Alcohol and Bone Turnover Markers among People Living with HIV and Substance Use Disorder

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Background: Although unhealthy alcohol use and low bone density are prevalent among people living with HIV (PLWH), it is not clear whether alcohol use is associated with bone turnover markers (BTMs), and if so, at what quantity and frequency. The study objective was to examine the association between alcohol and BTMs in PLWH with substance use disorder.

Methods: We studied a prospective cohort recruited from 2 HIV clinics who met criteria for DSM-IV substance dependence or reported ever injection drug use. Outcomes were BTM of (i) bone formation (serum procollagen type 1 N-terminal propeptide [P1NP]) and (ii) bone resorption (serum C-telopeptide type 1 collagen [CTx]). Alcohol consumption measures included (i) mean number of drinks/d (Timeline Follow-Back [TLFB]) (primary predictor), (ii) any alcohol use on ≥20 of the past 30 days, and phosphatidylethanol (PEth), a biomarker of recent alcohol consumption. Linear regression analysis examined associations between (i) each alcohol measure and each BTM and (ii) change in alcohol and change in BTM over 12 months.

Results: Among 198 participants, baseline characteristics were as follows: The median age was 50 years; 38% were female; 93% were prescribed antiretroviral medications; 13% had ≥20 drinking days/month; mean drinks/day was 1.93 (SD 3.89); change in mean drinks/day was −0.42 (SD 4.18); mean P1NP was 73.1 ng/ml (SD 34.5); and mean CTx was 0.36 ng/ml (SD 0.34). Higher drinks/day was significantly associated with lower P1NP (slope −1.09 ng/ml; 95% confidence interval [CI] −1.94, −0.23, per each additional drink). On average, those who drank on ≥ 20 days/month had lower P1NP (−15.45 ng/ml; 95% CI: −26.23, −4.67) than those who did not. Similarly, PEth level ≥ 8ng/ml was associated with lower P1NP. An increase in drinks/d was associated with a decrease in P1NP nonsignificantly (−1.14; 95% CI: −2.40, +0.12; $p = 0.08$, per each additional drink). No significant associations were detected between either alcohol measure and CTx.

Conclusions: In this sample of PLWH with substance use disorder, greater alcohol consumption was associated with lower serum levels of bone formation markers.

Key Words: HIV, Bone Turnover Markers, Alcohol, Substance Use Disorder.

THE IMPACT OF HIV infection (including antiretroviral medications [ARV]) on bone health is well established (Cotter et al., 2014; Escota et al., 2016; Yin et al., 2012). Low bone density is common among people living with HIV (PLWH); estimates span 22% to 67% even among those with virologic suppression (Brown et al., 2009; Carr et al., 2015; Escota et al., 2016). Fracture risk is increased for PLWH (Collin et al., 2009; Sharma et al., 2015; Womack et al., 2011; Yin et al., 2010b), in part, due to lower bone density and a higher risk of falls (Erlandson et al., 2016). Fracture can lead to impaired mobility and frailty (Wolinsky et al., 1997). Although not completely understood, the mechanism by which HIV impacts bone health involves dysregulated immune activation and chronic inflammation

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(Erlandson et al., 2014; Ofotokun et al., 2012; Titanji et al., 2014). Independent risk factors for low bone density—separate from the impact of the HIV itself—include tobacco use, low body mass index (BMI), hypogonadism, and liver disease, all of which are also associated with HIV (Bedimo et al., 2016; Carr et al., 2015; Ofotokun et al., 2016).

Another potentially significant factor for low bone density is alcohol use, which is particularly important because of the high prevalence of alcohol use in PLWH (Galvan et al., 2002; Williams et al., 2014) and its impact on HIV viral suppression, disease progression, and mortality (Deiss et al., 2016; Justice et al., 2016; Marshall et al., 2017; Williams et al., 2018). The metabolic effects of the systemic inflammatory reaction and oxidative stress induced by alcohol are widespread and include muscle wasting and lipoatrophy (Bagby et al., 2015; Gaddini et al., 2016; Molina et al., 2018). Although heavy alcohol consumption is associated with fracture (Kanis et al., 2005; Zhang et al., 2015), the effects of alcohol on bone mineral density (Gaddini et al., 2016) are unclear, particularly in PLWH (Brown et al., 2009; Saitz et al., 2018; Sharma et al., 2010; Ventura et al., 2017).

Bone is metabolically active throughout the life span—continually undergoing repair and remodeling. Under normal conditions, bone resorption (by osteoclasts) and bone formation (by osteoblasts) are synchronized to maintain adequate bone mass and strength; this process is referred to as bone remodeling. When bone resorption outpaces bone formation —as appears to be the case with HIV infection—the result is a decline in bone mass (Bedimo et al., 2016; Ofotokun et al., 2016; Titanji et al., 2018). In PLWH, the presence of proinflammatory cytokines, which include tumor necrosis factor-alpha and IL-6, stimulates bone resorption resulting in bone loss even in those with HIV viral suppression and high CD4+ cell counts (Bagby et al., 2015; Titanji et al., 2018; Yin et al., 2010a). Expression of the proresorptive cytokine RANKL (receptor activator of nuclear factor kappa-B ligand) is also increased in PLWH (Titanji et al., 2014). The degree to which alcohol augments these processes is not well understood (Molina et al., 2014; Williams et al., 2016).

Change in bone mineral density assessed by dual-energy X-ray bone densitometry (DXA) is typically small and takes years to detect. Serum bone turnover markers (BTMs), on the other hand, reflect short-term changes in bone metabolism and are associated with bone density changes in PLWH (Haskelberg et al., 2012; Yin et al., 2010a). In addition to heralding bone density changes, BTMs provide information about mechanisms of bone loss (i.e., bone formation and/or resorption). Abnormal levels of BTMs, independent of bone mineral density, are associated with higher fracture risk (Haskelberg et al., 2011; Vasikaran et al., 2011). Given these associations, BTMs are increasingly used to compare the effect of ARV medications on bone metabolism and to monitor the effectiveness of medications for osteoporosis (Bedimo et al., 2016; Haskelberg et al., 2012; Yin et al., 2019).

Despite the potential value of examining the impact of risk factors on BTMs and the association with osteoporosis in PLWH, what is known about alcohol consumption and BTMs is limited and has largely been studied in HIV-uninfected samples. While improvements in bone formation after cessation of alcohol consumption have been described in small studies of HIV-uninfected populations with alcohol use disorders (Laitinen et al., 1992; Malik et al., 2012; Nyquist et al., 1996), the effect of "moderate" amounts of alcohol in those without alcohol use disorders is less clear (Gaddini et al., 2016). It is not known whether these studies of alcohol and BTMs apply to PLWH. The amount of alcohol consumption associated with mortality for PLWH is lower than in uninfected adults (Justice et al., 2016). Similarly, the amount of alcohol consumption that impacts bone metabolism may also differ between HIV-infected and HIV-uninfected populations.

There are few studies of alcohol consumption and BTMs in which the quantity of alcohol is sufficiently specified using well-validated assessments in PLWH. One such study by Watt et al used several methods to quantify alcohol exposure including AUDIT score (Babor et al., 2001) and an alcohol biomarker (phosphatidylethanol or PEth) to examine the association between alcohol and BTMs in a subsample of participants with HIV infection (Watt et al., 2019). Many other studies dichotomize alcohol use comparing no use to any use. Assessment of BTM data across a range of alcohol consumption is necessary to adequately evaluate bone effects. Absence of these data makes it difficult to provide clinically relevant recommendations about alcohol use and bone health. Studies are also needed to systematically assess the use of other substances such as opioids that may affect bone metabolism given the frequency of polysubstance use in PLWH (Grey et al., 2011; Kim et al., 2006). If a relationship between alcohol and BTMs is found, measurement of BTMs could potentially be used to motivate reductions in alcohol use to mitigate risks related to abnormal bone metabolism.

The study objective was to determine the association of alcohol consumption with markers of bone formation and resorption in a sample of PLWH with substance use disorder.

MATERIALS AND METHODS

Study Design

We used data from the Boston ARCH Cohort study, a longitudinal study of HIV-infected adults with past-year substance dependence (DSM-4 criteria as assessed by the Mini-International Neuropsychiatric Interview Version 6.0 [MINI]) (Sheehan et al., 2010) or a lifetime history of any injection drug use. Ascertainment and recruitment methods have been previously published (Saitz et al., 2018). Briefly, eligible study participants were aged 18 or older, able to speak English, and willing to provide contact information for at least one other person to assist with follow-up. Exclusion criteria included pregnancy at time of enrollment, plans to leave the Boston area in the next year, and cognitive impairment such that the patient could not provide informed consent. Previous analyses examined the effect of alcohol on bone density in this cohort. Specific to the current study, the study sample consisted of Boston ARCH participants with serum collected at both baseline and 12-month follow-up study visits. Those with a past-year history of

fracture were excluded because BTMs remain elevated up to a year postfracture (Haskelberg et al., 2011).

Trained research associates administered standardized in-person interviews at study entry and a 12-month follow-up. Review of the electronic health record (EHR) was conducted to ascertain prescribed medications. Serum was collected at baseline and 12 months. Blood was tested for PEth at baseline visits only.

The Boston University Medical Campus Institutional Review Board approved the study. Participants provided written informed consent and received compensation for each study assessment completed. The National Institute on Alcohol Abuse and Alcoholism further protected participants with a Certificate of Confidentiality, and the U.S. Department of Health and Human Services approved follow-up assessments with participants who were incarcerated.

Measurements

Outcomes. The following 2 BTMs were examined: (i) serum procollagen type I N-terminal propeptide (P1NP), a marker of bone formation; and (ii) serum C-telopeptide of type 1 collagen (CTx), a marker of bone resorption. The International Osteoporosis Foundation, the International Federation of Clinical Chemistry and Laboratory Medicine, and others recommend using P1NP and CTx as BTMs in clinical studies (Szulc et al., 2017; Vasikaran et al., 2011). Serum P1NP and CTx were measured using IDS-iSYS CTX-1 (Crosslaps©) and IDS-iSYS Intact P1NP, respectively. Both assays were run sequentially for each participant using the ImmunoDiagnostic Systems IDS-iSYS multidiscipline platform.

Main Independent Variables and Covariates. We examined 3 self-report measures of alcohol consumption collected at baseline and 12-month follow-up. All alcohol measures were assessed using the TLFB method (Sobell and Sobell, 1992) and refer to alcohol use in the past 30 days: (i) mean number of drinks per day (primary predictor), (ii) any alcohol use on ≥ 20 or more days, and (iii) number of heavy drinking days, defined as ≥ 5 drinks in a day for men and ≥ 4 drinks in a day for women (National Institute on Alcohol Abuse and Alcoholism [NIAAA]).

We also examined alcohol exposure using PEth, a highly specific biomarker that correlates with alcohol consumption in the previous 2 to 3 weeks (Wurst et al., 2015). It was modeled as a binary measure, ≥8 ng/ml vs. <8, which is the limit of quantification for this specific test.

Covariates included the following: age, biological sex, race/ethnicity, BMI (underweight vs ideal/overweight/obese), CD4 cell count (<200 cells/μl), HIV viral load suppression (<200 copies), absence of menses for more than a year, serum vitamin D insufficiency (25-hydroxyvitamin D < 30 ng/ml), current opioid medication prescription (EHR review), current ARV known to affect bone (tenofovir [Bedimo et al., 2016] and/or a protease inhibitor [Moran et al., 2016]) (EHR review), prescribed medication associated with increased bone density, medication associated with decreased bone density (Saitz et al., 2018), current tobacco use, any past–30-day illicit opioid use (Addiction Severity Index [ASI]) (McLellan et al., 1992), any past–30-day cocaine use (ASI), and lifetime years of heavy drinking assessed by Lifetime Drinking History (NIAAA) (Skinner and Sheu, 1982).

Statistical Analysis

Given lack of consistent data on the threshold of alcohol consumption that impacts fracture risk, we first visually inspected plots of the association between drinks/d and each BTM noting the shape of any apparent relationship and threshold effect; no obvious threshold effects were identified.

We then used separate unadjusted linear regression models to examine cross-sectional associations between each alcohol measure and each BTM. We analyzed CTx as log marker value to account for the skewedness of the measure. Cross-sectional analyses used random-effects linear regression models on the pooled baseline and 12-month data for to examine associations between each alcohol measure and each BTM. To account for potential confounders, a series of adjusted models estimating BTM were built by entering core covariates (age, sex, and race/ethnicity) and one other covariate ("partially adjusted model"). For confirmation, we then examined one "fully adjusted model" with all covariates. As a general guide, we excluded covariates that were highly correlated (correlation coefficients r> 0.40) with other covariates. Lifetime years of heavy drinking and past-month heavy drinking days were the only highly correlated variables ($r = 0.43$). We decided to keep lifetime years of heavy drinking in all models because of its potential importance as a confounder and because it exceeded the arbitrary threshold minimally.

As confirmatory analyses, we performed similar cross-sectional analyses using PEth level (measured at baseline) to assess the association of recent alcohol exposure with each BTM.

We then examined the prospective association between a *change* in alcohol consumption and *change* in BTM over a 12-month period with separate linear regression models for each alcohol measure and each BTM. Changes in the continuous alcohol measures, mean drinks per day and number of heavy drinking days, were calculated by subtracting the amount of alcohol assessed at baseline from the amount assessed at the 12-month follow-up. Change in the alcohol measure "any alcohol use on ≥ 20 days in the past month" was modeled as a 4-level variable: drinking ≥ 20 days at (i) baseline and 12 months, (ii) baseline only, (iii) 12 months only, and (iv) neither baseline nor 12 months.

We again examined a series of adjusted models estimating the change in BTM. Models included the change in alcohol consumption, core covariates (age, sex, and race/ethnicity), and one covariate of interest ("partially adjusted model"). The same covariates included in the cross-sectional analyses were also included in the prospective models except that the following covariates were time-varying, indicating change from baseline to the 12-month follow-up: CD4 status, HIV viral load suppression, vitamin D insufficiency, prescribed opioid medication, prescribed bone-impacting medication, illicit opioid use, and cocaine use. All were modeled as 4-level variables. For example, HIV viral load suppression was modeled as HIV viral load suppressed at (i) baseline and 12 months, (ii) baseline only, (iii) 12 months only, and (iv) neither baseline nor 12 months. Similarly, vitamin D insufficiency was modeled as vitamin D insufficiency at (i) baseline and 12 months, (ii) baseline only, (iii) 12 months only, and (iv) neither baseline nor 12 months. We then examined one "fully adjusted model" with all covariates. We did not include a covariate for the baseline alcohol measure in the model of the corresponding change in alcohol use (e.g., baseline drinks/d was not included in the analysis of change in drinks/day from baseline to 12 months) because the correlation between the baseline alcohol measure and the change in alcohol use was high (correlation coefficients of 0.38 to 0.82).

We tested for potential interactions between each alcohol measure, sex, and HIV viral load suppression (separately) in the fully adjusted models. Finally, we removed vitamin D insufficiency, a covariate, from the fully adjusted models to determine if it was a mediator of the association between alcohol and bone marker.

RESULTS

Study Participants

The primary study had 250 participants, 233 of whom had baseline and 12-month follow-up data. Of the 233, 31 were excluded due to past-year fracture (14 at baseline and 15 at

12-month follow-up) and 4 were excluded due to missing data on bone turnover markers, resulting in a sample of 198 participants for this study.

Among the 198 participants (Table 1), baseline characteristics were as follows: The median age was 50 years (interquartile range [IQR] 44, 56); about a third (38%) were female; most were prescribed ARV (93%); and almost 3-quarters (72%) had HIV viral load suppression. Vitamin D insufficiency was common (62%). About half met criteria for both current alcohol and drug dependence (48%). Few (11%) participants had alcohol dependence alone.

Table 1. Baseline Characteristics of Participants With HIV Infection and Substance Dependence and/or a Lifetime History of Injection Drug Use (n = 198)[a]

Characteristic	% (n)
Age, median (IQR)	50 years (44, 56)
Female	38% (75)
Race/ethnicity	
Hispanic	25% (49)
Black, non-Hispanic	52% (103)
White, non-Hispanic	19% (37)
Other	4% (9)
CD4 count <200	10% (20)
Body mass index (kg/m^2)	
<20 (underweight)	10% (19)
20-30 (ideal and overweight)	66% (130)
>30 (obese)	25% (49)
HIV viral load <200 copies	72% (142)
Prescribed antiretroviral medications	93% (184)
Antiretroviral regimen includes tenofovir	77% (152)
Prescribed an opioid medication[b]	48% (95)
Hepatitis C infection (ever)	59% (116)
Vitamin D insufficiency[c]	62% (122)
Current tobacco	76% (150)
Ever injected drugs	57% (113)
DSM-IV substance dependence[d], past year	
Both alcohol and drug dependence	48% (96)
Drug dependence only	22% (44)
Alcohol dependence only	11% (21)
No dependence[e]	19% (37)
Past-month drug use, past 30 days [f]	
Any illicit opioid use[g]	19% (38)
Any illicit sedative use	8% (15)
Any cocaine use	29% (57)
P1NP, ng/ml, mean (standard deviation)[h]	
Baseline	73.1 (34.5)
Follow-up[i]	70.5 (37.6)
CTx, ng/ml, mean (standard deviation)[j]	
Baseline	0.36 (0.34)
Follow-up[i]	0.42 (0.57)

[a]The primary study had 233 participants with baseline and 12-month follow-up data. Of these, 31 were excluded due to past-year fracture (14 at baseline and 15 at follow-up) and 4 were excluded due to missing data on bone turnover markers.
[b]Includes opioid medications prescribed for pain, buprenorphine, and methadone.
[c]Serum 25-hydroxyvitamin D < 30 ng/ml.
[d]Mini-International Neuropsychiatric Interview (MINI) 6.0 DSM-IV criteria.
[e]Patients with no substance dependence in the past year were eligible for the study if s/he had a lifetime history of injection drug use.
[f]Addiction Severity Index.
[g]Includes use of medications without a prescription or more than prescribed.
[h]Serum procollagen type I N-terminal propeptide, a biomarker of bone formation.
[i]At 12 months.
[j]Serum C-terminal telopeptide of type I collagen, a biomarker of bone resorption.

Alcohol consumption is summarized in Table 2. The mean number of drinks/day at baseline was 1.93 (standard deviation [SD] 3.89). Participants drank, on average, 0.42 fewer drinks/day (SD 4.18) at the 12-month follow-up compared to baseline. A minority (13%) of the study participants had any alcohol on ≥20 days in the past month. The mean P1NP was 73.1 ng/ml (SD 34.5), and the mean CTx was 0.36 ng/ml (SD 0.34).

Main Findings

Bone Formation. Table 3 presents the results of the cross-sectional analyses. Higher average number of drinks/days was significantly associated with lower bone formation (P1NP slope −1.09 ng/ml; 95% confidence interval [CI]: −0.23, −1.94 per each additional drink) (Fig. 1). Those who drank on ≥ 20 days in the past month had lower bone formation (P1NP −15.45 ng/ml; 95% CI: −26.23, −4.67) than those who did not. Similarly, more heavy drinking days was associated with lower bone formation (slope −0.58; 95% CI −1.05, −0.12 per heavy drinking day). A PEth level ≥ 8 indicating recent alcohol consumption was associated with lower bone formation (P1NP −10.33 ng/ml; 95% CI: −20.07, −0.59).

Associations between drinks/d and alcohol use on ≥ 20 days in a month and P1NP remained significant in the "partially adjusted" models (in which each model included core covariates and one other covariate) and fully adjusted models. The association between the number of heavy drinking days and P1NP remained significant in all of the partially adjusted models but was no longer significant in the fully adjusted model (i.e., all covariates in one model).

The results of prospective analyses are presented in Table 4. An increase in mean drinks/day over 12 months was associated with a nonsignificant decrease in bone formation (P1NP −1.14; 95% CI: −2.40, +0.12; p = 0.08, per each additional drink) (Fig. 2). Neither change in the number of heavy drinking days nor change in whether a participant had alcohol use on ≥20 days in a month was significantly associated with bone formation. The adjusted models yielded similar results.

We tested interactions between each alcohol measure and sex and HIV viral load suppression (separately) using the

Table 2. Alcohol Consumption Measures at Baseline and 12 Months

Alcohol measure	Baseline	12 months
Drinks/day, mean (STD)[a]	1.93 (3.89)	1.51 (4.52)
Any alcohol use on 20+ days in the past 30 days	26 (13%)	21 (11%)
Number heavy drinking days, mean (STD)[a]	4.65 (8.13)	3.25 (7.45)

[a]In the past 30 days.
[b]NIAAA criteria for heavy alcohol use, ≥5 drinks in a day for men and ≥4 drinks in a day for women.

Table 3. Results of Separate Unadjusted and Adjusted Regression Models Examining the Cross-Sectional Association Between Alcohol Consumption and Bone Turnover Markers[a]

Higher alcohol consumption	P1NP[d] Beta (95% CI)	*p*-Value	Log CTx Beta (95% CI)	*p*-value
Average drinks/day (STD)[b]	−1.09 (−1.94, −0.23)	0.01	−0.01 (−0.03, 0.01)	0.22
Fully adjusted	−1.01 (−1.94, −0.07)	0.04	−0.01 (−0.04, 0.01)	0.25
Any alcohol use on 20+ days	−15.45 (−26.23, −4.67)	0.005	−0.19 (−0.45, 0.06)	0.14
Fully adjusted	−11.93 (−27.79, −3.69)	0.04	−0.17 (−0.42, 0.09)	0.20
Number heavy drinking days[c]	−0.58 (−1.05, −0.12)	0.01	−0.005 (−0.02, 0.01)	0.41
Fully adjusted	−0.43 (−0.94, 0.06)	0.11	−0.002 (−0.01, 0.01)	0.68

[a]Results of separate longitudinal regression models for each alcohol measure and bone formation (P1NP) as well as each alcohol measure and bone resorption (CTx). Unadjusted results are presented first, followed by the fully adjusted results in the next row. Covariates included age, biological sex, race/ethnicity, BMI (underweight vs ideal/overweight/obese), CD4 cell count (<200 cells/μL), HIV viral load suppression (<200 copies), absence of menses for more than a year, serum vitamin D insufficiency (25-hydroxyvitamin $D < 30$ ng/ml), opioid medication prescription (EHR review), ARV (tenofovir and/or a protease inhibitor (EHR review)), prescribed medication associated with increased bone density, medication associated with decreased bone density, current tobacco use, past–30-day illicit opioid use (Addiction Severity Index (ASI), past–30-day cocaine use (ASI), and lifetime years of heavy drinking assessed by Lifetime Drinking History).

[b]Per each additional drink.

[c]Per each additional day.

[d]Partially adjusted models yielded similar results for all alcohol measures. The results of fully adjusted models for drinks/day and alcohol use on 20 or more days of month were no different. The association between number of heavy drink days and P1NP was no longer significant in the fully adjusted model.

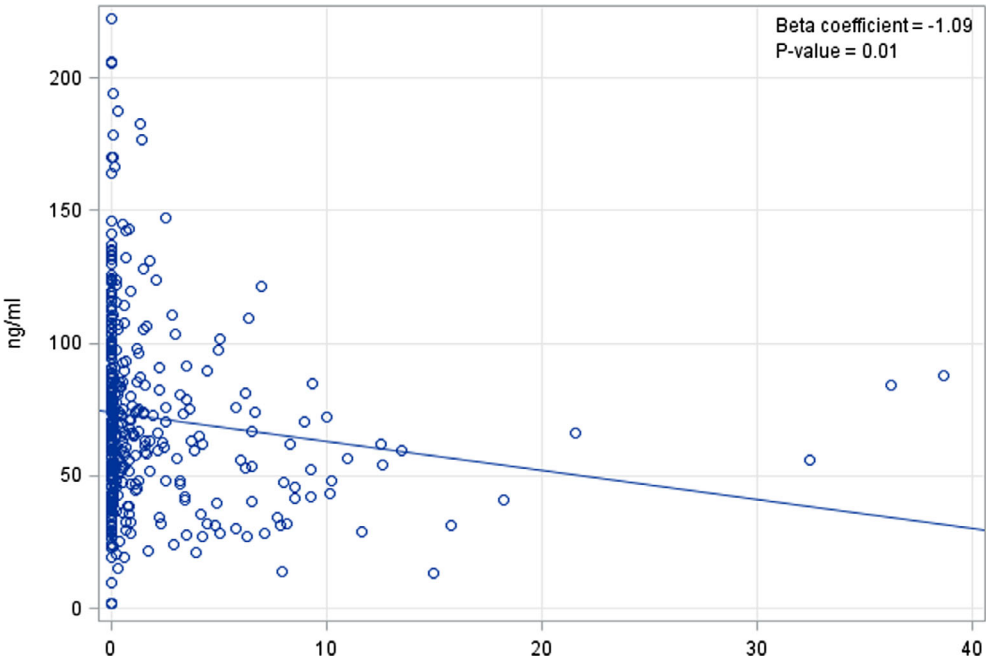

Fig. 1. Average drinks per day in past 30 days and P1NP.

fully adjusted models. None were significant. The results of the fully adjusted models were no different with and without vitamin D status.

Bone Resorption. There were no significant cross-sectional associations between any of the alcohol consumption measures (including PEth) nor change in alcohol consumption and bone resorption (CTx) (Tables 3 and 4). The results of adjusted analyses were similar. None of the interactions tested in the fully adjusted models were significant. As with the bone formation analyses, the results of the fully adjusted models were no different with and without vitamin D status.

DISCUSSION

We examined whether alcohol consumption was associated with bone formation and/or bone resorption serum

Table 4. Results of Separate Unadjusted and Adjusted Regression Models Examining the Prospective Association Between Change in Alcohol Consumption and Bone Turnover Markers[a]

Change in alcohol consumption	Change in P1NP[d] Beta (95% CI)	Change log CTx[d] Beta (95% CI)
Increased average drinks/day[b]	−1.14 (−2.40, 0.12)	−0.01 (−0.03, 0.02)
Fully adjusted	−1.24 (−2.65, 0.16)	−0.001 (−0.03, 0.03)
Any alcohol use on 20+ days		
Yes at baseline, Yes at follow-up	−0.15 (−23.35, 23.06)	−0.47 (−0.97, 0.03)
Fully adjusted	1.80 (−24.78, 28.38)	−0.44 (−0.99, 0.11)
Yes at baseline, No at follow-up	11.48 (−8.62, 31.58)	0.09 (−0.35, 0.52)
Fully adjusted	17.37 (−3.07, 37.80)	0.14 (−0.28, 0.56)
No at baseline, Yes at follow-up	−3.29 (−27.55, 20.98)	0.12 (−0.40, 0.64)
Fully adjusted	−1.13 (−30.55, 28.30)	0.30 (−0.31, 0.91)
No at baseline, No at follow-up	1	1
Increased number heavy drinking days[c]	−0.47 (−1.16, 0.23)	0.01 (−0.01, 0.02)
Fully adjusted	−0.71 (−1.48, 0.07)	0.01 (−0.01, 0.02)

[a]The table presents the results of separate longitudinal regression models for change in each alcohol measure and change in bone formation (P1NP) and in separate models, change in bone resorption (CTx) over 12 months. All alcohol measures reference the previous 30 days. The parameter and 95% confidence intervals from the unadjusted model are presented first, followed by the results from the fully adjusted regression model in the next row. Covariates included age, biological sex, race/ethnicity, BMI (underweight vs ideal/overweight/obese), absence of menses for more than a year, ARV (tenofovir and/or a protease inhibitor), current tobacco use, and lifetime years of heavy drinking assessed by Lifetime Drinking History. The following covariates were time-varying: CD4 status, HIV viral load suppression, serum vitamin D insufficiency, prescribed opioid medication, prescribed bone-impacting medication, illicit opioid use, and cocaine use.

[b]Per each additional drink.

[c]Per each additional day.

[d]Results of fully adjusted models were similar. HIV viral suppression was the only covariate significantly associated with P1NP in the adjusted models. Covariates significantly associated with CTx were baseline ARV, HIV viral load suppression, and serum vitamin D insufficiency.

markers using 3 measures of alcohol quantity and frequency in a sample of PLWH with substance use disorder or a lifetime history of injection drug use. Overall, we found that greater alcohol consumption was associated with lower levels of P1NP—a marker of bone formation. This was the case despite adjustment for a broad array of clinical and substance use indicators and regardless of the measure of alcohol consumption: drinks/day, any alcohol use on ≥ 20 days of the past month, number of heavy drinking days, or PEth level, a biological measure of recent alcohol use. Based upon plots of the association between drinks/day and each BTM, we did not find any threshold effects for the quantity of alcohol consumption and bone formation. In other words, the risk was linear starting with no alcohol use. Vitamin D insufficiency was not a mediator in these associations. There were no significant associations between alcohol consumption and bone resorption.

This is one of the few studies that have systematically assessed alcohol and other substances and BTMs among PLWH. This study's findings are consistent with Watt et al (2019) who found a cross-sectional association between several self-report alcohol measures, as well as PEth levels, and depressed osteocalcin, another biomarker of bone formation among 40 PLWH. They also did not find a significant association between alcohol and bone resorption using the same serum biomarker, CTx. The current study extends their findings with its relatively large sample size, prospective study design, and adjustment for exposure to other substances.

The current study results are also are in line with previous studies using the same cohort on the impact of alcohol on bone density which found that alcohol consumption was associated with lower bone density at baseline (Ventura et al., 2017). However, a *change* in alcohol consumption was not prospectively associated with *change* in bone density (Saitz et al., 2018). Previous studies in uninfected populations with alcohol use disorders have also found a negative association between any alcohol consumption and bone formation (Laitinen et al., 1992; Malik et al., 2012; Nyquist et al., 1996). We did not find a positive effect of "moderate" alcohol intake (up to 2 drinks a day) on BTMs which was demonstrated in a study of uninfected women *without* alcohol use disorders (Marrone et al., 2012). It is possible that our study findings are due to the fact that most of the sample consisted of adults with at least one substance use disorder, many of whom not only used alcohol in varying amounts but other substances as well. It is also possible that there are no positive effects of alcohol on bone formation for PLWH regardless of substance use history.

Contrary to expectations, we did not find an association between change in alcohol consumption and either BTM. Relatively narrow changes in alcohol consumption in the study sample may have limited detection of an association. Also it is possible that the interval between BTMs (12 months) was too narrow especially if increases in alcohol consumption induced an overall low rate of bone turnover. If this were the case, then bone resorption may end up outpacing bone formation but take more than a year to detect, even with BTMs (Gaddini et al., 2016). We also did not find significant interactions between any of the alcohol measures and sex or HIV viral suppression.

The study findings should be interpreted with the following limitations. We used measures of alcohol use that referenced the 30 days before each study interview (i.e., baseline and 12-month follow-up). A measure of alcohol use every day throughout the interval year may have been a more accurate measure of alcohol exposure. Additionally, alcohol consumption over a longer period of time prior to study entry may have had an impact on the relationship between recent alcohol and BTMs, although a previous analysis in this cohort did not find that lifetime alcohol consumption was associated with bone density (Ventura et al., 2017). We also adjusted for lifetime alcohol use in the cross-sectional analyses. Second, we did not have information about nadir CD4 count although the START Bone Mineral Density study did not find that nadir CD4 count was associated with bone mineral density (Carr et al., 2015). We accounted for use of

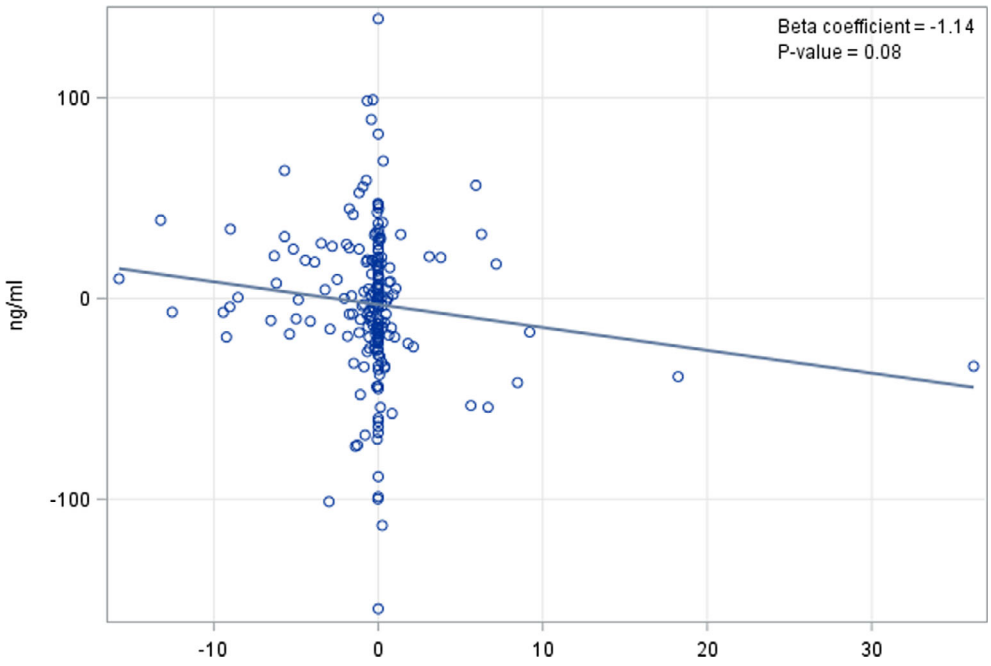

Fig. 2. Change in average drinks per day in past 30 days and change in P1NP.

bone-impacting ARVs (i.e., tenofovir and protease inhibitors) but did not take into account past use of older ARV medications known to affect bone, for example, stavudine and zidovudine, although BTMs reflect recent bone turnover. Next, we did not have data on other biomarkers used to estimate bone formation such as osteocalcin, although P1NP is used in many HIV studies and has been recommended for use in clinical studies (Szulc et al., 2017; Vasikaran et al., 2011). Without a HIV-uninfected comparator group, we cannot make conclusions about whether these findings are unique to PLWH. Given that 93% of the sample was prescribed ARVs, we also could not comment on the impact of initiation of ARV on the findings. These results may not generalize to PLWH without a substance use disorder or history of injection drug use.

Lastly, the clinical relevance of these findings is unclear. Although current guidelines do not include measurement of BTMs to assess need for treatment of osteoporosis (Bauer, 2019), BTMs have been used to inform treatment strategy and assess conditions that affect bone metabolism (Jain and Camacho, 2018). The negative association between alcohol and bone formation would be of interest to PLWH concerned about modifiable risk factors for poor bone health. The study findings also indicate that studies of bone health need to account for the quantity of alcohol use beyond the absence or presence of use.

In this study of PLWH with a substance use disorder or a lifetime history of injection drug use, greater alcohol consumption was associated with lower serum levels of bone formation markers. We did not observe a range of alcohol consumption ("moderate alcohol use") associated with higher levels of bone formation as other studies have observed. Low vitamin D was not a mediator in these findings. The effect of alcohol on bone formation is particularly important in the setting of accelerated bone resorption that occurs with HIV infection and aging and higher risk of fracture and functional decline (Dong et al., 2014; Erlandson et al., 2016). Information about low BTMs may provide motivation for PLWH to reduce alcohol use and mitigate risks related to abnormal bone metabolism.

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CONFLICT OF INTEREST

The authors report that Richard Saitz is the Principal Investigator of an NIH/NIAAA-supported (to Boston University [BU]) study of people with alcohol use disorder; BU receives injectable naltrexone for that study from Alkermes. The remaining authors have no conflicts of interest.

REFERENCES

Babor TF, Higgins-Biddle JC, Saunders JB, Monteiro MG (2001) The Alcohol Use Disorders Identification Test: Guidelines for Use in Primary Health Care, 2nd ed. World Health Organization, Geneva.

Bagby GJ, Amedee AM, Siggins RW, Molina PE, Nelson S, Veazey RS (2015) Alcohol and HIV effects on the immune system. Alcohol Res Curr Rev 37:287–297.

Bauer DC (2019) Clinical use of bone turnover markers. JAMA 332:569–570.

Bedimo R, Cutrell J, Zhang S, Drechsler H, Gao A, Brown G, Farukhi I, Castanon R, Tebas P, Maalouf NM (2016) Mechanisms of bone disease in HIV and hepatitis C virus: impact of bone turnover, tenofovir exposure, sex steroids and severity of liver disease. AIDS 30:601–608.

Brown TT, McComsey GA, King MS, Qaqish RB, Bernstein BM, da Silva BA (2009) Loss of bone mineral density after antiretroviral therapy initiation, independent of antiretroviral regimen. J Acquir Immune Defic Syndr 51:554–561.

Carr A, Grund B, Neuhaus J, Schwartz A, Bernardino Ji, White D, Badel-Faesen S, Avihingsanon A, Ensrud K, Hoy J, International Network for Strategic Initiatives in Global HIV Trials (INSIGHT) START Study Group (2015) Prevalence of and risk factors for low bone mineral density in untreated HIV infection: a substudy of the INSIGHT Strategic Timing of AntiRetroviral Treatment (START) trial. HIV Med 16(Suppl 1):137–146.

Collin F, Duval X, Le Moing V, Piroth L, Al Kaied F, Massip P, Villes V, Chêne G, Raffi F, ANRS CO8 APROCO-COPILOTE Study Group (2009) Ten-year incidence and risk factors of bone fractures in a cohort of treated HIV1-infected adults. AIDS 23:1021–1024.

Cotter AG, Sabin CA, Simelane S, Macken A, Kavanagh E, Brady JJ, McCarthy G, Compston J, Mallon PWG, HIV UPBEAT Study Group (2014) Relative contribution of HIV infection, demographics and body mass index to bone mineral density. AIDS 28:2051–2060.

Deiss RG, Mesner O, Agan BK, Ganesan A, Okulicz JF, Bavaro M, Lalani T, O'Bryan TA, Bebu I, Macalino GE (2016) Characterizing the association between alcohol and HIV virologic failure in a military cohort on antiretroviral therapy. Alcohol Clin Exp Res 40:529–535.

Dong HV, Cortés YI, Shiau S, Yin MT (2014) Osteoporosis and fractures in HIV/hepatitis C virus coinfection: a systematic review and meta-analysis. AIDS 28:2119–2131.

Erlandson KM, O'Riordan MA, Labbato D, McComsey GA (2014) Relationships between inflammation, immune activation, and bone health among HIV-infected adults on stable antiretroviral therapy. J Acquir Immune Defic Syndr 65:290–298.

Erlandson KM, Plankey MW, Springer G, Cohen HS, Cox C, Hoffman HJ, Yin MT, Brown TT (2016) Fall frequency and associated factors among men and women with or at risk for HIV infection. HIV Med 17:740–748.

Escota GV, Mondy K, Bush T, Conley L, Brooks JT, Önen N, Patel P, Kojic EM, Henry K, Hammer J, Wood KC, Lichtenstein KA, Overton ET (2016) High prevalence of low bone mineral density and substantial bone loss over 4 years among HIV-infected persons in the era of modern antiretroviral therapy. AIDS Res Hum Retroviruses 32:59–67.

Gaddini GW, Turner RT, Grant KA, Iwaniec UT (2016) Alcohol: a simple nutrient with complex actions on bone in the adult skeleton. Alcohol Clin Exp Res 40:657–671.

Galvan FH, Bing EG, Fleishman JA, London AS, Caetano R, Burnam MA, Longshore D, Morton SC, Orlando M, Shapiro M (2002) The prevalence of alcohol consumption and heavy drinking among people with HIV in the United States: results from the HIV Cost and Services Utilization Study. J Stud Alcohol 63:179–186.

Grey A, Rix-Trott K, Horne A, Gamble G, Bolland M, Reid IR (2011) Decreased bone density in men on methadone maintenance therapy. Addict 106:349–354.

Haskelberg H, Carr A, Emery S (2011) Bone turnover markers in HIV disease. AIDS Rev 13:240–250.

Haskelberg H, Hoy JF, Amin J, Ebeling PR, Emery S, Carr A, STEAL Study Group (2012) Changes in bone turnover and bone loss in HIV-infected patients changing treatment to tenofovir-emtricitabine or abacavir-lamivudine. PLoS ONE 7:e38377.

Jain S, Camacho P (2018) Use of bone turnover markers in the management of osteoporosis. Curr Opin Endocrinol Diabetes Obes 25:366–372.

Justice AC, McGinnis KA, Tate JP, Braithwaite RS, Bryant KJ, Cook RL, Edelman EJ, Fiellin LE, Freiberg MS, Gordon AJ, Kraemer KL, Marshall BDL, Williams EC, Fiellin DA (2016) Risk of mortality and physiologic injury evident with lower alcohol exposure among HIV infected compared with uninfected men. Drug Alcohol Depend 161:95–103.

Kanis JA, Johansson H, Johnell O, Oden A, De Laet C, Eisman JA, Pols H, Tenenhouse A (2005) Alcohol intake as a risk factor for fracture. Osteoporos Int 16:737–742.

Kim TW, Alford DP, Malabanan A, Holick MF, Samet JH (2006) Low bone density in patients receiving methadone maintenance treatment. Drug Alcohol Depend 85:258–262.

Laitinen K, Lamberg-Allardt C, Tunninen R, Härkönen M, Välimäki M (1992) Bone mineral density and abstention-induced changes in bone and mineral metabolism in noncirrhotic male alcoholics. Am J Med 93:642–650.

Malik P, Gasser RW, Moncayo R, Kemmler G, Wolfgang Fleischhacker W (2012) Markers of bone resorption and formation during abstinence in male alcoholic patients. Alcohol Clin Exp Res 36:2059–2064.

Marrone JA, Maddalozzo GF, Branscum AJ, Hardin K, Cialdella-Kam L, Philbrick KA, Breggia AC, Rosen CJ, Turner RT, Iwaniec UT (2012) Moderate alcohol intake lowers biochemical markers of bone turnover in postmenopausal women. Menopause 19:974–979.

Marshall BDL, Tate JP, McGinnis KA, Bryant KJ, Cook RL, Edelman EJ, Gaither JR, Kahler CW, Operario D, Fiellin DA, Justice AC (2017) Long-term alcohol use patterns and HIV disease severity. AIDS 31:1313–1321.

McLellan AT, Kushner H, Metzger D, Peters R, Smith I, Grissom G, Pettinati H, Argeriou M (1992) The Fifth Edition of the Addiction Severity Index. J Subst Abuse Treat 9:199–213.

Molina PE, Bagby GJ, Nelson S (2014) Biomedical consequences of alcohol use disorders in the HIV-infected host. Curr HIV Res 12:265–275.

Molina PE, Simon L, Amedee AM, Welsh DA, Ferguson TF (2018) Impact of alcohol on HIV pathogenesis, comorbidities, and aging: integrating preclinical and clinical findings. Alcohol Alcohol 53:439–447.

Moran CA, Weitzmann MN, Ofotokun I (2016) The protease inhibitors and HIV-associated bone loss. Curr Opin HIV AIDS 11:333–342.

Nyquist F, Ljunghall S, Berglund M, Obrant K (1996) Biochemical markers of bone metabolism after short and long- time ethanol withdrawal in alcoholics. Bone 19:51–54.

Ofotokun I, McIntosh E, Weitzmann MN (2012) HIV: Inflammation and bone. Curr HIV/AIDS Rep 9:16–25.

Ofotokun I, Titanji K, Vunnava A, Roser-Page S, Vikulina T, Villinger F, Rogers K, Sheth AN, Lahiri CD, Lennox JL, Weitzmann MN (2016) Antiretroviral therapy induces a rapid increase in bone resorption that is positively associated with the magnitude of immune reconstitution in HIV infection. AIDS 30:405–414.

Saitz R, Mesic A, Ventura AS, Winter MR, Heeren TC, Sullivan MM, Walley AY, Patts GJ, Meli SM, Holick MF, Kim TW, Bryant KJ, Samet JH (2018) Alcohol consumption and bone mineral density in people with HIV and substance use disorder: a prospective cohort study. Alcohol Clin Exp Res 42:1518–1529.

Sharma A, Flom PL, Weedon J, Klein RS (2010) Prospective study of bone mineral density changes in aging men with or at risk for HIV infection. AIDS 24:2337–2345.

Sharma A, Shi Q, Hoover DR, Anastos K, Tien PC, Young MA, Cohen MH, Golub ET, Gustafson D, Yin MT (2015) Increased fracture incidence in middle-aged HIV-infected and HIV-uninfected women: updated results from the Women's Interagency HIV Study. J Acquir Immune Defic Syndr 70:54–61.

Sheehan DV, Janavs J, Sheehan KH, Sheehan M, Gray C (2010) Mini-International Neuropsychiatric Interview 6.0: High Prevalence Disorders, English version. University of South Florida, Tampa, FL.

Skinner HA, Sheu WJ (1982) Reliability of alcohol use indices. The lifetime drinking history and the MAST. J Stud Alcohol 43:1157–1170.

Sobell LC, Sobell MB (1992) Timeline follow-back, in *Measuring Alcohol Consumption: Psychosocial and Biological Methods* (Litten RZ, Allen JP eds), pp 41–72. Humana Press, Totowa, NJ.

Szulc P, Naylor K, Hoyle NR, Eastell R, Leary ET, National Bone Health Alliance (2017) Use of CTX-I and PINP as bone turnover markers: National Bone Health Alliance recommendations to standardize sample handling and patient preparation to reduce pre-analytical variability. Osteoporos Int 28:2541–2556.

Titanji K, Vunnava A, Foster A, Sheth AN, Lennox JL, Knezevic A, Shenvi N, Easley KA, Ofotokun I, Weitzmann MN (2018) T-cell receptor activator of nuclear factor-κB ligand/osteoprotegerin imbalance is associated with HIV-induced bone loss in patients with higher CD4+ T-cell counts. AIDS 32:885–894.

Titanji K, Vunnava A, Sheth AN, Delille C, Lennox JL, Sanford SE, Foster A, Knezevic A, Easley KA, Weitzmann MN, Ofotokun I (2014) Dysregulated B Cell expression of RANKL and OPG correlates with loss of bone mineral density in HIV infection. PLoS Pathog 10:e1004497.

Vasikaran S, Cooper C, Eastell R, Griesmacher A, Morris HA, Trenti T, Kanis JA (2011) International Osteoporosis Foundation and International Federation of Clinical Chemistry and Laboratory Medicine position on bone marker standards in osteoporosis. Clin Chem Lab Med 49:1271–1274.

Ventura AS, Winter MR, Heeren TC, Sullivan MM, Walley AY, Holick MF, Patts GJ, Meli SM, Samet JH, Saitz R (2017) Lifetime and recent alcohol use and bone mineral density in adults with HIV infection and substance dependence. Medicine (Baltimore). 96:e6759.

Watt J, Schuon J, Davis J, Ferguson TF, Welsh DA, Molina PE, Ronis MJJ (2019) Reduced serum osteocalcin in high-risk alcohol using people living with HIV does not correlate with systemic oxidative stress or inflammation: Data from the New Orleans Alcohol Use in HIV Study. Alcohol Clin Exp Res 43:2374–2383.

Williams EC, Bradley KA, Balderson BH, McClure JB, Grothaus L, McCoy K, Rittmueller SE, Catz SL (2014) Alcohol and associated characteristics among older persons living with human immunodeficiency virus on antiretroviral therapy. Subst Abuse 35:245–253.

Williams EC, Hahn JA, Saitz R, Bryant K, Lira MC, Samet JH (2016) Alcohol use and human immunodeficiency virus (HIV) infection: current knowledge, implications, and future directions. Alcohol Clin Exp Res 40:2056–2072.

Williams EC, McGinnis KA, Bobb JF, Rubinsky AD, Lapham GT, Skanderson M, Catz SL, Bensley KM, Richards JE, Bryant KJ, Edelman EJ, Satre DD, Marshall BDL, Kraemer KL, Blosnich JR, Crystal S, Gordon AJ, Fiellin DA, Justice AC, Bradley KA (2018) Changes in alcohol use associated with changes in HIV disease severity over time: a national longitudinal study in the Veterans Aging Cohort. Drug Alcohol Depend 189:21–29.

Wolinsky FD, Fitzgerald JF, Stump TE (1997) The effect of hip fracture on mortality, hospitalization, and functional status: a prospective study. Am J Public Health 87:398–403.

Womack JA, Goulet JL, Gibert C, Brandt C, Chang CC, Gulanski B, Fraenkel L, Mattocks K, Rimland D, Rodriguez-Barradas MC, Tate J, Yin MT, Justice AC, Veterans Aging Cohort Study Project Team (2011) Increased risk of fragility fractures among HIV infected compared to uninfected male veterans. PLoS ONE 6:e17217.

Wurst FM, Thon N, Yegles M, Schruck A, Preuss UW, Weinmann W (2015) Ethanol metabolites: their role in the assessment of alcohol intake. Alcohol Clin Exp Res 39:2060–2072.

Yin MT, McMahon DJ, Ferris DC, Zhang CA, Shu A, Staron R, Colon I, Laurence J, Dobkin JF, Hammer SM, Shane E (2010a) Low bone mass and high bone turnover in postmenopausal human immunodeficiency virus-infected women. J Clin Endocrinol Metab 95:620–629.

Yin MT, Roy Choudhury A, Bucovsky M, Colon I, Ferris DC, Olender S, Agarwal S, Sharma A, Zeana C, Zingman B, Shane E (2019) A randomized placebo-controlled trial of low- versus moderate-dose Vitamin D3 supplementation on bone mineral density in postmenopausal women with HIV. J Acquir Immune Defic Syndr 80:342–349.

Yin MT, Shi Q, Hoover DR, Anastos K, Sharma A, Young M, Levine A, Cohen MH, Shane E, Golub ET, Tien PC (2010b) Fracture incidence in HIV-infected women: results from the Women's Interagency HIV Study. AIDS 24:2679–2686.

Yin MT, Zhang CA, McMahon DJ, Ferris DC, Irani D, Colon I, Cremers S, Shane E (2012) Higher rates of bone loss in postmenopausal HIV-infected women: a longitudinal study. J Clin Endocrinol Metab 97:554–562.

Zhang X, Yu Z, Yu M, Qu X (2015) Alcohol consumption and hip fracture risk. Osteoporos Int 26:531–542.