



# 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

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**Background:** HIV infection is now largely a chronic condition as a result of the success of antiretroviral therapy. However, several comorbidities have emerged in people living with HIV (PLWH), including alcohol use disorders and musculoskeletal disorders. Alcohol use has been associated with lower bone mineral density, alterations to circulating bone turnover markers, and hypocalcemia. The pathophysiological basis of bone loss in the PLWH population is unclear but has been suggested to be linked to oxidative stress and inflammation. To test the hypothesis that PLWH consuming excessive alcohol have altered markers of bone turnover and/or calcium homeostasis in association with oxidative stress, we correlated measurements of alcohol consumption with markers of oxidative stress and inflammation, serum calcium concentrations, and measurements of bone turnover, including c-terminal telopeptide cross-links (CTX-1) and osteocalcin.

**Methods:** Data were drawn from cross-sectional baseline data from the ongoing New Orleans Alcohol Use in HIV (NOAH) study, comprised of 365 in care PLWH. Alcohol consumption measures (Alcohol Use Disorders Test, 30-day timeline follow-back calendar, and phosphatidylethanol [PEth]) were measured in a subcohort of 40 subjects selected based on highest and lowest PEth measurements. Multivariate linear regression was performed to test the relationships between alcohol consumption and systemic oxidative stress (4-hydroxynonenal; 4-HNE) and inflammation (c-reactive protein; CRP).

**Results:** Serum calcium and CTX-1 did not differ significantly between the high and low-PEth groups. Individuals in the high-PEth group had significantly lower serum osteocalcin (median low-PEth group: 13.42 ng/ml, inter-quartile range [IQR] 9.26 to 14.99 ng/ml; median high-PEth group 7.39 ng/ml, IQR 5.02 to 11.25 ng/ml;  $p = 0.0005$ , Wilcoxon rank-sum test). Osteocalcin negatively correlated with PEth (Spearman  $r = -0.45$ ,  $p = 0.05$ ) and self-reported measures after adjusting for covariates. Alcohol consumption showed mild, but significant, positive associations with serum 4-HNE, but not with CRP. Osteocalcin did not correlate with either 4-HNE or CRP.

**Conclusions:** In this subcohort of PLWH, we detected significant associations between at-risk alcohol use and osteocalcin, and at-risk alcohol use and serum 4-HNE, suggesting suppression of bone formation independent of increased systemic oxidative stress with increasing alcohol consumption.

**Key Words:** HIV, Alcohol, Bone, Oxidative Stress.

THE USE OF antiretroviral therapy (ART) among people living with HIV/AIDS (PLWH) has effectively reduced mortality from the disease and extended lifespans, but as a result, the incidence rates of chronic and age-related

diseases have increased among this population. Osteoporosis and osteopenia (low bone density) rates are higher among PLWH compared with the general population (Bruera et al., 2003; Dolan et al., 2006; Paton et al., 1997). These conditions heavily contribute to the higher rates of bone fracture observed in PLWH (Sharma et al., 2015; Shiao et al., 2013; Triant et al., 2008).

Bone health is maintained via a balance of bone formation by osteoblasts and bone resorption by osteoclasts (Henriksen et al., 2009). The dominant mechanism by which HIV directly affects bone tissue to disrupt this homeostasis and compromise bone health is not known. It has been hypothesized that HIV-associated chronic inflammation contributes by indirectly stimulating osteoclast differentiation and resorptive activity (Fakruddin and Laurence, 2003; Gazzola et al., 2013; Hileman et al., 2014). Although HIV infection independently is associated with reduced bone mass (Bruera

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et al., 2003), ART has been found to accelerate bone loss (Tebas et al., 2000), potentially by exacerbating chronic inflammation coincident with immune cell restoration and promoting bone osteoclastogenesis (Ofotokun et al., 2015; Pan et al., 2004). In addition, in vitro studies have shown HIV to directly impair osteoblast differentiation and function (Cotter et al., 2007). Together or independently, these mechanisms could act to shift the balance of bone turnover to favor resorption and a net loss of bone.

Alcohol use disorders (AUDs) are prevalent among a rising incidence of chronic conditions affecting PLWH (Conigliaro et al., 2003; Galvan et al., 2002; Williams et al., 2016). Alcohol overuse is also a known risk factor for low bone mass and fracture in healthy populations (Berg et al., 2008; González-Reimers et al., 2011; Santori et al., 2008). Disruptions to calcium homeostasis have also been reported among people consuming hazardous levels of alcohol (Laitinen et al., 1992; Míguez et al., 2012). Mechanistically, animal models have demonstrated that alcohol-induced oxidative stress in osteoblasts suppresses proosteoblastic signaling while increasing osteoclast differentiation to promote resorption (Chen et al., 2010; Mercer et al., 2014), and dietary antioxidants protect against ethanol (EtOH)-induced bone loss (Alund et al., 2017; Chen et al., 2011).

Osteocalcin and c-terminal telopeptide cross-links (CTX-1) are soluble biomarkers of bone formation and bone resorption, respectively, commonly used to assess bone homeostasis in human serum and in animal models of bone pathology (Greenblatt et al., 2017; Szulc et al., 2017). Circulating levels of CTX-1 have been found to be unaltered or decreased in the case of mild alcohol consumption (Marrone et al., 2012; Sripanyakorn et al., 2009) but increased in cases of high consumption or diagnosis of alcoholism (Alvisa-Negrín et al., 2009; Nyquist et al., 1996). Animal models of alcohol exposure have linked decreases in circulating osteocalcin to changes in bone structure (e.g., cortical thinning and loss of trabecular bone; Mercer et al., 2014; Shankar et al., 2008), and heavy alcohol use in humans is associated with decreases in serum osteocalcin (Laitinen et al., 1991; Nyquist et al., 1996; Santori et al., 2008), implying a suppressive effect of alcohol on bone formation. Serum osteocalcin has also been reported to be decreased in PLWH, negatively correlating to disease severity (Serrano et al., 1995; Teichmann et al., 2000).

Although both HIV and AUD increase the incidence of adverse skeletal outcomes, it is not known mechanistically how these 2 conditions interact to exacerbate risk. The objective of this cross-sectional study was to determine whether PLWH consuming excessive alcohol (representing AUD) had altered calcium and/or altered levels of bone turnover markers, and whether these were associated with changes in systemic oxidative stress and markers of inflammation. Data were collected from baseline participant visits in the New Orleans Alcohol Use in HIV (NOAH) study, a prospective cohort study of 365 predominately African American, low income in care PLWH in the New Orleans, Louisiana area

(Welsh et al., 2019). A subcohort of 40 PLWH with lowest and highest levels of alcohol consumption as measured by phosphatidylethanol (PEth), a plasma marker of recent EtOH consumption (Isaksson et al., 2011), was selected for analysis. Alcohol consumption was also estimated by self-report (Alcohol Use Disorders Identification Test [AUDIT], timeline follow-back; Saunders et al., 1993; Sobell and Sobell, 1992).

## MATERIALS AND METHODS

### *Study Population*

The NOAH study is a bidirectional investigation of AUD, HIV/AIDS, and ART in aging and exacerbated comorbid conditions in an underserved cohort of PLWH. A total of 365 HIV-infected individuals, under care at a HIV specialty clinic, were enrolled in NOAH. The study population was oversampled toward individuals with AUDIT scores  $\geq 8$ , suggestive of potentially hazardous drinking habits. Exclusion criteria included acute illness within the preceding 6 weeks (defined by unscheduled healthcare utilization for a new or exacerbated illness), nonprophylaxis administration of antibiotics, pregnancy, or acute intoxication. Louisiana State University Health Sciences Center, New Orleans Human Research Protection Program and Institutional Review Board approved and oversee the study. Further study details are described in Welsh et al. (2019).

As a pilot analysis to detect effects at the extreme ends of the alcohol consumption spectrum, a subset of the study population based on recent alcohol consumption was selected for analyses of bone turnover markers. This consisted of the 20 participants with the highest PEth values and a random sample of 20 participants from 128 NOAH study subjects with PEth values  $< 8$  ng/ml, equivalent to a negative PEth result.

### *Data Collection*

Enrolled participants completed a baseline visit where data were collected via survey questionnaires, physical examination, function performance tests, and specimen and blood collection. Interviewer-administered surveys were collected through LSUHSC-NO's online Research Electronic Data Capture (REDCap) system by trained research staff. Fasting blood samples were collected as part of a baseline examination for clinical laboratory tests (including total calcium (as part of a comprehensive metabolic panel), 4-hydroxynonenal (4-HNE, via ELISA, Cell Biolabs Inc, San Diego, CA, cat# STA-838), c-reactive protein (CRP, via clinical lab panel), and PEth (measured by USDTL, Inc., Des Plaines, IL) for recent alcohol use). A complete list of sample collection and analysis protocols is provided in Welsh et al. (2019) (Table S1).

Multidimensional characterization of alcohol exposure and psychosocial stressors was carried out by interviewer-administered questionnaire, which included the AUDIT (Babor et al., 2001) and a 30-day timeline follow-back (TLFB) calendar (Sobell and Sobell, 1992). The AUDIT questionnaire is a 10-item tool, and responses are summed for an overall score ranging from a possible 0 to 40 (Saunders et al., 1993). The first 3 questions' scores were summed for an AUDIT-C score ranging from 0 to 12.

### *Serum Analysis*

Serum was separated by centrifugation and stored at  $-80$  until analysis. Samples were assayed for total osteocalcin (Invitrogen, Waltham, MA cat# KAQ1381) and c-terminal telopeptide cross-links (CTX-1; Immunodiagnostic Systems, Tyne & Wear, UK, cat#

AC-02F1) by enzyme-linked immunosorbent assay (ELISA). Serum biomarkers levels were interpolated from curves generated by manufacturer-provided osteocalcin or CTX-1 standards, respectively, included in the assay.

### Data Analysis

Data were analyzed using Microsoft Excel 2016, Prism v.8.0 (GraphPad, Inc., San Diego, CA) and SAS v.9.4 (Statistical Analysis Systems, Cary, NC). Pairwise comparisons of circulating calcium, c-terminal telopeptide cross-links (CTX-1), and osteocalcin were performed by PEth category. Wilcoxon rank-sum tests were used due to the lack of normality to compare the means of the continuous alcohol measures by PEth category. Additional covariates (age, gender, education, race, smoking status, viral load, and cluster of differentiation-4 (CD4) count) were adjusted for in multivariate linear regression analyses based on known association with alcohol and oxidative stress and/or bone health, as well as preliminary analyses of alcohol consumption in the study population. Serum 4-HNE and C-reactive protein data were log-transformed prior to analysis.

## RESULTS

Table 1 reports select characteristics of the NOAH cohort and the 40-subject subsample used in the current analysis to examine the relationship between bone turnover markers and alcohol consumption. The distributions of the variables in the subsample reflected the distributions of each variable in the full study population, with the exception of smoking status: The subcohort drawn herein contained a higher proportion of individuals who are former smokers, whereas the study population as a whole (at baseline) is predominately current smokers ( $p < 0.0001$ , chi-square test). Greater than 90% of the study participants reported taking ART.

We began by determining whether increases in plasma PEth—as a measurement of recent drinking—were correlated with hypocalcemia in the total study cohort of 365 individuals. Spearman's correlation was very weak and not statistically significant ( $r = -0.052$ ,  $p = 0.32$ ; Fig. 1), indicating that serum calcium level was not associated with recent alcohol consumption in this study population. In the subsample, those with the highest PEth measures ( $n = 20$ ) had a median PEth of 1268.5 ng/ml (interquartile range 703.7 ng/ml). As expected, subsampling these 2 extreme groups did not reveal a significant difference in circulating calcium (mean low-PEth group: 9.4 mg/dl, SD 0.34 mg/dl; mean high-PEth group: 9.3 mg/dl, SD 0.39 mg/dl;  $p = 0.40$ , unpaired  $t$ -test; Fig. 2A), nor did the 2 groups significantly differ in CTX-1 (median low-PEth group: 0.29 ng/ml, interquartile range [IQR] 0.40 ng/ml; median high-PEth group: 0.24 ng/ml, IQR 0.73 ng/ml;  $p = 0.33$ , Wilcoxon rank-sum test; Fig. 2B). On the contrary, we detected a significant difference in circulating total osteocalcin between the low and high-PEth groups (median low-PEth group: 13.42 ng/ml, IQR 5.73 ng/ml; median high-PEth group 7.39 ng/ml, IQR 6.23 ng/ml;  $p = 0.0005$ , Wilcoxon rank-sum test; Fig. 2C).

Within the high-PEth group, linear increases in PEth were not significantly correlated with calcium (Pearson  $r = 0.04$ ,

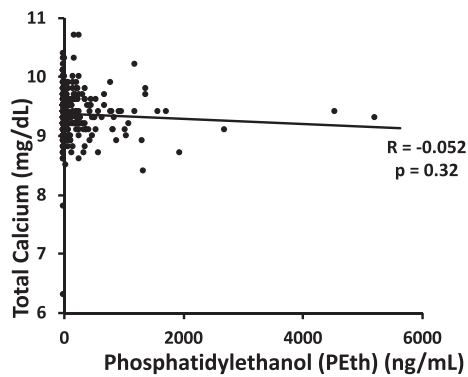
**Table 1.** Demographic Distributions and Alcohol Measures of the NOAH Cohort ( $N = 365$ ) and Subsample of 40 Individuals Selected Based on Low and High Phosphatidylethanol (PEth) Levels

Demographics	Full study ( $N = 365$ )		Cohort subsample ( $n = 40$ )	
	Frequency	Percent	Frequency	Percent
Gender				
Women	113	31.0	10	25.0
Men	252	69.0	30	75.0
$p$ -value <sup>a</sup>		0.4702		
Education				
<High school	149	40.8	12	30.0
High school graduate	114	31.2	17	42.5
Some college/ Community College, Vocational/Trade School	81	22.2	10	25.0
College graduate or graduate school	21	5.7	1	2.5
$p$ -value <sup>a</sup>		0.2508		
Race				
Black	305	83.6	34	85.0
Other	60	16.4	6	15.0
$p$ -value <sup>a</sup>		>0.9999		
Smoking status				
Nonsmoker	84	23.0	7	17.5
Former smoker	59	16.2	29	72.5
Current smoker	222	60.8	4	10.0
$p$ -value <sup>a</sup>		<0.0001		
Age				
20 to 29	19	5.2	0	0.0
30 to 39	60	16.4	12	30.0
40 to 49	88	24.1	9	22.5
50 to 59	152	41.6	15	37.5
≥60	46	21.6	4	10.0
$p$ -value <sup>a</sup>		0.0976		
Viral load (copies/ml)				
<20	247	67.7	26	65.0
≥20	118	32.3	14	35.0
$p$ -value <sup>a</sup>		0.7219		
CD4 count (cells/ $\mu$ l)				
<200	47	13.2	5	12.5
200 to 350	47	13.2	7	17.5
>350	263	73.7	28	70.0
$p$ -value <sup>a</sup>		0.6906		
Alcohol measures	Full study ( $N = 365$ )	Low-PEth ( $n = 20$ )	High-PEth ( $n = 20$ )	
PEth (ng/ml)				
Median (IQR)	34.0 (179.0)	0	1,269 (703.7)	
AUDIT (score, 0 to 40 points)				
Median (IQR)	6 (10.5)	1 (4.75)	15 (9.5)	
$p$ -value <sup>b</sup>			<0.0001	
AUDIT-C (score, 0 to 12 points)				
Median (IQR)	4.0 (4.0)	1 (4.0)	7.0 (4.5)	
$p$ -value			<0.0001	
Timeline follow-back (TLFB), total grams (g)				
Median (IQR)	158.2 (738.5)	0 (182.0)	2,216 (2386.1)	
$p$ -value <sup>b</sup>			<0.0001	
TLFB, 30 days (# of d/30 d)				
Median (IQR)	3 (11)	0 (2.75)	19.5 (21)	
$p$ -value <sup>b</sup>			<0.0001	

AUDIT, Alcohol Use Disorders Identification Test; PEth, Phosphatidylethanol; TLFB, Time-linked follow-back.

<sup>a</sup> $p$ -value for comparison between distributions of the 40-subject subsample and the remaining subjects (chi-squared test or Fisher's exact test).

<sup>b</sup> $p$ -value for comparison between low-PEth subjects and high-PEth subjects (Wilcoxon rank-sum pairwise comparison).



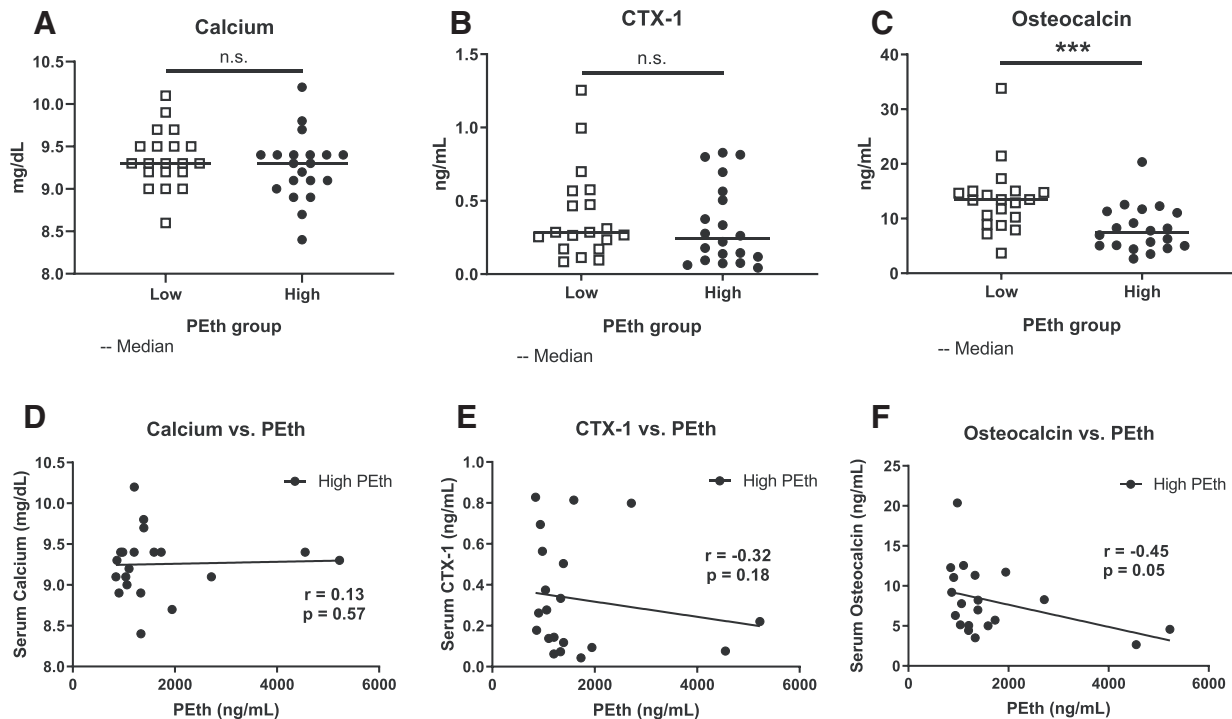
**Fig. 1.** Total serum calcium (mg/dl) by plasma PEth concentration (ng/ml) in the entire NOAH study population at baseline. Each data point represents 1 participant ( $n = 365$ ). Solid line: linear trend;  $R$ : Spearman correlation coefficient.

$p = 0.88$ ,  $n = 20$ ; Fig. 2D) or CTX-1 (Spearman  $r = -0.32$ ,  $p = 0.18$ ,  $n = 20$ ; Fig. 2E). Consistent with the pairwise difference between high and low-PEth groups, PEth was significantly, negatively correlated with circulating osteocalcin within the high-PEth group (Spearman  $r = -0.45$ ,  $p = 0.05$ ,  $n = 20$ ; Fig. 2F), indicating that in this sample, recent alcohol consumption is associated with a dose-dependent suppression of bone formation.

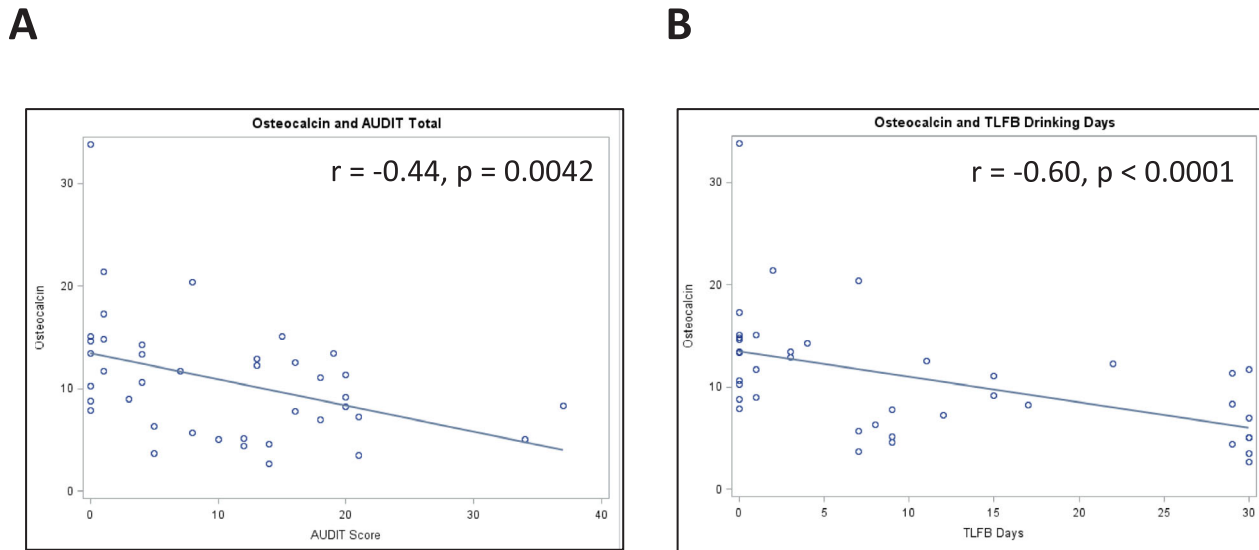
In order to account for covariates that may contribute to the observed variability between PEth and osteocalcin, and to better understand the relationship between osteocalcin

and self-reported alcohol consumption, we performed multivariate linear regression analyses on the combined high- and low-consumption groups ( $n = 40$ ). Independent variables for alcohol consumption included PEth, AUDIT (point scale of 0 to 40), AUDIT-C (point scale of 0 to 12), TLFB (number of drinks in past 30 days), and TLFB (total grams of EtOH, last 30 days; Table 1). Each of the consumption measures was highly correlated with one another (Table S1). Additional covariates included age, gender, race, education, smoking status, viral load, and CD4 count (Table 1). Results of the crude (consumption variable only) and adjusted (consumption variable plus covariates) are presented in Fig. 3 (crude analysis) and Table 2. In addition to a significant, negative association with PEth ( $\beta = -0.00291$ ,  $p = 0.0007$ ), each of the self-reported measures of alcohol consumption was associated with osteocalcin (Table 2).

We hypothesized that alcohol consumption would be a significant predictor of general oxidative stress (measured by serum 4-HNE) and inflammation (measured by circulating CRP). Results of the crude and adjusted analyses (as above) are presented in Table 3. We found small, but significant, positive associations between all measures of alcohol consumption and circulating 4-HNE before and after adjusting for the above covariates, suggesting that individuals in this study with higher alcohol consumption have increased systemic oxidative stress. There were no significant associations between the measures of alcohol consumption and inflammation, as measured by CRP (Table 4).



**Fig. 2.** Pairwise differences in serum markers of bone turnover and plasma PEth in subjects with low-PEth (20 random samples below LOD, open squares) and high-PEth (20 highest measurements, closed circles) (A–C), and linear correlations within the high-PEth group between markers and PEth (D–F). (A) and (D): total calcium; (B) and (E): c-terminal telopeptide cross-links (CTX-1; bone resorption); (C) and (F): osteocalcin (bone formation). \*\*\*Significant pairwise difference ( $p < 0.001$ ), n.s.: not significant, Wilcoxon rank-sum test.  $r$ : Spearman correlation,  $p$ :  $p$ -value for Spearman correlation.



**Fig. 3.** Linear correlations between serum osteocalcin (ng/ml) and **(A)** AUDIT score (0 to 40), **(B)** timeline follow-back (TLFB), number of drinking days in the past 30 days. Each data point is 1 subject within the 40-sample low/high-PEth subsample. *r*: Spearman correlation, *p*: *p*-value for Spearman correlation.

We did not find osteocalcin to be associated with 4-HNE (Fig. 4A, Spearman  $r = -0.036$ ,  $p = 0.83$ ), suggesting that the observed suppression of osteocalcin is independent of systemic oxidative stress. As expected based on the linear regression results, there was no significant correlation between osteocalcin and CRP (Fig. 4B, Spearman  $r = -0.181$ ,  $p = 0.27$ ).

**DISCUSSION**

This study determined relationships between biomarkers of bone turnover and multiple assessments of alcohol consumption, including self-report (timeline follow-back, AUDIT) and a biomarker of recent consumption (PEth; Isaksson et al., 2011), to test the hypothesis that at-risk alcohol use adversely affects bone health in adult PLWH.

Additionally, we performed linear regression analyses to determine associations between alcohol consumption and markers of oxidative stress and inflammation. Subdividing the subject population by extreme levels of alcohol consumption (20 subjects with PEth levels greater than 845 ng/ml and a random sample of 20 nondetectable PEth scores) revealed a pairwise significant difference in the serum bone formation marker osteocalcin, suggesting a lower rate of bone formation in subjects with greater alcohol consumption. Within this subcohort, osteocalcin negatively correlated with multiple assessments of both self-report and biochemically determined alcohol consumption (PEth) after controlling for several related covariates. The range of serum osteocalcin concentrations in this subset of 40 individuals (2.65 to 33.82 ng/ml) is comparable to reported intervals in other adult populations of similar age or older (Ardawi et al.,

**Table 2.** Multivariate Linear Regression of Serum Osteocalcin Versus Alcohol Consumption Measures

Mean: 10.77 ng/ml Median: 10.44 ng/ml Range: 2.65 to 33.82 ng/ml IQR: 7.01 ng/ml n = 40	Serum osteocalcin (ng/ml)			
	Crude model		Adjusted model <sup>a</sup>	
	$\beta$	<i>p</i> -value	$\beta$	<i>p</i> -value
PEth (ng/ml)	-0.0023	0.0026	-0.00291	0.0007
TLFB (total grams)	-0.00151	0.004	-0.00183	0.0066
TLFB (d/30 d)	-0.2494	0.0012	-0.30221	0.0011
AUDIT (total)	-0.25551	0.0089	-0.28282	0.0189
AUDIT-C	-0.74756	0.0021	-0.76564	0.0044

Descriptive statistics of serum osteocalcin listed in left panel. AUDIT, Alcohol Use Disorders Identification Test; TLFB, timeline follow-back. <sup>a</sup>Model adjusted for age, gender, education, race, smoking status, viral load, and CD4 count.

**Table 3.** Multivariate Linear Regression of Serum 4-Hydroxynonenal (4-HNE) Versus Alcohol Consumption Measures

Mean: 6.65 $\mu$ g/ml Median: 5.31 ng/ml Range: 1.83 to 21.59 ng/ml IQR: 4.41 ng/ml n = 40	Serum 4-HNE (log $\mu$ g/ml)			
	Crude model		Adjusted model <sup>a</sup>	
	$\beta$	<i>p</i> -value	$\beta$	<i>p</i> -value
PEth (ng/ml)	0.000168	0.0648	0.000153	0.0067
TLFB (total grams)	$7.91 \times 10^{-5}$	0.0167	$7.44 \times 10^{-5}$	0.0072
TLFB (d/30 d)	0.01319	0.0047	0.0141	0.0016
AUDIT (total)	0.01399	0.0091	0.0129	0.0059
AUDIT-C	0.04642	0.0005	0.0465	0.0005

Descriptive statistics of serum 4-HNE listed in left panel. TLFB, timeline follow-back; AUDIT, Alcohol Use Disorders Identification Test. <sup>a</sup>Model adjusted for age, gender, education, race, smoking status, viral load, and CD4 count.

**Table 4.** Multivariate Linear Regression of Serum c-Reactive Protein Versus Alcohol Consumption Measures

	Serum c-reactive protein (units)			
	Crude model		Adjusted model <sup>a</sup>	
	$\beta$	<i>p</i> -value	$\beta$	<i>p</i> -value
Mean: 0.72 mg/dl				
Median: 0.5 mg/dl				
Range: 0.5 to 2.8 mg/dl				
IQR: 0.20 mg/dl				
<i>n</i> = 39				
PEth (ng/ml)	$-5.13 \times 10^{-5}$	0.4030	$-4.10 \times 10^{-5}$	0.5554
TLFB (total grams)	$-1.86 \times 10^{-5}$	0.6584	$-4.48 \times 10^{-4}$	0.3955
TLFB (d/30 d)	$6.84 \times 10^{-5}$	0.9913	$-7.91 \times 10^{-4}$	0.9163
AUDIT (total)	$5.77 \times 10^{-4}$	0.9413	$-3.91 \times 10^{-4}$	0.9670
AUDIT-C	$-1.97 \times 10^{-3}$	0.9212	$-3.00 \times 10^{-3}$	0.8908

TLFB, timeline follow-back; AUDIT, Alcohol Use Disorders Identification Test.

Descriptive statistics of serum c-reactive protein listed in left panel.

<sup>a</sup>Model adjusted for age, gender, education, race, smoking status, viral load, and CD4 count.

2010; Chubb et al., 2015; Shou et al., 2017) but with a higher central tendency value (median 10.77 ng/ml) compared with previously published values in PLWH (Serrano et al., 1995; Teichmann et al., 2000).

A strength of this study is that it measured alcohol consumption in several dimensions. Self-reported alcohol consumption can be biased (Devaux and Sassi, 2016; Stockwell et al., 2004). However, measurement of PEth as a biomarker of recent alcohol consumption correlated well with the self-reported consumption estimates, and PEth is considered a valid measure to protect against consumption underestimation (Bajunirwe et al., 2014; Littlefield et al., 2017; Schröck et al., 2017; Stewart et al., 2014). Further assessments of the full cohort as the study progresses will add validity to these consumption estimates via repeat measurements.

The strongest predictor of the association between osteocalcin and consumption was AUDIT-C, a 3-question subset of the AUDIT scale that specifically addresses consumption measures (Bush et al., 1998). In our sample, changes in

**Table 5.** Study Highlights

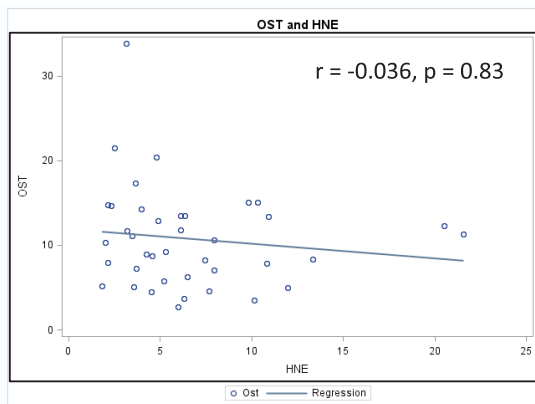
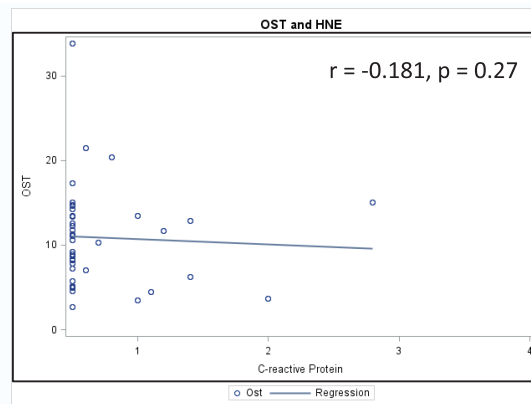
## Study highlights

- PLWH study participants with elevated PEth levels (representative of recent excessive alcohol consumption) had lower levels of serum bone formation marker osteocalcin compared to subjects with nondetectable PEth
- Self-reported alcohol consumption (timeline follow-back, AUDIT, AUDIT-C), in addition to PEth, was negatively associated with serum osteocalcin after adjusting for several relevant covariates
- Adjusted analyses found alcohol consumption to be associated with a significant increase in circulating oxidative stress marker 4-HNE
- 4-HNE was not independently associated with osteocalcin, implying independent effects of alcohol on oxidative stress and altered bone turnover in PLWH

AUDIT, Alcohol Use Disorders Identification Test; HNE, 4-hydroxynonenal.

osteocalcin from the lowest quartile to the highest correspond to a 3-point increase per AUDIT-C question, which may correspond to a difference between safe and hazardous drinking. That this association should be the most striking is not surprising in light of the association with PEth (Schröck et al., 2017), as levels of osteocalcin (and other biochemical markers) are more likely altered by consumption patterns rather than social consequences of drinking as examined in other dimensions of the AUDIT scale.

These results agree with prior findings that levels of the bone-specific protein osteocalcin in serum are dose-dependently and negatively associated with alcohol use (González-Reimers et al., 2011; Laitinen et al., 1991; Santori et al., 2008). Levels increase following periods of abstinence from alcohol (Laitinen et al., 1991; Marrone et al., 2012; Nyquist et al., 1996), suggesting a reversible effect that is responsive to intervention measures. Bone structural changes as measured by dual-energy X-ray absorptiometry are the best measures of bone mineral density. We did not perform these measures in our study. However, decreased osteocalcin has

**A.****B.**

**Fig. 4.** Linear correlations between serum osteocalcin (ng/ml) and (A) serum 4-hydroxynonenal (HNE) ( $\mu\text{g/ml}$ ) and (B) c-reactive protein (mg/dl). Each data point is 1 subject within the 40-sample low/high-PEth subsample. *r*: Spearman correlation, *p*: *p*-value for Spearman correlation.

been reported to be predictive of lower BMD (Tian et al., 2017). Outside of skeletal health, osteocalcin has been subject of increased interest for its putative role as a circulating signaling factor that increases insulin production and sensitivity (Ducy, 2011; Lee et al., 2007). Given that PLWH have higher rates of metabolic complications (Willig and Overton, 2016), our results warrant consideration of osteocalcin as a marker linking alcohol use and greater susceptibility to metabolic outcomes among PLWH.

Additional studies have found alterations to bone formation markers and BMD in PLWH who consume alcohol. Saitz et al. (2018), using self-reported drinking, found decreases in serum bone formation marker PINP, but no association with changes in BMD in HIV-infected individuals, the majority of which had low BMD at baseline. In the same cohort, Ventura et al. (2017) reported a cross-sectional, negative association between BMD and recent alcohol consumption, with the strongest association during the postdiagnosis/pre-ART phase of the HIV care continuum. We did not examine alcohol consumption during different phases (i.e., pre-HIV, pre-ART, post-ART). However, it is important to note that the majority of our study population is virally suppressed (83.8% <200 copies/ml with 75.6% <50 copies/ml) with a high adherence to ART. ART has been shown to be an independent stressor to bone health (Bruera et al., 2003). Further analyses into precise consumption patterns, particularly into the second phase of HIV care, will help illuminate associations between alcohol consumption and bone turnover markers that are modified by ART.

Despite observing significant differences in osteocalcin, this study did not identify an effect on the bone turnover marker CTX-1. These findings are in line with those reported by Saitz et al. (2018). Results in the literature are equivocal regarding the effect of alcohol on net bone turnover markers in humans (Alvisa-Negrin et al., 2009; Marrone et al., 2012; Nyquist et al., 1996; Sripanyakorn et al., 2009). In the cohort subset herein, subjects are older and predominately male. Accelerated bone turnover (i.e., increases in both osteocalcin and CTX-1) may be more pronounced in postmenopausal women (Garnero et al., 1996). Our finding that serum calcium is not affected by recent alcohol use is in line with other studies that found no change to circulating calcium levels with alcohol, despite decreased osteocalcin (Laitinen et al., 1991; Nyquist et al., 1996), suggesting perhaps tighter control of calcium homeostasis compared with secretion of bone turnover markers.

Increased resorption markers are associated with inflammation and immune cell dysregulation in PLWH (Gazzola et al., 2013; Titanji et al., 2018). Our results did not show an increase in the inflammatory marker CRP in subjects with the highest PEth values. Our finding that alcohol consumption was positively associated with oxidative stress (via increased 4-HNE) agrees with previously reported mechanisms of alcohol (Alund et al., 2017; Ronis et al., 2005). However, these changes were modest and not correlated with

osteocalcin. That these 2 factors do not correlate in this study suggests independent mechanisms of alcohol-induced systemic oxidative stress and suppression of bone formation. Our measures would not be able to detect bone-localized oxidative stress, and this could be the underlying cause of the suppressed osteocalcin secretion by osteoblasts. Suppressed osteocalcin is consistent with NADPH oxidase-dependent ROS production observed in bone from rodents exposed to EtOH *in vivo*, and in mesenchymal stem cells and osteoblasts exposed to EtOH *in vitro* (Alund et al., 2017; Chen et al., 2011; Watt et al., 2018).

Several limitations to our study bear noting. The present analysis is cross sectional, and thus, associations are not necessarily causative. Our ongoing longitudinal study will collect prospective data that will help elucidate the interaction between HIV, ART, and alcohol use with bone health. As noted, several measures of alcohol consumption are self-reported (AUDIT, TLFB). Although PEth (which is most effective as a very recent measure of consumption) can help validate these responses, by selecting subjects with high recent drinking levels we may be selecting participants with greater recall bias toward higher AUDIT responses. Subjects were not excluded based on concurrent recreational drug use, which has been considered a confounder in similar analyses (Saitz et al., 2018; Ventura et al., 2017). Thus, additional factors related to adverse health effects (with the exception of smoking) have not been fully characterized in this analysis. The study population as a whole is HIV positive and majority low income, presenting complications in comparing these results to the general US population. On the other hand, African Americans are currently the most affected HIV-infected population in the United States (CDC, 2018), a demographic reflected in our NOAH cohort (Welsh et al., 2019).

A summary of key study findings is presented in Table 5. This study helps clarify a complex picture of the relationship between HIV and bone health as modified by alcohol consumption, demonstrating a decrease in the bone formation marker osteocalcin that is associated with high levels of drinking but not increased oxidative stress. Additional self-reported measures describing recent and month-long drinking habits supported this relationship. Studies of HIV's impact on late endpoints of bone pathologies are lacking due to the relatively recent advent of improved ART and prolonged lifespan of individuals with HIV. However, in uninfected populations, fracture late in life is heavily associated with excess mortality and lower quality of life, and individuals suffering a fracture typically require surgical intervention and acquire severe risk for a second fracture (Haentjens et al., 2010; Johansson et al., 2017; Willig et al., 2001). Given the combinatorial risks of both HIV and alcohol use toward adverse bone outcomes, early biomarker research in longitudinal studies such as NOAH will serve as valuable predictors of risk and determinants of intervention, particularly in underserved populations that make up the majority of HIV cases in the United States.

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## CONFLICTS OF INTEREST

No competing interests to disclose.

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## SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

**Table S1.** Spearman Correlation coefficients for alcohol use measures.