-Verboom et al. (2012) different scanners were used for baseline and follow-up investigations. In a sensitivity analysis we adjusted the multivariate analysis for scanner, and this did not change the estimates significantly.

7. Conclusion
The frequency of low BMD among well-treated PLWH in Denmark was 57 %, in line with what has previously been reported. Our data supports the protective effect of higher BMI and exercise and found a negative effect of current smoking and trend towards a negative effect of TDF usage. Results differed according to how low BMD was defined, which calls for greater uniformity in the mode of analysis.

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Author details
Terese L. Katzenstein1
E-mail: terese.katzenstein@regionh.dk
ORCID ID: http://orcid.org/0000-0002-2233-500X
Maria Wessman3
E-mail: MARW@ssi.dk
Ellen Moseholm3
E-mail: ellen.moseholm@gmail.com
Haakon Sandholdt1
E-mail: haakon.sandholdt@regionh.dk
Ann-Brit E Hansen1
E-mail: ann-brit.e.hansen.02@regionh.dk
Anne-Mette Lebech2
E-mail: anne-mette.lebech@regionh.dk
Niklas R Jørgensen1
E-mail: niklas.rye.joergensen@regionh.dk
ORCID ID: http://orcid.org/0000-0001-9624-5210
Nina Weis3,4
E-mail: nina.weis@regionh.dk

1 Department of Infectious Diseases, Copenhagen University Hospital, Rigshospitalet, Copenhagen, Denmark.
2 Department of Infectious Diseases, Copenhagen University Hospital, Hvidovre Hospital, Copenhagen, Denmark.
3 Department of Clinical Biochemistry, Copenhagen University Hospital, Rigshospitalet, Glostrup, Copenhagen, Denmark.
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