Alcohol Use and HIV Disease Progression in an Antiretroviral Naive Cohort

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Background: Alcohol use has been shown to accelerate disease progression in experimental studies of simian immunodeficiency virus in macaques, but the results in observational studies of HIV have been conflicting.

Methods: We conducted a prospective cohort study of the impact of unhealthy alcohol use on CD4 cell count among HIV-infected persons in southwestern Uganda not yet eligible for antiretroviral treatment (ART). Unhealthy alcohol consumption was 3-month Alcohol Use Disorders Identification Test—Consumption positive (≥3 for women, ≥4 for men) and/or phosphatidylethanol (PEth—an alcohol biomarker) ≥50 ng/mL, modeled as a time-dependent variable in a linear mixed effects model of CD4 count.

Results: At baseline, 43% of the 446 participants were drinking at unhealthy levels and the median CD4 cell count was 550 cells/mm^3 (interquartile range 416–685). The estimated CD4 cell count decline per year was −14.5 cells/mm^3 (95% confidence interval: −38.6 to 9.5) for unhealthy drinking vs. −24.0 cells/mm^3 (95% confidence interval: −43.6 to −4.5) for refraining from unhealthy drinking, with no significant difference in decline by unhealthy alcohol use (*P* value 0.54), adjusting for age, sex, religion, time since HIV diagnosis, and HIV viral load. Additional analyses exploring alternative alcohol measures, participant subgroups, and time-dependent confounding yielded similar findings.

Conclusion: Unhealthy alcohol use had no apparent impact on the short-term rate of CD4 count decline among HIV-infected ART naive individuals in Uganda, using biological markers to augment self-report and examining disease progression before ART initiation to avoid unmeasured confounding because of misclassification of ART adherence.

Key Words: HIV progression, phosphatidylethanol, Uganda, antiretroviral treatment adherence

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INTRODUCTION

A substantial proportion (between 8% and 42%) of persons living with HIV/AIDS worldwide has been reported to drink at unhealthy levels (ie, at risk drinking or meeting criteria for an alcohol use disorder).[1] Alcohol use has been shown to be a consistent independent risk factor for HIV acquisition,[2] and among persons with HIV, alcohol use is associated with decreased retention in care[3] and worse antiretroviral treatment (ART) adherence,[4] with a dose–response relationship.[5,6]

Chronic alcohol use impacts both innate and adaptive immune functioning,[7] and chronic alcohol use and HIV independently damage the intestinal mucosa, enabling increased microbial translocation with subsequent increased inflammation.[8] Experimental studies in which high doses of alcohol were administered to macaques before and after infection with simian immunodeficiency virus (SIV) found increased levels of SIV viremia and mortality compared with control macaques who were infected with SIV but who received a sucrose control.[9–12] Thus alcohol use might be an important factor in HIV disease progression.

Despite the high biologic plausibility of an effect of alcohol use on HIV disease progression, the results of human observational studies have been mixed. No prospective study conducted in the period before the advent of ART found an association between alcohol consumption and the onset of AIDS,[13] and a retrospective analysis of persons not yet on ART participating in a large clinical HIV cohort found no association between risky alcohol use and CD4 cell count.[14] However, 2 studies conducted since the advent of ART suggested a detrimental effect of alcohol use before ART use, with one study reporting a difference in mean CD4 cell count of 49 cells/mm^3 among those reporting heavy drinking compared with those abstaining[15] and another reporting a strong association between frequent alcohol use (≥2 drinks daily) and time to CD4 cell count below 200 cells/mm.[3,16]

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Neither study found an association of alcohol use measures with HIV viral load.

Among longitudinal studies of persons on ART, the findings have been mixed as well. Several studies among persons on ART have found no association between high levels of alcohol use (variously defined as heavy, hazardous, problem, or severe risk alcohol use) and CD4 cell count and/or HIV viral load after controlling for ART adherence.[14,15,17–19] Two recent studies conducted mediation analyses to separate out effects of alcohol use on CD4 cell counts because of reduced adherence vs. other pathways. One found direct effects of heavy alcohol use on CD4 cell counts,[20] whereas the other found only indirect effects of alcohol use through adherence.[21]

Several methodological considerations might explain these inconsistent findings. First, inability to accurately measure alcohol use, due to recall bias, especially social desirability bias, may impact the results.[2] Biomarkers of alcohol use, such as phosphatidylethanol (PEth), a direct metabolite of alcohol use that is highly specific and reasonably sensitive for measuring previous 2–3 weeks' alcohol use,[22] can provide an objective measure of alcohol intake. Second, in some populations, alcohol use may be associated with illicit drug use, which may be associated with more rapid HIV progression (ie, illicit stimulant use[23,24]), and thus a spurious association of alcohol use with HIV progression may occur. A solution to this is to exclude other substance use, or conduct studies in settings with very little substance use, such as in Uganda.[25] Third, studies of persons on ART may be susceptible to residual confounding because of imperfect measurement of adherence, such as in the case of exaggerated self-reported adherence.[26] Thus, restricting the sample to those who are not yet on ART avoids this potential pitfall. Last, the relationship between unhealthy alcohol use and HIV disease progression may be confounded over time, if individuals who engage in unhealthy drinking experience declines in their health and thus reduce their subsequent drinking. This circumstance may spuriously reduce the apparent relationship between unhealthy drinking and HIV disease progression[27]; previous analyses of this issue have not accounted for this possibility.

The main goal of our study was to determine the biological impact of Unhealthy alcohol consumption was defined as on HIV disease progression, to clarify the previous inconsistent results. We conducted a prospective cohort study among persons not yet on ART in southwestern Uganda, a population with little other substance use, and used PEth, an objective measure of alcohol consumption, to augment self-reported unhealthy alcohol consumption.

METHODS

Study Participants

This was a longitudinal prospective cohort study conducted in Mbarara, Uganda. Participants were recruited from the Immune Suppression Syndrome Clinic of the Mbarara Regional Referral Hospital of the Mbarara University of Science and Technology. Study enrollment was conducted from September 2011 to August 2014. Eligibility criteria were adult (age ≥18) patient of the Mbarara Immune Suppression Syndrome Clinic; living within 60 km (or 120 km for men to increase male enrollment); fluent in either Runyankole (the local language) or English; and not yet meeting eligibility criteria for ART [ie, CD4 cell count <350 cells/mm^3 (cutoff changed to <500 cells/mm^3 beginning March 1, 2014), World Health Organization disease stage III or IV, or AIDS-defining illnesses]. We aimed to include equal numbers of persons drinking at unhealthy levels to those not drinking at such levels. To increase the proportion of unhealthy drinkers recruited, reporting any previous year alcohol consumption became a further eligibility criterion beginning in September 2013; the definition of unhealthy drinking used for analysis (see below) was unchanged.

Study Procedures

Study visits (baseline and follow-up) included a structured interviewer-administered assessment and phlebotomy for laboratory testing. Follow-up visits were conducted every 6 months, until loss to follow-up, death, ART initiation, study withdrawal, or the end of the study period (December 2015). Those who became eligible to start ART received a final interview and blood draw before initiating ART. All procedures were approved by the institutional review boards of the Boston University/Boston Medical Center, Mbarara University of Science and Technology, and the University of California, San Francisco, as well as the Uganda National Council for Science and Technology.

Laboratory Testing

Whole blood specimens were tested from all study visits to determine the CD4 cell count, and baseline specimens were tested to determine HIV viral RNA level (<40 copies/mL) in batches from frozen (−80°C) plasma. For PEth testing, whole blood was pipetted on the day of collection onto Whatman 903 cards and stored at −80°C before shipment in batches at room temperature to the United States Drug Testing Laboratories. PEth testing was performed measuring the most common PEth homologue, PEth 16:0/18:1, as previously described.[28] The limit of quantification was 8 ng/mL. All baseline dried blood spots were tested for PEth level. No further PEth testing was conducted for participants whose baseline PEth level was <8 ng/mL and who denied current (previous 3 months) alcohol use at all study visits; the PEth level was assumed to be <8 ng/mL for all visits. PEth was tested at all visits for participants who tested PEth positive (≥8 ng/mL) at any visit or who reported any alcohol consumption at any visit.

Measures

The study assessment included demographics, alcohol consumption [using the Alcohol Use Disorders Identification Test—Consumption (AUDIT-C),[29] modified to measure alcohol consumption in the previous 3 months]. Although the interval between study visits was 6 months to maximize

available funds and minimize participant fatigue, we chose a 3-month interval for self-report of alcohol use because recall for frequent behaviors may be better for shorter recall periods.[30] This period was also chosen to be roughly comparable with the maximum time period PEth can be detected after drinking ceases.[31] We also measured physical health functioning (using the Medical Outcomes Study HIV Survey[32,33]), symptoms of HIV, and symptoms of depression (using the Center for Epidemiologic Studies Depression Scale[34]).

We defined unhealthy alcohol use, the primary independent variable, as unhealthy drinking detected through self-report (AUDIT-C $\geq$3 for women and $\geq$4 for men) and/or PEth, as follows. We used a cutoff of PEth $\geq$50 ng/mL to indicate unhealthy drinking, a cutoff that was highly sensitive (93%) and reasonably specific (83%) for detecting daily drinking of 2 or more drinks per day on average (S. Stewart, personal communication). Thus, our primary measure of unhealthy alcohol use was either AUDIT-C positive or PEth $\geq$50 ng/mL.

Statistical Analysis

We calculated descriptive statistics to characterize study participants overall and by unhealthy drinking status. We used χ^2 or Fisher exact tests for categorical variables and *t*-tests or the Wilcoxon rank-sum test for continuous variables, as appropriate, to compare baseline characteristics between groups.

We evaluated the association between unhealthy alcohol consumption and CD4 cell count over time as our primary analysis. We used linear mixed effects models with subject-specific random intercepts and slopes (to account for within-subject correlation over time) and included a time by unhealthy alcohol use (as a time-dependent variable) interaction term to evaluate the main hypothesis that unhealthy alcohol use is associated with the rate of HIV disease progression. The primary outcome variable was CD4 cell count, assessed every 6 months from baseline to the final study visit (just before ART initiation or the end of the study). We adjusted for baseline age, religion, sex, time since HIV diagnosis, and $\log_{10}$ HIV RNA viral load as potential confounders. Before regression modeling, we calculated Spearman correlation coefficients for independent variables and covariates (all correlations $r < 0.40$).

We conducted several confirmatory analyses. These included analyses limited to those who were recruited and followed before the change in ART eligibility (from CD4 cell count <350 to <500 cells/mm^3) to assess the impact of this change. In an analysis to assess the impact of possible "sick quitters," we included only those participants reporting any previous 3-month alcohol use or PEth $\geq$8 ng/mL at baseline, and lifetime abstainers (ie, excluding past drinkers). In another analysis, we restricted the sample to those diagnosed with HIV in the past year, to be more comparable with the macaque models that focused on early infection.[9] We also reran the primary analysis excluding $\log_{10}$ HIV RNA viral load to assess potential overfitting by including viral load as a covariate. We also examined using alternative measures of alcohol consumption, such as using PEth alone, as a 3-level variable (PEth <50 ng/mL, PEth $\geq$50 to <210 ng/mL, and PEth $\geq$210 ng/mL; 210 ng/mL is a suggested PEth cutoff for excessive drinking[35]), as a continuous variable (log PEth), and modeling self-report, using 3 AUDIT-C categories (low-level drinking: <3 for women, <4 for men; medium-level drinking: $\geq$3 and <6 for women, $\geq$4 and <6 for men; and high-level drinking: $\geq$6 for men and women). We also applied pattern mixture models to the main analyses to explore departures from the assumption that data are missing at random.[36] For these analyses, we classified participants' visit patterns as complete (completing all scheduled study visits), monotone (missing one and all after visits), or intermittent (returning at least once after a missed visit), and we assessed interactions between these patterns and the parameters in the mixed effects model.

We additionally conducted analyses using marginal structural models (MSMs) to account for time-dependent covariates that may potentially be both confounders and mediators of the relationship between unhealthy alcohol consumption and HIV disease progression,[27,37,38] to determine whether an effect of unhealthy alcohol use on HIV disease progression could be masked if participants reduced their drinking in response to disease progression that was the result of previous unhealthy alcohol use. The parameters of the MSM were estimated using a Generalized Estimating Equation model of CD4 cell count using inverse probability of treatment weights, to balance the joint distribution of all covariables at each time point, thus eliminating both time-independent and time-dependent confounding. Weights were estimated using a logistic GEE model and accounted for time-independent and time-dependent variables (age, sex, marital status, education, literacy, overall health status, nausea, physical functioning, number of HIV symptoms, depression, and months since HIV diagnosis).

We examined a secondary outcome, time from enrollment to CD4 cell count below the threshold for ART initiation, using the Cox proportional hazards model using the exact method for handling tied event times.[39] Participants were censored at the earliest of the following: ART start, loss to follow-up, study withdrawal, or end of study. For this model, we conducted the additional analyses described for the mixed models above, and also conducted a sensitivity analysis by including a time-varying covariate for date of the guideline change for the CD4 threshold to initiate ART (ie, before vs. after March 1, 2014). In addition, we fit a separate model with time to CD4 <500 as the outcome, including only those with CD4 $\geq$500 at enrollment. Last, we used an MSM approach to examine the association of unhealthy alcohol use with time to CD4 cell count below the threshold of ART initiation, accounting for time-dependent confounding. All analyses were conducted using 2-sided tests and a significance level of 0.05.

Sample Size

A priori, we estimated the sample size needed to detect the expected differences in CD4 cell count decline between

TABLE 1. ART Naive HIV-Infected Persons in Southwestern Uganda: Participant Characteristics at Baseline (N = 446)

Variable	Response	Overall	Unhealthy Drinking * = Yes	Unhealthy Drinking * = No	*P*
All		446 (100.0%)	193 (43.3%)	253 (56.7%)	
Age	<30	164 (36.8%)	66 (40.2%)	98 (59.8%)	0.59
	30–40	173 (38.8%)	79 (45.7%)	94 (54.3%)	
	>40	109 (24.4%)	48 (44.0%)	61 (56.0%)	
Religion	Catholic	157 (35.2%)	80 (51.0%)	77 (49.0%)	<0.01
	Moslem	41 (9.2%)	8 (19.5%)	33 (80.5%)	
	Saved/Other	29 (6.5%)	3 (10.3%)	26 (89.7%)	
	Protestant	219 (49.1%)	102 (46.6%)	117 (53.4%)	
Sex	Male	144 (32.3%)	85 (59.0%)	59 (41.0%)	<0.01
	Female	302 (67.7%)	108 (35.8%)	194 (64.2%)	
Months since HIV diagnosis	N	446	193	253	0.17
	Mean (SD)	37.2 (44.0)	33.9 (39.1)	39.7 (47.3)	
	Median (25th, 75th)	18.5 (1.9, 64.6)	18.6 (1.4, 56.7)	18.4 (2.5, 72.9)	
Viral Load (log_{10})	N	442	191	251	0.12
	Mean (SD)	3.7 (1.0)	3.8 (1.1)	3.6 (1.0)	
	Median (25th, 75th)	3.7 (3.0, 4.3)	3.8 (3.0, 4.5)	3.7 (3.0, 4.3)	
CD4 Count	N	446	193	253	0.23
	Mean (SD)	570.0 (206.3)	556.5 (197.6)	580.2 (212.5)	
	Median (25th, 75th)	550.0 (416.0, 685.0)	541.0 (415.0, 666.0)	553.0 (421.0, 705.0)	
Alcohol use					
When last consumed alcohol (self-report)	In the past 3 days	114 (26.0%)	96 (50.8%)	18 (17.2%)	<0.01
	3 days–3 weeks ago	68 (15.5%)	48 (25.4%)	20 (8.0%)	
	3 weeks–3 months ago	57 (13.0%)	32 (16.9%)	25 (10.0%)	
	3 months–5 years ago	77 (17.5%)	7 (3.7%)	70 (28.0%)	
	Never or >5 years ago	123 (28.0%)	6 (3.2%)	117 (46.8%)	
AUDIT-C	Positive (≥3 for women, ≥4 for men)	133 (30.0%)	133 (100.0%)	0 (0.0%)	<0.01
	Negative	310 (70.0%)	59 (19.0%)	251 (81.0%)	
AUDIT-C score	N	443	192	251	<0.01
	Mean (SD)	2.1 (2.8)	4.4 (2.9)	0.4 (0.7)	
	Median (25th, 75th)	1.0 (0.0, 3.0)	4.0 (2.0, 6.0)	0.0 (0.0, 1.0)	
PEth level	≥50 ng/mL	153 (34.5%)	153 (100.0%)	0 (0.0%)	<0.01
	<50 ng/mL	290 (65.5%)	39 (13.4%)	251 (86.6%)	
PEth level (ng/mL)	N	446	193	253	<0.01
	Mean (SD)	160.7 (393.0)	365.4 (532.6)	4.5 (9.0)	
	Median (25th, 75th)	8.5 (BLQ†, 109.0)	148.0 (60.4, 403.0)	(BLQ†, BLQ†)	
AUDIT-C by PEth level	AUDIT-C positive and PEth ≥50 ng/mL	94 (21.2%)			
	AUDIT-C positive and PEth <50 ng/mL	39 (8.8%)			
	AUDIT-C negative and PEth ≥50 ng/mL	59 (13.3%)			
	AUDIT-C negative and PEth <50 ng/mL	251 (56.7%)			

*Unhealthy drinking = Yes defined as AUDIT-C+ and/or PEth ≥50 ng/mL; Unhealthy drinking = No defined as AUDIT-C− and PEth <50 ng/mL.
†Below the limit of quantification.

unhealthy vs. no unhealthy drinking, with 80% power and a 2-sided test with a significance level of 0.05. We considered the change in CD4 cell count from baseline to the 12-month time point (corresponding to testing an alcohol by time interaction), which is a conservative approach, given our analyses based on repeated measures. The SD of change in CD4 cell count over 1 year was previously 166 cells/mm^3,[15] with an expected retention rate of 90%, and a sample size of 450 would detect a difference in the 1-year decline in CD4 count between the groups of 50 cells/mm^3 or greater, similar to the difference in CD4 count previously found.[15]

TABLE 2. Unadjusted and Adjusted Models of CD4 Cell Count in ART Naive HIV-Infected Persons in Southwestern Uganda

	Model 1		Model 2	
	Unadjusted, n = 447		Adjusted for Age, Religion, Sex, Time Since HIV Diagnosis, HIV Viral Load, n = 443	
	β (95% CI)	*P*	β (95% CI)	*P*
Unhealthy drinking* (main effect)				
Yes	−16.25 (−50.07 to 17.58)	0.35	−7.26 (−42.22 to 27.70)	0.68
No (ref)	—		—	
Months since baseline (main effect)	−2.27 (−3.92 to −0.63)	0.01	−2.00 (−3.63 to −0.37)	0.02
Interaction term				
Unhealthy drinking* (yes vs. no) × months since baseline	0.82 (−1.75 to 3.39)	0.53	0.79 (−1.76 to 3.34)	0.54
Age				
<30			−19.10 (−65.03 to 26.83)	0.65
30–40			−17.79 (−59.21 to 23.63)	
>40 (ref)			—	
Religion				
Catholic			2.63 (−32.36 to 37.62)	0.53
Moslem			−22.64 (−79.65 to 34.36)	
Saved/other			−42.43 (−109.37 to 24.50)	
Protestant (ref)			—	
Sex				
Male			−24.07 (−60.50 to 12.36)	0.20
Female (ref)			—	
Months since HIV diagnosis			−0.17 (−0.57 to 0.24)	0.41
HIV viral load (log_{10})			−49.09 (−65.14 to −33.04)	<0.01

*Unhealthy drinking = Yes defined as AUDIT-C+ and/or PEth >=50 ng/mL; Unhealthy drinking = No defined as AUDIT-C- and PEth <50 ng/mL.
CI, confidence interval.

RESULTS

Of 1096 persons approached for enrollment, 484 persons enrolled, 445 persons were initially deemed ineligible, and 167 persons declined enrollment. Reasons for declining enrollment included not having time (n = 70), not wanting to have blood drawn (n = 30), not wanting to participate (n = 20), worries about stigma or disclosure (n = 15), feeling too weak to participate (n = 13), needing permission from someone to participate (n = 11), and other unspecified reasons (n = 8). Declining participation did not differ by sex. After enrollment and baseline testing, we found that several participants were not eligible for this study, based on testing of stored specimens of participants whose HIV viral load was found to be low or undetectable (<500 copies/mL). We thus excluded 32 participants who were not HIV antibody positive, 5 who tested positive for the presence of nevirapine or efavirenz (the 2 most commonly used HIV drugs in Uganda), and one participant who was missing alcohol use data, leaving 446 participants for analysis.

At baseline, 30 percent (30%) of the participants were AUDIT-C positive and 35% had PEth level ≥50 ng/mL (Table 1). Most were concordant on AUDIT-C and PEth (57% concordant negative, 21% concordant positive), 13% were AUDIT-C negative but PEth ≥50 ng/mL, and 9% were AUDIT-C positive but PEth <50 ng/mL. Thus 43% of the cohort were defined as drinking at unhealthy levels (AUDIT-C positive and/or PEth ≥50 ng/mL). Two-thirds (68%) of participants were women; all reported lifetime abstention from heroin, methamphetamine, and cocaine, and 7 (1.6%) persons reported any lifetime marijuana use and 7 reported any lifetime khat use (1.6%). The median CD4 cell count at baseline was 550 cells/mm^3 [interquartile range (IQR) 416–685], and the median time since HIV diagnosis was 18.5 months (IQR 1.9–64.6).

The median duration of follow-up was 12.4 months (IQR 6.5–22.5), and the median number of study visits per participant was 3 (IQR 2–5). Over the course of the study, two-thirds (67%) graduated from the cohort because of starting ART, 25% were followed until the end of the study, whereas 8% were lost to follow-up or withdrew from the study.

Primary Outcome: CD4 Cell Count

The unadjusted and adjusted mixed models showed declines in CD4 cell count over time, but no statistically significant difference in the rate of the decline by unhealthy drinking (Table 2). The estimated decline in CD4 cell count from baseline over 1 year was −14.5 cells/mm^3 (95% confidence interval: −38.6 to 9.5) for unhealthy drinking vs. −24.0 cells/mm^3 (95% confidence interval: −43.6 to −4.5) for not drinking at unhealthy levels in the adjusted model (Table 3), and the *P* value for interaction was not statistically significant (*P* = 0.54). We did not find a significant

TABLE 3. Estimated 12-Month Change in CD4 Cell Count by Drinking Status for Primary and Additional Analyses of CD4 Cell Count in ART Naive HIV-Infected Persons in Southwestern Uganda

Model Descriptionx	Unadjusted Estimate (95% CI)	*P* for Interaction	Adjusted Estimate (95% CI) (Adjusted for Age, Religion, Sex, Time Since HIV Diagnosis, HIV Viral Load)	*P* for Interaction
Primary model		0.53		0.54
Unhealthy drinking*				
Yes	−17.43 (−41.73 to 6.87)		−14.53 (−38.58 to 9.52)	
No	−27.30 (−47.02 to −7.57)		−24.02 (−43.56 to −4.49)	
Model limited to observations before change in ART eligibility in 2014 (n = 379)		0.20		0.13
Unhealthy drinking*				
Yes	−66.31 (−117.48 to −15.13)		−61.07 (−112.86 to −9.29)	
No	−107.27 (−143.29 to −71.25)		−109.63 (−145.97, −73.28)	
Model excluding past drinkers (n = 338)		0.32		0.38
Unhealthy drinking*				
Yes	−18.35 (−43.46 to 6.76)		−16.23 (−41.14 to 8.68)	
No	−36.49 (−62.93 to −10.05)		−32.27 (−58.54 to −6.00)	
Model limited to persons diagnosed in the past year (n = 191)		0.45		0.49
Unhealthy drinking*				
Yes	−44.44 (−84.14 to −4.74)		−40.23 (−79.94 to −0.52)	
No	−24.91 (−58.96 to 9.15)		−22.50 (−56.69 to 11.70)	
Viral load excluded from model				0.54
Unhealthy drinking*				
Yes	—		−16.86 (−41.16 to 7.44)	
No	—		−26.56 (−46.29 to −6.83)	
Model using PEth categories to represent drinking level		0.69		0.71
PEth ≥210 ng/mL	−9.06 (−49.10 to 30.98)		−5.77 (−45.49 to 33.95)	
PEth ≥50 ng/mL and <210 ng/mL	−28.56 (−71.96 to 14.83)		−23.11 (−66.38 to 20.15)	
PEth <50 ng/mL or confirmed abstainer	−27.79 (−47.78 to −7.81)		−24.05 (−43.82 to −4.28)	
Model using continuous PEth to represent drinking level				
Log PEth (per 1 unit log10 PEth change, per 12 months)	−31.57 (−54.53 to −8.62)	0.47	−23.14 (−46.57 to 0.29)	0.49
Model using AUDIT-C categories to represent self-reported drinking level		0.26		0.29
High: AUDIT-C ≥6	5.98 (−34.60 to 46.56)		7.74 (−32.65 to 48.13)	
Medium: AUDIT-C positive† and AUDIT-C <6	−20.18 (−57.79 to 17.43)		−15.91 (−53.45 to 21.63)	
Low: AUDIT-C negative	−30.52 (−48.47 to −12.56)		−27.04 (−44.82 to −9.25)	
Marginal structural model‡				0.22
Unhealthy drinking*				
Yes	—		−2.85 (−69.17 to 63.47)	
No	—		46.32 (−11.09 to 103.72)	

*Unhealthy drinking = Yes defined as AUDIT-C+ and/or PEth ≥50 ng/mL; Unhealthy drinking = No defined as AUDIT-C− and PEth <50 ng/mL.
†AUDIT-C positive defined as ≥3 for women, ≥4 for men.
‡Estimate from weighted model.
CI, confidence interval.

relationship between level of drinking and CD4 cell count decline over time in any of the additional analyses (limiting visits to those that occurred before ART eligibility changes, excluding past drinkers, excluding those diagnosed more than 1 year before enrollment, not including HIV viral load in the model, examining alternative measures for unhealthy alcohol use, and using MSM techniques to account for potential time-dependent confounding) (Table 3). We did not detect

TABLE 4. Cox Proportional Hazard Models of Time to CD4 Count Below Level for ART Eligibility in ART Naive HIV-Infected Persons in Southwestern Uganda

	Model 1: Unadjusted, n = 446		Model 2: Adjusted for Age, Religion, Sex, Time Since HIV Diagnosis, HIV Viral Load, n = 442	
	Hazard Ratio (95% CI)	*P*	Hazard Ratio (95% CI)	*P*
Unhealthy drinking*				
Yes	1.17 (0.91 to 1.49)	0.22	1.10 (0.84 to 1.43)	0.49
No (ref)	—		—	
Age				
<30			0.84 (0.59 to 1.19)	0.52
30–40			0.97 (0.71 to 1.33)	
>40 (ref)			—	
Religion				
Catholic			1.02 (0.78 to 1.34)	0.12
Moslem			1.60 (1.08 to 2.39)	
Saved/Other			1.02 (0.61 to 1.72)	
Protestant (ref)			—	
Sex				
Male			1.08 (0.82 to 1.42)	0.60
Female (ref)			—	
Months since HIV diagnosis			1.00 (1.00 to 1.00)	0.52
HIV viral load (log_{10})			1.33 (1.18 to 1.51)	<0.01

*Unhealthy drinking = Yes defined as AUDIT-C+ and/or PEth ≥50 ng/mL; Unhealthy drinking = No defined as AUDIT-C− or PEth ≥50 ng/mL.
CI, confidence interval.

significant differences across patterns of missing data in the pattern mixture models (*P* value for interaction: 0.68) nor did we detect a relationship between unhealthy alcohol use and HIV disease progression within any missing data pattern group (all $P > 0.30$).

Secondary Outcome: Time to ART Eligibility

The unadjusted and adjusted hazard ratios for unhealthy drinking in examining time to ART eligibility (ie, CD4 cell count <350 cells/mm^3 before March 1, 2014, CD4 cell count <500 cells/mm^3 thereafter) were 1.17 (0.91–1.49) and 1.10 (0.84–1.43), respectively (Table 4). When we included a time-dependent indicator to reflect when the threshold for starting ART changed, when we examined time to CD4 cell count to 500 cells/mm^3, and when we limited the data to visits completed before the change in ART eligibility, the hazard ratios for unhealthy alcohol use were not substantially different from above. The additional analyses of time to ART eligibility (eg, excluding past drinkers, as above) yielded similar results (Table 5).

DISCUSSION

We conducted a large prospective study of CD4 cell count over time in persons with HIV who were not yet on ART and found no significant difference in the rate of CD4 decline for unhealthy vs. no unhealthy drinking in the primary analysis, nor in any of the extensive confirmatory analyses. The clinical significance of this is that unhealthy alcohol use does not seem to have a direct short-term impact on CD4 cell counts among persons with HIV who are not on ART. This is consistent with several prospective studies conducted in East Africa[18,19,21] and elsewhere[14,17] but differs from some others.[15,16,20] The gender distribution (68% women) was representative of that of HIV in eastern and southern Africa.[40] The PEth levels revealed some discordance with self-report, consistent with previous studies of persons with HIV.[41–45] The methodological strengths of this study are notable. We recruited a large number of unhealthy drinkers, incorporated biological measures of drinking, restricted the sample to persons not on ART, and did not include persons using other substances. In addition, we conducted multiple confirmatory analyses (ie, examining alternative measures of alcohol use, including using higher AUDIT-C and PEth cutoffs to examine very heavy drinking, key subgroups of participants, and accounting for potential time-dependent confounding) that were consistent with the primary results, arguing for the robustness of these findings.

Despite finding no impact on CD4 cell count decline, there is evidence that alcohol use negatively impacts the HIV epidemic and individuals with HIV in ways other than CD4 decline. First, alcohol use has been a consistent risk factor for HIV acquisition,[46–48] and thus drinkers make up a disproportionate number of those infected with HIV. Alcohol use can impact the risk of onward transmission of HIV as well, presumably as a consequence of increased sexual risk behavior. The literature strongly suggests that persons with HIV who consume alcohol have lower ART adherence,[4] putting viral suppression and HIV outcomes at risk. Drinkers also may be more likely to transmit HIV due to increased vaginal shedding.[49–51] In addition to acquisition and transmission of HIV, some evidence suggests that alcohol use adversely impacts gut factors related to microbial translocation, despite the absence of consequences of the latter on CD4 cell count decline.[52]

This study was limited by the changes in the ART initiation criterion that occurred during the course of the study. These changes restricted our ability to examine CD4 cell count decline to a narrow range of starting values (ie, above 500 cells/mm^3) and decreased the amount of follow-up time, because, by design, we were following participants only until they started ART. The relatively short follow-up duration (median 12.5 months) may have limited our ability to find associations of alcohol use with longer-term outcomes, such as a multifactor measure of HIV morbidity.[53] Because this study was observational, unmeasured confounding may have obscured our results. For example, we did not measure exercise or nutritional status.

We conclude from these results, despite some past literature to the contrary, that there is no clinically meaningful

TABLE 5. Cox Proportional Hazards Models for Time to CD4 Count Below ART Threshold, for Secondary Variable and Additional Analyses in ART Naive HIV-Infected Persons in Southwestern Uganda

	Unadjusted Hazard Ratio (95% CI)	*P*	Adjusted Hazard Ratioz (95% CI) (Adjusted for Age, Religion, Sex, Time Since HIV Diagnosis, HIV Viral Load)	*P*
Primary model		0.22		0.49
Unhealthy drinking*				
Yes	1.17 (0.91 to 1.49)		1.10 (0.84 to 1.43)	
No (Ref)	—		—	
Model limited to person-time before change in ART eligibility in 2014 (n = 379)		0.28		0.86
Unhealthy drinking*				
Yes	1.20 (0.86 to 1.67)		1.03 (0.72 to 1.49)	
No (Ref)	—		—	
Model excluding past drinkers (n = 337)		0.51		1.00
Unhealthy drinking*				
Yes	1.10 (0.83 to 1.45)		1.00 (0.74 to 1.35)	
No (Ref)	—		—	
Model limited to persons diagnosed in the past year (n = 190)		0.33		0.92
Unhealthy drinking*				
Yes	1.20 (0.83 to 1.73)		0.98 (0.64 to 1.49)	
No (Ref)	—		—	
Viral load excluded from model				0.32
Unhealthy drinking*				
Yes	—		1.14 (0.88 to 1.48)	
No	—		—	
Model of time to CD4 <500 (n = 263)		0.50		0.52
Unhealthy drinking*				
Yes	0.90 (0.65 to 1.24)		0.89 (0.62 to 1.27)	
No (Ref)	—		—	
Model including indicator for change in threshold for starting ART		0.80		0.63
Unhealthy drinking*				
Yes	1.03 (0.80 to 1.33)		0.94 (0.72 to 1.22)	
No (Ref)	—		—	
Model using continuous $\log_{10}$ PEth to represent drinking level	1.09 (0.97 to 1.21)	0.13	1.06 (0.94 to 1.20)	0.36
Model using PEth categories to represent drinking level		0.44		0.76
PEth ≥210 ng/mL	1.22 (0.90 to 1.67)		1.07 (0.76 to 1.50)	
PEth ≥50 ng/mL and <210 ng/mL	1.01 (0.71 to 1.44)		0.94 (0.65 to 1.36)	
PEth <50 ng/mL (Ref)	—		—	
Model using AUDIT-C categories to represent self-reported drinking level		0.56		0.73
AUDIT-C ≥6	1.01 (0.70 to 1.45)		0.98 (0.66 to 1.44)	
AUDIT-C positive and AUDIT-C <6	0.82 (0.56 to 1.19)		0.86 (0.58 to 1.26)	
AUDIT-C negative† (Ref)	—		—	
Marginal structural model‡				0.69
Unhealthy drinking*				
Yes			1.10 (0.79 to 1.52)	
No (Ref)			—	

*Unhealthy drinking = Yes defined as AUDIT-C+ and/or PEth ≥50 ng/mL; Unhealthy drinking = No defined as AUDIT-C− and PEth <50 ng/mL.
†AUDIT-C negative defined as AUDIT-C <3 for women, <4 for men.
‡Estimate from weighted model, estimates at 12 months.
CI, confidence interval.

biological impact of unhealthy alcohol use on the CD4 cell count decline among persons with HIV not yet on ART. However, unhealthy alcohol use has been previously shown to decrease ART adherence and increase HIV transmission, thus adversely impacting both individual- and population-level outcomes. In addition, there is suggestive evidence that

unhealthy alcohol use may negatively impact inflammation; for these reasons, unhealthy alcohol use should be strongly discouraged for persons with HIV. However, unhealthy alcohol use does not seem to have a short-term direct biological impact on CD4 cell count.

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