

UCSF

UC San Francisco Previously Published Works

Title

Alcohol Consumption and Tryptophan Metabolism Among People with HIV Prior to Antiretroviral Therapy Initiation: The Uganda ARCH Cohort Study.

Permalink

<https://escholarship.org/uc/item/4367x86q>

Journal

Alcohol and alcoholism (Oxford, Oxfordshire), 57(2)

ISSN

0735-0414

Authors

Pierre, Frantz
Forman, Leah S
Winter, Michael
et al.

Publication Date

2022-03-01

DOI

10.1093/alcalc/agab033

Peer reviewed

Article

Alcohol Consumption and Tryptophan Metabolism Among People with HIV Prior to Antiretroviral Therapy Initiation: The Uganda ARCH Cohort Study

Frantz Pierre¹, Leah S. Forman², Michael Winter^{2,3}, Debbie Cheng³, Christine Ngabirano⁴, Nneka Emenyonu⁵, Peter W. Hunt⁶, Yong Huang⁷, Winnie Muyindike⁸, Jeffrey Samet⁹, Judith A. Hahn¹⁰, and Kaku So-Armah^{11,*}

¹Department of Epidemiology and Biostatistics, Boston University School of Public Health, 72 E Concord St, Boston, MA 02118, USA, ²Biostatistics and Epidemiology Data Analytics Center, Boston University School of Public Health, 85 East Newton Street, M921, Boston, MA 02118, USA, ³Department of Biostatistics, Boston University School of Public Health, 801 Massachusetts Ave Boston, MA 02119, USA, ⁴Mbarara University of Science and Technology Department of Internal Medicine P.O Box 1410 Mbarara Uganda, Uganda, ⁵Department of Medicine, University of California San Francisco, 1001 Potrero Ave, San Francisco, CA 94110, USA, ⁶Division of HIV/AIDS, San Francisco General Hospital, San Francisco, CA, USA, ⁷Department of Bioengineering and Therapeutics, University of California San Francisco, 1700 4th Street, San Francisco, CA 94158, USA, ⁸Mbarara University of Science and Technology Department of Internal Medicine P.O Box 1410 Mbarara, Uganda, ⁹Department of Medicine, Boston University School of Medicine and Boston Medical Center, 801 Massachusetts Ave Crosstown, 2nd Floor Boston, MA 02118, USA, ¹⁰Departments of Medicine and Epidemiology & Biostatistics, University of California San Francisco, 550 16th St., 3rd floor San Francisco, CA 94158-2549, USA, and ¹¹Department of Medicine, Boston University School of Medicine, 801 Massachusetts Ave, 2nd Floor Boston, MA 02118, USA

*Corresponding author: Department of Medicine, Boston University School of Medicine, 801 Massachusetts Ave, 2nd Floor Boston, MA 02118, USA. Tel.: 617 414 6624; E-mail: kaku@bu.edu

Received 14 December 2020; Revised 2 April 2021; Editorial Decision 6 April 2021; Accepted 6 April 2021

Abstract

Aims: Alcohol is hypothesized to have effects on the kynurenine pathway of tryptophan catabolism, a potential mechanism for alcohol-induced depression and aggression. A biomarker of this pathway, the plasma kynurenine to tryptophan ratio (K/T ratio), has been associated with HIV progression, mortality and depression. Our aim was to assess whether hazardous alcohol consumption is associated higher K/T ratio among people with HIV.

Methods: Participants were a subset of the Uganda Alcohol Research Collaboration on HIV/AIDS Cohort. Alcohol consumption was categorized (abstinent, moderate and hazardous alcohol use) using the Alcohol Use Disorders Identification Test—Consumption and phosphatidylethanol (PEth). K/T ratio was the primary outcome. We used linear regression adjusted for age, sex, FIB-4, hepatitis B surface antigen, log (HIV viral load) to estimate the association between alcohol consumption and K/T ratio.

Results: Compared to abstinent participants, hazardous drinkers and moderate drinkers had higher K/T ratio but these differences did not reach statistical significance.

Conclusions: Our results suggest that hazardous alcohol consumption, in the context of untreated HIV infection, may not significantly alter kynurenine to tryptophan ratio as a measure of activity of the kynurenine pathway of tryptophan metabolism.

INTRODUCTION

Heavy alcohol use among people with human immunodeficiency virus infection is common (Galvan *et al.*, 2002; Nahvi and Cooperman, 2009) (Samet and Walley, 2010) and associated with immunologic, neurologic and cardiovascular complications (Freiberg *et al.*, 2013; Szabo and Mandrekar, 2009), but pathways for alcohol-associated increases in morbidity and mortality have not been fully explained (Williams *et al.*, 2016).

Tryptophan is an essential amino acid that is primarily metabolized by enzymes called hepatic tryptophan 2,3-dioxygenase (TDO) and indoleamine 2,3-dioxygenase-1 (IDO-1). Normally, 90% of tryptophan degradation occurs via TDO pathway. However, under conditions of immune activation (e.g. untreated HIV infection), the IDO-1 pathway of tryptophan degradation is upregulated (Schrocksadel *et al.*, 2006). TDO and IDO-1 break down tryptophan to kynurenine and by-products, including 3-hydroxyanthranilic acid, a free radical generator, quinolinic acid, an excitotoxin and anthranilic acid that are potentially neurotoxic and immunomodulatory (Byakwaga *et al.*, 2014; Favre *et al.*, 2010; Guillemin *et al.*, 2001; Jones *et al.*, 2013; Schrocksadel *et al.*, 2006). This makes tryptophan degradation relevant for HIV-infected drinkers, who may have increased tryptophan catabolism due to both HIV and alcohol, and thus may be at high risk for inflammation and ultimately neurodegenerative diseases and immune dysfunction.

HIV-associated immune activation increases IDO-1 activity. This catabolizes tryptophan via the kynurenine pathway, leading to elevations in the ratio of kynurenine to tryptophan in plasma (K/T ratio). Higher K/T ratio is associated with clinical progression in untreated HIV disease, poor CD4+ T-cell recovery during HIV antiretroviral therapy (ART), and increased mortality (Byakwaga *et al.*, 2014; Huengsberg *et al.*, 1998; Lee *et al.*, 2017; Zuo *et al.*, 2016). Tryptophan is a precursor of serotonin, a multifunctional neurotransmitter involved in regulation of behavior, including depression. Alcohol has major effects on tryptophan metabolism providing a potential mechanism for alcohol-induced depression and aggression (Badawy, 2002). Thus, among people with HIV, tryptophan catabolism may be a pathway through which heavy alcohol use exerts negative health consequences.

However, little is known about the effects of alcohol use on the kynurenine pathway of tryptophan metabolism among people with HIV. Our primary hypothesis is that the level of alcohol consumption is associated with higher K/T ratio, among people with HIV. Since immune activation is characteristic of HIV infection and upregulates an important enzyme in tryptophan degradation, we also explored whether monocyte activation, measured by soluble CD14 in our previous study (Carrico *et al.*, 2015), modifies the association between alcohol consumption and tryptophan metabolism.

MATERIALS AND METHODS

Ethics statement

The study procedures were all approved by the Institutional Review Boards of the University of California—San Francisco, Boston University Medical Center, the Mbarara University of Science and Technology and the Uganda National Committee on Science

and Technology. We obtained informed consent obtained from all participants at the enrollment visit.

Study design and participants

This was a secondary analysis of existing data and biospecimens. The participants in this investigation were a subset of participants in the Uganda ARCH (Alcohol Research Collaboration on HIV/AIDS) Cohort who were eligible for inclusion in the current study. The Uganda ARCH cohort is an ongoing prospective cohort study conducted in Mbarara, Uganda. This cohort study was formed to investigate the effects of heavy alcohol consumption on HIV disease progression prior to the initiation of ART. Inclusion criteria for the cohort have been previously published (Carrico, 2015). Briefly, adults aged 18 and over who had HIV and had not initiated ART were recruited from the Immune Suppression Syndrome (ISS) Clinic of the Mbarara Regional Referral Hospital in Mbarara, Uganda. The subset of participants were those chosen for our previous examination of alcohol consumption and monocyte activation. (Carrico *et al.*, 2015) In this prior study, 60 Uganda ARCH participants who reported unhealthy alcohol use (defined below) were selected along with a random subset of participants (frequency matched on sex) from the same cohort with lower risk alcohol use ($N = 60$) and abstinent alcohol use ($N = 60$). Frequency matching on sex was achieved by performing random selection separately among men and women in the lower risk and abstinent alcohol use groups in order to achieve a similar proportion of men and women in each alcohol exposure group. Participants from our prior study who did not have available serum/plasma samples for tryptophan and kynurenine testing ($N = 33$) or were later found to have been exposed to ART ($N = 11$) were excluded.

Exposure assessment and definitions

Alcohol consumption was assessed using self-report via the 3-month Alcohol Use Disorders Identification Test—Consumption (AUDIT-C) and phosphatidylethanol (PEth), an abnormal phospholipid that forms only in the presence of alcohol (Bradley *et al.*, 2007; Hahn *et al.*, 2012). The primary definition of alcohol consumption was a combined measure using both AUDIT-C and PEth. By combining AUDIT-C with PEth, we aimed to increase the sensitivity for detecting unhealthy alcohol use, by combining two measures that are highly specific but not completely sensitive.

The three categories of alcohol consumption, as defined in our prior study were: abstinent, moderate and hazardous alcohol use (Carrico *et al.*, 2015). The abstinent group, defined as AUDIT-C = 0 and PEth < 8 ng/ml, was used as the referent group. Moderate alcohol use was defined as PEth ≥ 8 ng/ml and AUDIT-C < 3 for women and < 4 for men. Participants with an AUDIT-C ≥ 3 for women, or ≥ 4 for men or a PEth of 50 ng/ml or greater were classified as hazardous drinkers. This conservative cutoff for PEth maximizes specificity, which minimizes the likelihood of false positive results (Stewart *et al.*, 2010).

Secondary definitions of alcohol consumption included AUDIT-C alone or PEth alone categorizing each into tertiles. We used these measures in secondary analyses to explore whether a purely

biological method of unhealthy alcohol use (PEth) would pick up associations that may be missed by using self-reported unhealthy alcohol use (AUDIT-C), which may be more prone to recall and social desirability biases.

Outcome assessment and definition

K/T ratio was the primary outcome. The measurement of tryptophan and kynurenine concentrations in nonfasting plasma samples was done according to protocols using liquid chromatography–tandem mass spectrometry (LC–MS/MS) described elsewhere in detail (Favre *et al.*, 2010; Huang *et al.*, 2013). Briefly, plasma (100 μ l) is mixed with Kyn-d4 and Trp-d5 internal standards and then precipitated with trifluoroacetic acid. The supernatant was directly analyzed by LC–MS/MS. The assay using surrogate matrix calibrators is validated for precision, accuracy, matrix effect, extraction efficiency and stability. Plasma was not ultrafiltered prior to freezing and it was stored at -80°C .

We conducted analyses of secondary outcomes kynurenine concentration and tryptophan concentration separately to assess whether kynurenine or tryptophan concentration was driving the hypothesized association of alcohol and K/T ratio. Logarithmic transformation was performed for K/T ratio and kynurenine to better approximate a normal distribution.

Covariate assessment and definition

Covariates included demographic characteristics (age, sex), liver disease (Fibrosis Index for Liver Fibrosis (FIB-4) (Sterling *et al.*, 2006), hepatitis B surface antigen [HBsAg]), smoking status and HIV characteristics (HIV viral load [log transformed], time since HIV diagnosis) and marker of monocyte activation (sCD14). We measured sCD14 using quantitative sandwich enzyme immunoassay method (R&D Systems; Minneapolis, MN, USA) as described previously (Carrico *et al.*, 2015). We selected these covariates because prior work indicates associations with alcohol use and K/T ratio (Badawy, 2017; Bilal *et al.*, 2018; Byakwaga *et al.*, 2015; Williams *et al.*, 2019). By adjusting for them, we account for demographic differences between alcohol groups; differences in the level of HIV control and immune dysfunction between alcohol groups; and the role of the liver in tryptophan catabolism. Although we did not have data on nutritional status of participants, we assessed food insecurity scores (Coates, 2007) and body mass index (BMI) for nutritional context.

Statistical analysis

We tabulated demographic characteristics of the study participants using frequencies and proportions for categorical variables. Means, medians, standard deviation and percentiles were used for continuous variables. For descriptive purposes, we used ANOVA for continuous variables, and chi-square tests for categorical variables to perform bivariate comparisons for each variable with the primary independent variable (3-level drinking group: hazardous; moderate; abstinent). In order to minimize chance of collinearity, the spearman correlation was also used to identify pairs of variables with correlation >0.40 . Such pairs were not included in the same regression model.

We used linear regression models to estimate the unadjusted association between alcohol consumption and K/T ratio. The regression models were then adjusted for age, sex, FIB-4, hepatitis B surface antigen, log (HIV viral load). To assess whether sCD14 modified the association between alcohol consumption and K/T ratio, the adjusted regression model was repeated to include an interaction

term (alcohol consumption by sCD14 dichotomized at the median of its distribution). Since K/T ratio declines with liver damage, we performed a sensitivity analysis excluding people with evidence of liver fibrosis (FIB-4 > 3.25) or who were hepatitis B surface antigen reactive.

Regression analyses were repeated for secondary exposure (AUDIT-C alone and PEth alone). Regression estimates for kynurenine to tryptophan ratio and kynurenine were back-transformed for ease of interpretation. Two-sided *P*-values, 95% confidence intervals and betas, were calculated and reported for all regression models.

RESULTS

The mean age of the 136 participants included in the study was 36 years, 56% were female, and all participants were black Africans. Forty-seven (35%) were abstinent, 28 (21%) had moderate drinking, and 61 (45%) had hazardous drinking. Among those abstinent, 14 (30%) reported never drinking, 7 (15%) reported their last drink was between 3 months to 1 year prior; 10 (21%) reported 1–5 years prior and 16 (34%) reported >5 years ago for the last drink. Median BMI was 22.6 kg/m^2 and food insecurity was common but neither differed across alcohol groups. Overall, few (10%) reported current smoking with hazardous drinking participants reporting the highest smoking prevalence (16%). Advanced liver fibrosis was uncommon (3%) and 10% of participants were positive for hepatitis B surface antigen. Almost half the participants had a CD4+ T-cell count <500 cells/mm^3 and mean HIV viremia was 3.8 log copies/ml. Participants who were hazardous drinkers had significantly higher sCD14 levels compared to those who were abstinent from alcohol as previously reported (Carrico *et al.*, 2015) (Table 1). Median (25th, 75th percentile) kynurenine, tryptophan and K/T ratio ($\times 1000$) were 1.9 (1.6, 2.3) μM , 43 (35, 50) μM and 47 (37, 62), respectively, and did not differ significantly by alcohol status.

In unadjusted linear regression models, no significant associations between alcohol consumption and K/T ratio were detected (Table 2). Compared to abstinent participants, hazardous drinkers (ratio of means: 1.09 95% confidence interval: (0.91, 1.29); $P = 0.35$) and moderate drinkers (1.05 (0.84, 1.30); $P = 0.68$) had higher K/T ratio but these differences did not reach statistical significance. The ratio of means is interpreted as follows for hazardous drinkers as an example: on average, mean K/T ratio for hazardous drinkers was 9% higher than that for those abstinent. Likewise, we did not detect statistically significant differences in kynurenine concentration or tryptophan concentration between abstainers and hazardous or moderate drinkers (Table 3).

In adjusted models, we did not detect significant associations between alcohol consumption and K/T ratio. Compared to abstaining, both moderate and hazardous drinking were associated with a 3% increase in K/T ratio (CI: 0.85, 1.24 $P = 0.80$; 0.88, 1.21 $P = 0.69$, respectively). HIV viremia and female sex were significantly associated with higher K/T ratio (Table 2).

We did not detect statistically significant interactions between alcohol group and sCD14 on kynurenine to tryptophan ratio (interaction *P*-value = 0.14). Heavy drinking with sCD14 above the median was associated with 33% higher K/T ratio (1.33 (0.96–1.83; $p_{\text{interaction}} = 0.09$) whereas moderate drinking with sCD14 above the median was only 1% lower K/T ratio (0.99 (0.67, 1.45; $p_{\text{interaction}} = 0.95$). Excluding people with FIB-4 > 3.25 or hepatitis B did not alter the associations of heavy drinking and K/T ratio.

Table 1. Characteristics of UGANDA ARCH Cohort of people living with HIV by level of alcohol use

	Overall (<i>n</i> = 136)	Abstinent (<i>n</i> = 47)	Moderate Drinking (<i>n</i> = 28)	Hazardous Drinking (<i>n</i> = 61)	<i>P</i> -value
Mean age, years	35.5 (10.2)	35.0 (10.4)	33.3 (9.2)	37.0 (10.4)	0.25
Female	76 (55.9%)	27 (57.4%)	17 (60.7%)	32 (52.5%)	0.74
Household food insecurity access scale					0.90
Food secure	22 (16.3%)	10 (21.3%)	4 (14.3%)	8 (13.3%)	
Mildly food insecure	20 (14.8%)	5 (10.6%)	5 (17.9%)	10 (16.7%)	
Moderately food insecure	55 (40.7%)	18 (38.3%)	12 (42.9%)	25 (41.7%)	
Severely food insecure	38 (28.1%)	14 (29.8%)	7 (25.0%)	17 (28.3%)	
Median (p25, p75) body mass index/kg/m ²	22.6 (20.7, 25.2)	23.1 (20.6, 25.5)	23.9 (21.6, 26.1)	21.8 (19.8, 25.0)	0.28
Time since last drink					
Never drank	—	14 (29.8%)	—	1 (1.6%)	
More than 5 years ago	—	16 (34.0%)	—	1 (1.6%)	
1–5 years ago	—	10 (21.3%)	1 (3.6%)	2 (3.3%)	
6 months–1 year ago	—	3 (6.4%)	1 (3.6%)	—	
3–6 months ago	—	4 (8.5%)	2 (7.1%)	2 (3.3%)	
3 weeks–3 months ago	—	—	8 (28.6%)	3 (4.9%)	
4 days–3 weeks ago	—	—	7 (25.0%)	12 (19.7%)	
In the past 3 days	—	—	9 (32.1%)	40 (65.6%)	
Current smoker (past 3 months)	14 (10.3%)	1 (2.1%)	3 (10.7%)	10 (16.4%)	0.04
FIB-4 < 1.45	96 (71.1%)	33 (70.2%)	23 (82.1%)	40 (66.7%)	0.66
FIB-4 1.45–3.25	35 (25.9%)	12 (25.5%)	5 (17.9%)	18 (30.0%)	
FIB-4 > 3.25	4 (3.0%)	2 (4.3%)	0 (0.0%)	2 (3.3%)	
Hepatitis B positive	13 (9.6%)	4 (8.5%)	1 (3.6%)	8 (13.1%)	0.38
Median (25th, 75th) years since first positive HIV test	3.0 (0.5, 6.9)	2.8 (0.6, 6.6)	1.1 (0.1, 6.5)	3.6 (0.7, 6.9)	0.55
CD4 count <350	13 (9.6%)	5 (10.6%)	1 (3.6%)	7 (11.5%)	0.77
CD4 count 350–499	46 (33.8%)	15 (31.9%)	9 (32.1%)	22 (36.1%)	
CD4 count ≥500	77 (56.6%)	27 (57.4%)	18 (64.3%)	32 (52.5%)	
HIV viral load (log ₁₀ transformed)	3.8 (1.0)	3.7 (1.0)	3.8 (0.8)	3.9 (1.1)	0.59
Median (25th, 75th) sCD14, ng/ml	1466.5 (1072.3, 1765.7)	1244.2 (1073.9, 1670.0)	1400.1 (958.2, 1664.6)	1613.2 (1128.0, 1901.5)	0.04
Median (25th, 75th) Kynurenine, μM	1.9 (1.6, 2.3)	1.9 (1.6, 2.3)	1.8 (1.7, 2.1)	1.9 (1.5, 2.3)	0.57
Median (25th, 75th) Tryptophan, μM	42.7 (34.6, 49.5)	43.4 (35.8, 49.5)	37.1 (33.4, 47.1)	42.7 (34.1, 48.5)	0.40
Median (25th, 75th) K/T ratio × 1000	46.5 (36.6, 61.9)	45.6 (37.2, 56.7)	50.4 (36.8, 58.2)	46.3 (35.4, 67.8)	0.41

Table 2. Association of alcohol use and K/T ratio in the Uganda ARCH cohort of people living with HIV

Independent variable	Unadjusted back-transformed betas (95% CI)	<i>P</i> -value
Abstinent	1 (ref)	—
Moderate drinking	1.05 (0.84, 1.30)	0.68
Heavy drinking	1.09 (0.91, 1.29)	0.35
Independent variable	Adjusted back-transformed betas (95% CI)	<i>P</i> -value
Abstinent	1 (ref)	—
Moderate drinking	1.03 (0.85, 1.24)	0.80
Heavy drinking	1.03 (0.88, 1.21)	0.69
Female	1.18 (1.00, 1.39)	0.04
Hepatitis B surface antigen reactive	1.16 (0.92, 1.47)	0.21
FIB4	1.03 (0.96, 1.11)	0.43
HIV viral load (log), copies/ml	1.26 (1.18, 1.36)	<0.01
Age, years	1.00 (0.99, 1.01)	0.61

Hazardous or moderate alcohol use was not significantly associated with kynurenine or tryptophan concentrations when these biomarkers were considered separately (Table 3).

In exploratory analyses, no significant associations between AUDIT-C score alone or PEth alone were detected for kynurenine to tryptophan ratio (Table 4).

Table 3. Association of alcohol use with kynurenine and tryptophan (separately) in the Uganda ARCH cohort of people living with HIV

Independent variable	Kynurenine		Tryptophan	
	Adjusted back-transformed betas (95% CI)	P-value	Adjusted betas (95% CI)	P-value
Abstinent	1 (ref)	—	1 (ref)	—
Moderate drinking	0.94 (0.80, 1.11)	0.45	−2.95 (−7.69, 1.79)	0.22
Heavy drinking	0.97 (0.85, 1.11)	0.62	−1.13 (−5.01, 2.74)	0.56

Table 4. Association of AUDIT-C and PEth tertiles (separately) with K/T ratio in the Uganda ARCH cohort of people living with HIV

	AUDIT-C		PEth	
	Adjusted back-transformed betas (95% CI)	P-value	Adjusted back-transformed Betas (95% CI)	P-value
Lowest tertile	1 (ref)	—	1 (ref)	—
Middle tertile	1.05 (0.88, 1.25)	0.59	1.09 (0.92, 1.29)	0.34
Highest tertile	0.98 (0.84, 1.16)	0.85	1.10 (0.94, 1.30)	0.22

DISCUSSION

Among Ugandans with HIV, neither hazardous nor moderate drinking, compared to abstaining from alcohol were associated with the kynurenine pathway of tryptophan catabolism.

Although prior studies have found significant associations between alcohol consumption and tryptophan catabolism (Badawy *et al.*, 1995; Eriksson *et al.*, 1983; Friedman *et al.*, 1988; Markus *et al.*, 2004; Pietraszek *et al.*, 1991; Siegel *et al.*, 1964; Walsh *et al.*, 1966), none of them have been conducted among people with HIV. This unique aspect of our study may explain some of the discrepancy between our findings and prior work. HIV-associated immune activation is associated with higher kynurenine, lower tryptophan and thus higher K/T ratio (Huengsborg *et al.*, 1998). Higher K/T ratio is associated with clinical progression in untreated HIV disease and poor CD4+ T-cell recovery during ART (Byakwaga *et al.*, 2014; Huengsborg *et al.*, 1998; Lee *et al.*, 2017). The effects of alcohol on tryptophan metabolism may be partly mediated through alcohol-related liver disease and/or NAD(P)H (reduced nicotinamide adenine dinucleotide [phosphate] production) impacting the TDO pathway of tryptophan catabolism (Badawy, 2017). In this case, HIV related increases in the IDO-1 pathway of tryptophan catabolism may be offset by liver cirrhosis-related decreases in the TDO pathway such that there would be no apparent association of heavy drinking and K/T ratio among people with HIV. However, there was little evidence of cirrhosis in our cohort and our sensitivity analyses excluding people with high likelihood of liver fibrosis or hepatitis B did not alter the association of heavy drinking and K/T ratio. Conversely to cirrhosis-related TDO inhibition, alcohol-related consumption of NAD(P)H may limit TDO inhibition thus increasing the TDO pathway of tryptophan catabolism (Berg *et al.*, 2002). These opposing effects underscore the complexity of teasing apart potential alcohol effects among people with HIV or HIV coinfection with viral hepatitis.

Other potential explanations for these discrepant findings involve differences in the degree of alcohol exposure in prior work compared to the current study. Prior studies linking alcohol consumption and tryptophan metabolism involved people with a clinical diagnosis

of alcohol use disorder or acute alcohol administration to healthy volunteers. Alcohol use disorder status in our study was not known and we did not assess the acute effects of alcohol administration. It is feasible that one or both of these conditions is required to observe an association between alcohol consumption and tryptophan metabolism.

Another possible explanation is that chronic alcohol consumption and acute alcohol consumption may have opposing effects on tryptophan metabolism. Chronic alcohol consumption among people with alcohol use disorders is associated with inhibition of a rate-limiting enzyme (TDO) required for tryptophan catabolism, which may or may not lower the K/T ratio, depending on other associated factors, e.g. extrahepatic IDO induction (Eriksson *et al.*, 1983; Friedman *et al.*, 1988; Pietraszek *et al.*, 1991; Siegel *et al.*, 1964; Walsh *et al.*, 1966). On the other hand, acute alcohol administration to healthy volunteers decreases tryptophan concentration, which would result in higher K/T ratio (Badawy *et al.*, 1995; Friedman *et al.*, 1988; Markus *et al.*, 2004; Siegel *et al.*, 1964; Walsh *et al.*, 1966).

In our population of people with untreated HIV infection and unknown alcohol use disorder status, these opposing effects could explain why we did not detect significant associations in contrast to studies in non-HIV populations. As discussed earlier, most tryptophan degradation occurs via TDO pathway but under conditions of immune activation (e.g. untreated HIV infection), the IDO-1 pathway of tryptophan degradation is upregulated (Schrocksnadel *et al.*, 2006). In people with HIV who drink alcohol, there are other determinants of K/T ratio beyond IDO-1 pathway activation. Diet, liver function and renal function contribute to the variability in K/T ratio and are simultaneously impacted by HIV status (Badawy and Guillemin, 2019). Excluding people with high likelihood of liver fibrosis or hepatitis B did not alter the associations of heavy drinking and K/T ratio. Our regression models do not include information on diet or renal function, which were not obtained in this study.

These results suggest important implications for research in this area. Future studies should assess alcohol use disorders, and detailed alcohol use data (e.g. type and patterns of use via the 90-day Timeline Followback or Ecological Momentary Assessment).

There are limitations to this analysis that warrant discussion. This study is a cross-sectional observational study that does not permit inference of causality and may be limited by residual and unmeasured confounding. We did not have data on alcohol use disorders or granular data on types and patterns of alcohol use. We were also limited by lack of data on nutritional status in the cohort to put baseline tryptophan levels into context. However, we did not detect statistical differences in BMI or food insecurity across alcohol groups. Blood samples used for K/T ratio determination were not necessarily obtained as fasting samples, which could impact plasma amino acid levels including tryptophan. Since ultrafiltration was not used when quantifying K/T ratio, we measured total tryptophan, majority of which is bound to albumin (Badawy and Guillemin, 2019). Differences in albumin levels (or free fatty acids which displace tryptophan from albumin) between study participants were not available and may have impacted our estimation of K/T ratio. The generalizability of our study findings may be limited to populations similar to those receiving care at the ISS Clinic in Mbarara, Uganda.

CONCLUSION

In conclusion, we were unable to detect an association of hazardous or moderate drinking, compared to abstaining from alcohol with the kynurenine pathway of tryptophan catabolism in Ugandans with HIV. Additional studies including people with and without HIV will help isolate HIV-specific from alcohol-specific effects on tryptophan metabolism and investigate how these effects may interact in people with HIV who drink alcohol. Future studies should also assess alcohol use disorders, and more granular alcohol use data (e.g. type and patterns of alcohol use via the 90-day Timeline Followback or Ecological Momentary Assessment).

DATA AVAILABILITY STATEMENT

All data are available on request excluding identifiable information. Please contact Dr. Kaku So-Armah.

ACKNOWLEDGEMENTS

Funding for this study was provided by the National Institutes of Health: R01 AA018631, U01AA020776, K24AA022586, U24AA020778, U24AA020779 and K01HL13414704. This work was facilitated by the Providence/Boston Center for AIDS Research (P30AI042853) Center for AIDS Research (CFAR).

CONFLICT OF INTEREST STATEMENT

Authors have no conflicts to disclose except Debbie Cheng serves on Data Safety Monitoring Boards for Janssen Research & Development.

REFERENCES

- Badawy AA. (2002) Tryptophan metabolism in alcoholism. *Nutr Res Rev* 15:123–52. doi: [10.1079/NRR200133](https://doi.org/10.1079/NRR200133).
- Badawy AA, Guillemin G. (2019) The plasma [kynurenine]/[tryptophan] ratio and indoleamine 2,3-dioxygenase: time for appraisal. *Int J Tryptophan Res* 12:1178646919868978.
- Badawy AA, Morgan CJ, Lovett JW, et al. (1995) Decrease in circulating tryptophan availability to the brain after acute ethanol consumption by normal volunteers: implications for alcohol-induced aggressive behaviour and depression. *Pharmacopsychiatry* 28:93–7. doi: [10.1055/s-2007-979626](https://doi.org/10.1055/s-2007-979626).
- Badawy AAB. (2017) Kynurenine pathway of tryptophan metabolism: regulatory and functional aspects. *International journal of tryptophan research: IJTR* 10:1178646917691938.
- Berg JM, Tymoczko JL, Stryer L. (2002) *Biochemistry*. New York: W H Freeman.
- Bilal U, McCaul ME, Crane HM, et al. (2018) Predictors of longitudinal trajectories of alcohol consumption in people with HIV. *Alcohol Clin Exp Res* 42:561–70. doi: [10.1111/acer.13583](https://doi.org/10.1111/acer.13583).
- Bradley KA, DeBenedetti AF, Volk RJ, et al. (2007) AUDIT-C as a brief screen for alcohol misuse in primary care. *Alcohol Clin Exp Res* 31:1208–17. doi: [10.1111/j.1530-0277.2007.00403.x](https://doi.org/10.1111/j.1530-0277.2007.00403.x).
- Byakwaga H, Boum Y 2nd, Huang Y, et al. (2014) The kynurenine pathway of tryptophan catabolism, CD4+ T-cell recovery, and mortality among HIV-infected Ugandans initiating antiretroviral therapy. *J Infect Dis* 210:383–91. doi: [10.1093/infdis/jiu115](https://doi.org/10.1093/infdis/jiu115).
- Byakwaga H, Hunt PW, Laker-Oketta M, et al. (2015) The kynurenine pathway of tryptophan catabolism and AIDS-associated Kaposi sarcoma in Africa. *J Acquir Immune Defic Syndr* 70:296–303. doi: [10.1097/QAI.0000000000000747](https://doi.org/10.1097/QAI.0000000000000747).
- Carrico AW, Hunt PW, Emenyonu NI, et al. (2015) Unhealthy alcohol use is associated with monocyte activation prior to starting antiretroviral therapy. *Alcohol Clin Exp Res* 39:2422–6. doi: [10.1111/acer.12908](https://doi.org/10.1111/acer.12908).
- Coates JASPB. (2007) *Household Food Insecurity Access Scale (HFIAS) for Measurement of Household Food Access: Indicator Guide (v. 3)*. Washington DC: FHI 360/FANTA.
- Eriksson T, Magnusson T, Carlsson A, et al. (1983) Decrease in plasma amino acids in man after an acute dose of ethanol. *J Stud Alcohol* 44:215–21. doi: [10.15288/jsa.1983.44.215](https://doi.org/10.15288/jsa.1983.44.215).
- Favre D, Mold J, Hunt PW, et al. (2010) Tryptophan catabolism by indoleamine 2,3-dioxygenase 1 alters the balance of TH17 to regulatory T cells in HIV disease. *Sci Transl Med* 2:32ra36.
- Freiberg MS, Chang CC, Kuller LH, et al. (2013) HIV infection and the risk of acute myocardial infarction. *JAMA Intern Med* 173:614–22. doi: [10.1001/jamainternmed.2013.3728](https://doi.org/10.1001/jamainternmed.2013.3728).
- Friedman MJ, Krstulovic AM, Severinghaus JM, et al. (1988) Altered conversion of tryptophan to kynurenine in newly abstinent alcoholics. *Biol Psychiatry* 23:89–93. doi: [10.1016/0006-3223\(88\)90109-6](https://doi.org/10.1016/0006-3223(88)90109-6).
- Galvan FH, Bing EG, Fleishman JA, et al. (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* 63:179–86. doi: [10.15288/jsa.2002.63.179](https://doi.org/10.15288/jsa.2002.63.179).
- Guillemin GJ, Kerr SJ, Smythe GA, et al. (2001) Kynurenine pathway metabolism in human astrocytes: a paradox for neuronal protection. *J Neurochem* 78:842–53. doi: [10.1046/j.1471-4159.2001.00498.x](https://doi.org/10.1046/j.1471-4159.2001.00498.x).
- Hahn JA, Dobkin LM, Mayanja B, et al. (2012) Phosphatidylethanol (PEth) as a biomarker of alcohol consumption in HIV-positive patients in sub-Saharan Africa. *Alcohol Clin Exp Res* 36:854–62. doi: [10.1111/j.1530-0277.2011.01669.x](https://doi.org/10.1111/j.1530-0277.2011.01669.x).
- Huang Y, Louie A, Yang Q, et al. (2013) A simple LC-MS/MS method for determination of kynurenine and tryptophan concentrations in human plasma from HIV-infected patients. *Bioanalysis* 5:1397–407. doi: [10.4155/bio.13.74](https://doi.org/10.4155/bio.13.74).
- Huengsborg M, Winer JB, Gompels M, et al. (1998) Serum kynurenine-to-tryptophan ratio increases with progressive disease in HIV-infected patients. *Clin Chem* 44:858–62. doi: [10.1093/clinchem/44.4.858](https://doi.org/10.1093/clinchem/44.4.858).
- Jones SP, Guillemin GJ, Brew BJ. (2013) The kynurenine pathway in stem cell biology. *Int J Tryptophan Res* 6:57–66. doi: [10.4137/IJTR.S12626](https://doi.org/10.4137/IJTR.S12626).
- Lee S, Byakwaga H, Boum Y, et al. (2017) Immunologic pathways that predict mortality in HIV-infected Ugandans initiating antiretroviral therapy. *J Infect Dis* 215:1270–4. doi: [10.1093/infdis/jix113](https://doi.org/10.1093/infdis/jix113).
- Markus CR, Sierksma A, Verbeek C, et al. (2004) Moderate whisky consumption in combination with an evening meal reduces tryptophan availability to the brain but does not influence performance in healthy volunteers. *Br J Nutr* 92:995–1000. doi: [10.1079/BJN20041287](https://doi.org/10.1079/BJN20041287).
- Nahvi S, Cooperman NA. (2009) Review: the need for smoking cessation among HIV-positive smokers. *AIDS Educ Prev* 21:14–27. doi: [10.1521/aeap.2009.21.3_suppl.14](https://doi.org/10.1521/aeap.2009.21.3_suppl.14).

- PIETRASZEK MH, URANO T, SUMIOSHI K, *et al.* (1991) Alcohol-induced depression: involvement of serotonin. *Alcohol Alcohol* **26**:155–9. doi: [10.1093/oxfordjournals.alcalc.a045096](https://doi.org/10.1093/oxfordjournals.alcalc.a045096).
- Samet JH, Walley AY. (2010) Interventions targeting HIV-infected risky drinkers: drops in the bottle. *Alcohol Res Health* **33**: 267–79.
- Schrocksadel K, Wirleitner B, Winkler C, *et al.* (2006) Monitoring tryptophan metabolism in chronic immune activation. *Clin Chim Acta* **364**:82–90. doi: [10.1016/j.cca.2005.06.013](https://doi.org/10.1016/j.cca.2005.06.013).
- Siegel FL, Roach MK, Pomeroy LR. (1964) Plasma amino acid patterns in alcoholism: the effects of ethanol loading. *Proc Natl Acad Sci USA* **51**:605–11. doi: [10.1073/pnas.51.4.605](https://doi.org/10.1073/pnas.51.4.605).
- Sterling RK, Lissen E, Clumeck N, *et al.* (2006) Development of a simple noninvasive index to predict significant fibrosis in patients with HIV/HCV coinfection. *Hepatology* **43**:1317–25. doi: [10.1002/hep.21178](https://doi.org/10.1002/hep.21178).
- Stewart SH, Law TL, Randall PK, *et al.* (2010) Phosphatidylethanol and alcohol consumption in reproductive age women. *Alcohol Clin Exp Res* **34**:488–92. doi: [10.1111/j.1530-0277.2009.01113.x](https://doi.org/10.1111/j.1530-0277.2009.01113.x).
- Szabo G, Mandrekar P. (2009) A recent perspective on alcohol, immunity, and host defense. *Alcohol Clin Exp Res* **33**:220–32. doi: [10.1111/j.1530-0277.2008.00842.x](https://doi.org/10.1111/j.1530-0277.2008.00842.x).
- WALSH MP, HOWORTH PJN, MARKS V. (1966) Pyridoxine deficiency and tryptophan metabolism in chronic alcoholics. *Am J Clin Nutr* **19**:379–83. doi: [10.1093/ajcn/19.5.379](https://doi.org/10.1093/ajcn/19.5.379).
- Williams EC, Hahn JA, Saitz R, *et al.* (2016) Alcohol use and human immunodeficiency virus (HIV) infection: current knowledge, implications, and future directions. *Alcohol Clin Exp Res* **40**:2056–72. doi: [10.1111/acer.13204](https://doi.org/10.1111/acer.13204).
- Williams EC, McGinnis KA, Edelman EJ, *et al.* (2019) Level of alcohol use associated with HIV care continuum targets in a National U.S. sample of persons living with HIV receiving healthcare. *AIDS Behav* **23**:140–51. doi: [10.1007/s10461-018-2210-6](https://doi.org/10.1007/s10461-018-2210-6).
- Zuo H, Ueland PM, Ulvik A, *et al.* (2016) Plasma biomarkers of inflammation, the kynurenine pathway, and risks of all-cause, cancer, and cardiovascular disease mortality: The Hordaland health study. *Am J Epidemiol* **183**:249–58. doi: [10.1093/aje/kwv242](https://doi.org/10.1093/aje/kwv242).