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Effect of Testosterone Use on Bone Mineral Density in HIV-Infected Men

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Abstract

HIV-infected men have increased rates of osteoporosis and fracture compared to HIV-uninfected men. Testosterone use among HIV-infected men is common. In HIV-uninfected men, testosterone increases bone mineral density (BMD), but its effects have not been evaluated in HIV-infected men. In a substudy of Multicenter AIDS Cohort Study (MACS), the Bone Strength Substudy (BOSS) enrolled 202 HIV-infected and 201 HIV-uninfected men aged between 50 and 69 years. Study participants underwent dual-energy X-ray absorptiometry (DXA) at the lumbar spine (LS), total hip (TH), and femoral neck (FN) and detailed assessment of osteoporosis risk factors. We used multivariable linear regression to determine associations and 95% confidence intervals (CIs) between self-reported testosterone use and T-scores at the LS, TH, and FN after adjustment for demographics, behavioral covariates, comorbidities, and other traditional osteoporosis risk factors. HIV-infected men reported more frequent testosterone use (22% vs. 4%; $p < .001$) and had lower median BMD T-score at TH than HIV-uninfected men (0.0 vs. 0.3; $p = .045$) but similar T-scores at LS and FN. In the overall study population, testosterone use was associated with significantly greater BMD T-score at LS (0.68; 95% CI: 0.22–1.13). In HIV-infected men with virologic suppression, testosterone was significantly associated with higher BMD T-score at LS (0.95; 95% CI: 0.36–1.54) and TH (0.45; 95% CI: 0.04–0.86). Current testosterone use is common in HIV-infected men and was associated with higher BMD, compared to those not taking testosterone. Testosterone's role in reducing fracture risk in HIV-infected men should be investigated.

Keywords: HIV infection, bone density, testosterone, anti-HIV agents, humans

Introduction

Rates of osteoporosis and fragility fractures are higher in HIV-infected individuals than in HIV-uninfected individuals.[1–4] The etiology of low bone mineral density (BMD) is multifactorial, with contributions from HIV infection, antiretroviral therapy (ART),[5] HIV-associated immunodeficiency and inflammation,[6,7] and traditional osteoporosis risk factors such as low body mass index (BMI), hypogonadism, and vitamin D deficiency.[8]

Serum testosterone levels are often low in HIV-infected men,[9,10] which could contribute to low BMD. In the general population testosterone replacement has been shown to not only increase BMD[11] but also, potentially, the risk of cardiovascular disease.[12] Men with low testosterone levels are frequently prescribed testosterone for issues such as low libido, muscle mass, or energy, but the effect of testosterone replacement in HIV-infected men has not been systematically evaluated. In this study, we investigated the relationship between testosterone use and BMD in a well-characterized cohort of older HIV-infected and HIV-uninfected men.

Materials and Methods

Study participants

Initiated in 1984, the Multicenter AIDS Cohort Study (MACS) is an ongoing prospective cohort study of HIV-infected and HIV-uninfected men who have sex with men

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(MSM). MACS enrolled participants from the following four sites in the United States: Los Angeles, CA; Pittsburgh, PA; Baltimore, MD/Washington, DC, and Chicago, IL.[13] The current analysis used data collected in the Bone Strength Substudy (BOSS), a substudy of the MACS designed to better understand the contributions of aging, chronic HIV infection, and ART to bone fracture risk.

Between 2012 and 2015, BOSS enrolled HIV-infected men on ART and HIV-uninfected men between the ages of 50 and 69 years from each of the four MACS sites with recruitment practices to ensure a balance of older and younger HIV-infected and HIV-uninfected men. Exclusion criteria included a history of osteoporosis medication use (e.g., bisphosphonates, teriparatide, and denosumab), weight >300 pounds precluding safe dual-energy X-ray absorptiometry (DXA), and a plasma HIV RNA level ≥200 copies/mL.

Study procedures

At a single study visit, we measured height and weight. In addition, participants completed questionnaires to characterize fracture risk, including self-reported alcohol, cigarette, and injection drug use, and physical activity levels.[14] We captured the use of testosterone and other concomitant medications known to affect BMD (i.e., corticosteroids, proton pump inhibitors, and antidepressants) by self-report. Participants underwent morning phlebotomy to determine free testosterone levels determined by equilibrium dialysis (Brigham Research Assay Core, Boston, MA) and 25-hydroxy vitamin D levels by chemiluminescence assay (DiaSorin, Stillwater, MN) at the Johns Hopkins Advanced Chemistry Laboratory (Baltimore, MD). Participants underwent DXA to determine BMD T-scores at the lumbar spine (LS), total hip (TH), and femoral neck (FN). DXA scans were standardized at each site and then read at the central reading site (Tufts Body Composition Laboratory, Boston, MA).

Other covariates

Age was analyzed as a dichotomous variable as ≥60 or <60 years. Self-reported race was categorized as White versus Black versus Other. Alcohol use was categorized as ≥14 versus <14 drinks/week, smoking as current tobacco smoker versus former versus never, and injection drug use as current or past use versus none. Viral hepatitis B or C infection was defined by the presence of viral DNA or RNA, respectively, in the serum. Diabetes was defined as fasting glucose ≥126 mg/dL or the self-reported diagnosis of diabetes with use of medications for glucose control. Kidney disease was defined as a calculated glomerular filtration rate of <60 mL/min (Cockcroft-Gault Formula) or urine protein in milligrams per gram of creatinine of ≥200. HIV-specific factors included current and nadir $CD4^+$ T-lymphocyte cell count/μL (CD4), current and percentage of MACS visits with HIV RNA levels <50 copies/mL, cumulative years of combination ART use, cumulative years of tenofovir disoproxil fumarate (TDF) and protease inhibitors (PIs), and ever thymidine analog use (i.e., zidovudine and stavudine).

Statistics

We used the Wilcoxon Rank-Sum test to compare continuous variables and the Chi-squared test to compare categorical variables between HIV-infected and HIV-uninfected men. Multivariable linear regression was used to determine the relationship between self-reported testosterone use and BMD T-score at the LS, TH, and FN, adjusting for HIV serostatus, age, race, MACS site, BMI, self-reported alcohol, smoking and history of injection drug use, hepatitis B virus (HBV) infection, hepatitis C virus (HCV) infection, diabetes, concomitant medications that affect BMD, history of parental hip fracture, physical activity level, and free testosterone and 25-hydroxy vitamin D levels.

In addition, limiting the analysis to virologically suppressed HIV-infected men (i.e., plasma HIV RNA <50 copies/mL), we performed multivariable analysis to determine the relationship between testosterone use and BMD T-score at the LS, TH, and FN, adjusting for the same factors as in the overall analysis, as well as for the HIV-specific factors listed above.

Multiple imputation was used to complete missing covariate data (9%) in multivariable linear models. Five imputation data sets were created using a multivariable normal model, including all variables in the linear regression. The final estimates of the association between BMD T-scores (at the LS, TH, and FN) and factors examined were obtained by averaging the estimates from the five imputation data sets. All analysis testing was two sided with a type I error of 5%; thus p-values of <.05 were considered statistically significant with no adjustment for multiple comparisons. All analyses were done using SAS 9.2 (SAS Institute, Cary, NC).

Ethics

The institutional review board at each site approved the study. Each participant provided written informed consent before entry into BOSS.

Results

Participant characteristics

Among 202 HIV-infected and 201 HIV-uninfected men, age, race, and BMI were similar between the two groups (Table 1). HIV-infected men were significantly more likely to report a history of injection drug use (16% vs. 7%), be HCV infected (10% vs. 3%), and have chronic kidney disease (30% vs. 9%). Otherwise there were no significant differences in demographic and clinical characteristics between the groups.

Among HIV-infected participants, the median current CD4 was 642 cells/μL, and 90% were virologically suppressed (HIV RNA <50 copies/mL). HIV-infected men had received ART for a median of 13 years with 5 years of TDF and 8 years of PI exposure. Eighty-seven percent of participants previously had received a thymidine analog.

Testosterone use and BMD in HIV-infected and -uninfected men

Compared to HIV-uninfected men, a significantly higher proportion of HIV-infected men in both the 50–59 and 60–69 years age categories reported testosterone use (17% vs. 3%, $p < .001$; 27% vs. 5%, $p < .001$, respectively).

Table 2 displays the median BMD T-scores at the LS, TH, and FN. The TH BMD T-score was significantly lower in

Table 1. Baseline Characteristics

Characteristic[a]	*HIV infected* (n = *202*)	*HIV uninfected* (n = *201*)
Age, years	60 (55–64)	60 (56–65)
Race, %		
White	69	79
Black	25	17
Other	5	4
MACS site, %		
Baltimore	25	25
Chicago	27	24
Pittsburgh	24	25
Los Angeles	24	25
Body mass index, kg/m^2	25 (23–28)	25 (23–29)
Alcohol use ≥14 drinks/week, %	8	8
Smoking, %		
Current	18	17
Former	53	56
Never	28	28
History of injection drug use, %	16	7
HBV infection, %	4	2
HCV infection	10	3
Diabetes, %	17	10
Kidney disease, %	30	9
Familial history of hip fracture, %	9	10
Physical activity, %		
Low	18	18
Moderate	29	32
High	52	50
Free testosterone level, % FT	2.6 (2.2–3.1)	2.7 (2.3–3.2)
25-OH vitamin D level, ng/mL	30.2 (23.0–37.5)	30.9 (23.5–39.6)
Current CD4 T cell count, cells/μL	642 (502–835)	
Nadir CD4 T cell count, cells/μL	272 (164–372)	
Plasma HIV RNA <50 copies/mL, %	90	
Cumulative years of combination antiretroviral therapy use	13 (9–15)	
Cumulative years of TDF use	5 (1–9)	
Cumulative years of PI use	8 (2–13)	
Ever used thymidine analog, %	87	

[a]Median (IQR) or %.

HBV, hepatitis B virus; HCV, hepatitis C virus; IQR, interquartile range; MACS, Multicenter AIDS Cohort Study; PI, protease inhibitor; TDF, tenofovir disoproxil fumarate.

HIV-infected compared to the HIV-uninfected men (0.0 vs. 0.3; $p = .045$). LS and FN BMD T-scores did not differ significantly by HIV serostatus.

Relationship between testosterone use and BMD T-score in HIV-infected and -uninfected men

In multivariate analysis, adjusting for age, race, MACS site, BMI, alcohol, smoking and history of injection drug use, HBV infection, HCV infection, diabetes, concomitant medications that affect BMD, history of parental hip fracture, physical activity level, and free testosterone and 25-hydroxy vitamin D levels, testosterone use was significantly associated with higher BMD T-score at the LS ($p = .003$) (Fig. 1). There was no significant association between testosterone use and BMD at the TH and FN ($p = .30$ and $p = .10$, respectively), although numerically mean BMD T-score was higher in testosterone users than in nonusers.

Relationship between testosterone use and BMD T-score in virologically suppressed HIV-infected men

In the HIV-infected men who were virologically suppressed at the BOSS visit ($n = 182$), adjusting for the

Table 2. Median (Interquartile Range) Bone Mineral Density T-Score by HIV Status

Site	*HIV infected*	*HIV uninfected*	p-*Value for difference*
Lumbar spine	−0.6 (−1.4 to 0.5)	−0.5 (−1.4 to 0.5)	.77
Total hip	0.0 (−0.7 to 0.8)	0.3 (−0.5 to 1.1)	.045
Femoral neck	−0.7 (−1.2 to 0.2)	−0.5 (−1.2 to 0.4)	.32

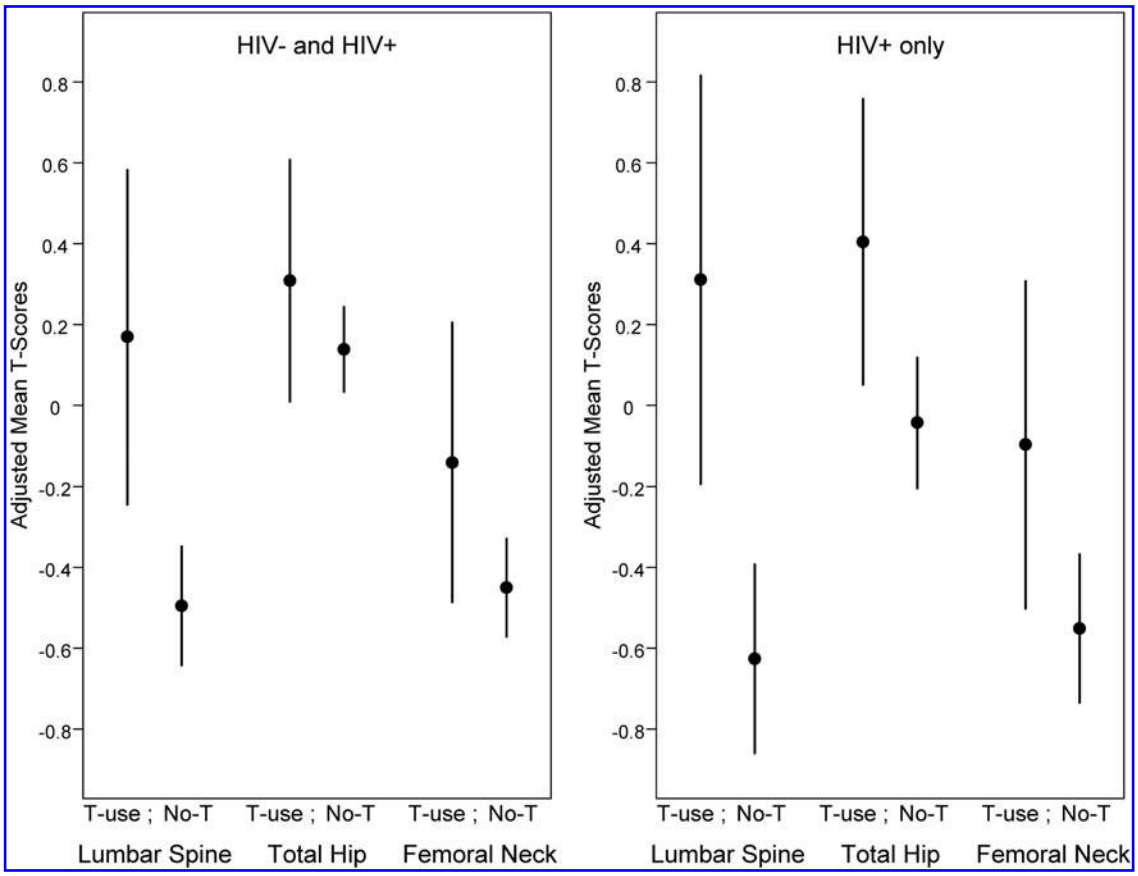

FIG. 1. Relationship between testosterone use and BMD T-score. Adjusted for age, race, MACS site, BMI, alcohol, smoking and history of injection drug use, HBV infection, HCV infection, diabetes, concomitant medications that affect BMD, history of parental hip fracture, physical activity level, and free testosterone and 25-hydroxy vitamin D levels current, and in virologically suppressed HIV-infected men also adjusted for current and nadir $CD4^+$ T cell count, current and percentage of MACS visits with suppressed HIV RNA level, duration of combination of antiretroviral therapy use, cumulative tenofovir disoproxil fumarate and protease inhibitor use, and ever thymidine analog use. BMD, bone mineral density; BMI, body mass index; HBV, hepatitis B virus; HCV, hepatitis C virus; MACS, Multicenter AIDS Cohort Study.

multivariate factors listed above, as well as for HIV-related factors, testosterone use was associated with higher BMD T-scores at all three sites examined (Fig. 1); this association was significant at the LS and TH ($p = .002$ and $p = .03$, respectively) and of borderline significance at the FN ($p = .06$). The point estimates for the effect size at the three sites ranged from 0.45 to 0.95.

Discussion

In this substudy of older men enrolled in MACS, we found that HIV-infected men had lower BMD at the TH but not at the LS or FN compared to HIV-uninfected men. A high proportion of HIV-infected men reported current testosterone use, over five times higher than HIV-uninfected MSM. In HIV-infected men with a suppressed viral load, we found testosterone use to be significantly associated with an increased BMD T-score at both the LS and TH. These findings suggest that testosterone replacement may preserve BMD in HIV-infected men.

Testosterone replacement is indicated for men with low AM serum testosterone levels and signs or symptoms consistent with hypogonadism, including low libido, low BMD, gynecomastia, and small testes. However, men with nonspecific symptoms such as fatigue, depression, and increased fat mass sometimes also are often prescribed testosterone. Given the overlap of many of the symptoms of hypogonadism with symptoms commonly seen among HIV-infected persons, the high frequency of testosterone usage among HIV-infected men in the present study (22% overall) is not surprising. To our knowledge, no comprehensive studies have evaluated the effects of testosterone replacement in HIV-infected individuals. In the general population, recent studies suggest a potentially increased risk of cardiovascular disease with testosterone use,[12,15] although the data are conflicting.[16] Given the high rates of testosterone use and the increased rates of cardiovascular disease in HIV-infected individuals, randomized trials that evaluate the relative risks and benefits of testosterone treatment in older HIV-infected men should be performed.

Lower BMD T-scores in HIV-infected men than in HIV-uninfected men have been reported elsewhere,[1,2,17] although it should be noted that we identified this association only at the TH, but not the LS or FN, and the observed

difference was not large. The HIV-uninfected men in this study were well matched to the HIV-infected study participants. Consistent with what has been reported previously in MSM populations,[18,19] the HIV-uninfected men in our study had lower BMD than the general population mean.

This is the first study to our knowledge that has evaluated the relationship between testosterone use and BMD in HIV-infected individuals. We found a strong association between testosterone use and BMD in HIV-infected individuals with point estimates on the effect on the BMD T-score ranging from 0.45 to 0.95. There has been a recent reevaluation of the risks and benefits of testosterone replacement in HIV-uninfected men,[12,20] with some experts viewing testosterone as overused and overmarketed.[21] Similar to our findings in HIV-infected men, in HIV-uninfected men testosterone supplementation was associated with increased BMD.[22] However, testosterone usage has not been shown to reduce fracture risk in HIV-uninfected men.

There are several limitations to our study. Given the observational nature of the study, we cannot determine causation between testosterone use and BMD increase. Unmeasured variables could have accounted for this association, although we did control for numerous potential confounders using the comprehensive data available on study participants in the MACS. We did not have access to pharmacy records and, therefore, relied on participant self-report of testosterone use. Given the multiple comparisons, marginally significant associations should be interpreted cautiously. Measured testosterone levels were not associated with BMD in multivariate models. Additional studies should be performed to determine the relationship between testosterone use and fracture risk.

In conclusion, testosterone usage was high in this cohort of older HIV-infected men and was associated with increased BMD. Given the frequency of testosterone's use in this population and its diverse biologic effects, more extensive examinations of the risks and benefits of testosterone supplementation in HIV-infected individuals should be performed.

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Author Disclosure Statement

No competing financial interests exist.

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