Published in final edited form as:

Alcohol Clin Exp Res. 2018 March ; 42(3): 531–539. doi:10.1111/acer.13592.

Investigating the relationships between alcohol consumption, cannabis use and circulating cytokines: a preliminary analysis

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Abstract

Background—In recent years, human and animal studies have converged to support altered inflammatory signaling as one molecular mechanism underlying the pathophysiology of alcohol use disorders (AUDs). Alcohol binds to receptors on immune cells, triggering signaling pathways that produce pro-inflammatory cytokines. Chronic inflammation is associated with tissue damage, which may contribute to negative effects of AUD. Conversely, cannabis is associated with decreased inflammatory signaling, and animal studies suggest that cannabinoids may impact alcohol-induced inflammation. Thus, the impact of cannabis on inflammation in AUDs in humans warrants examination.

Methods—We explored the relationship between self-reported alcohol and cannabis use and circulating levels of the pro-inflammatory cytokines IL-6, IL-8 and IL-1 β in the blood. Among $N=66$ regular drinkers (mean age=30.08), we examined circulating cytokines and administered questionnaires assessing alcohol consumption and days of cannabis use over the past 90 days. We examined whether alcohol consumption, cannabis use and gender were associated with changes in circulating cytokines, and whether there was a significant interaction between alcohol and cannabis use predicting blood levels of circulating cytokines.

Results—A positive association between alcohol and IL-6 emerged. We also observed a negative association between cannabis and IL-1 β . Follow-up moderation analyses indicated a cannabis by alcohol interaction predicting circulating IL-6, such that cannabis non-users showed a stronger relationship between alcohol and IL-6 compared to cannabis users.

Conclusions—These preliminary findings suggest that cannabinoid compounds may serve to mitigate inflammation associated with alcohol use. In addition, the present results provide data to inform future investigations, with the goal of ultimately leveraging knowledge of the role of inflammation in AUDs to develop more effective treatments focused on novel immune targets.

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The authors have no conflicts of interest or financial disclosures to report.

Keywords

Inflammation; Cytokines; Cannabinoids

Introduction

Recent evidence from human and animal research has converged to support the role of alcohol in promoting deleterious adaptations in inflammatory signaling cascades. It is likely that perturbation of the immune system is a critical mechanism in the etiology of AUD (Alfonso-Loeches and Guerri, 2011; Collier and Hutchinson, 2012; Leclercq et al., 2012; Leclercq et al., 2014a; Mayfield et al., 2013), as alcohol appears to modulate inflammation, specifically through actions on the nuclear factor- κ B (NF- κ B) pathway (Lenhert et al., 2014; Maraslioglu et al., 2014). Briefly, inflammation is a component of the innate immune system, which relies on germline-encoded, pattern-recognition receptors, such as Toll-like receptors (TLRs), to identify invading pathogens and activate various immune cells. Of particular importance to the discussion of alcohol and immune signaling is TLR4, a cell-surface receptor found in numerous peripheral immune cells as well as microglial cells in the brain. Activation of the TLR4-mediated pathway by alcohol is associated with the aberrant production of inflammatory mediators, such as pro-inflammatory cytokines, (Fernandez-Lizarbe et al., 2009).

Alcohol-related damage to the brain and other organs (e.g., the liver), is likely mediated in part by peripheral inflammation, which can be measured by quantifying the levels of circulating cytokines in the blood. Chronic alcohol use in humans impairs the function of the intestinal barrier (Parlesak et al., 2000), and increases translocation of lipopolysaccharide (LPS; an endotoxin recognized by TLR4) from the gut (Wang et al., 2010), thereby causing LPS levels and pro-inflammatory cytokines in the blood to increase (Achur et al., 2010; Bode et al., 1987; Fujimoto et al., 2000; Leclercq et al., 2012; Leclercq et al., 2014a; Parlesak et al., 2000). In general, higher circulating LPS in the blood indicates greater gut permeability, and is associated with inflammation (e.g., increased cytokine production) in the context of alcohol use and alcoholic liver injury (Szabo and Lippai, 2014; Szabo et al., 2010).

Recently, circulating cytokines, such as the interleukins IL-6, IL-8 and IL-1 β , have been proposed as potential biomarkers for AUDs (Achur et al., 2010). One human study found increased circulating levels of IL-6, IL-8 and IL-1 β in blood samples among alcohol dependent patients compared to healthy controls (Leclercq et al., 2014a). Another study collected whole blood following an acute binge-alcohol administration in humans, and found an increase in circulating endotoxin in the blood, as well as increased inflammatory cytokines (e.g., IL-6) following an in vitro LPS stimulation procedure (Bala et al., 2014). In addition, blood levels of pro-inflammatory cytokines (e.g., IL-6) were correlated with alcohol craving in non-cirrhotic alcohol dependent patients (Leclercq et al., 2012), suggesting that peripheral cytokines may be able to directly influence the central nervous system, and underscoring their utility as a possible AUD biomarker. The mechanism through which this peripheral-to-central nervous system cytokine signaling occurs is not completely

understood, but may involve the ability of certain cytokines to penetrate the blood brain barrier (BBB) via circumventricular organs or active transport across the blood-brain barrier (Quan and Banks, 2007). Thus, although neuroinflammation cannot be measured *in vivo* in human subjects, assays of peripheral inflammation may still provide useful information regarding the impact of alcohol on inflammatory signaling.

Importantly, the endocannabinoid system has also been implicated as playing a critical role in the neurobiological mechanisms underlying alcohol use disorders (Erdozaid & Callado, 2011). Specifically, endocannabinoids have been shown to ameliorate inflammation associated with traumatic brain injury (TBI) in rodent models (Mayeux et al., 2017), while acute alcohol intoxication at the time of a TBI prolongs inflammation (e.g., upregulation of IL-6) (Teng & Molina, 2014). Similarly, cannabinoids (the active compounds in cannabis), such as cannabidiol (CBD) and tetrahydrocannabinol (THC), have been shown to regulate production of cytokines such as IL-6 and IL-1 β in response to inflammatory signals and CBD appears to mitigate the effects of heavy alcohol exposure on liver and brain inflammation (Hamelink et al., 2005; Liput et al., 2013; Yang et al., 2014) in animal models. Cannabinoid agonists have also demonstrated anti-inflammatory effects that confer protective benefits in individuals with co-occurring HIV and alcohol abuse (Perdisky et al., 2011). In addition, alcohol-users show upregulation of cannabinoid receptors (CNR2 and GPCR55) in human monocyte-derived dendritic cells (MDDCs) compared to non-users, and after *in vitro* ethanol exposure, MDDCs that were siRNA transfected to silence CNR2 and GPCR55 demonstrated higher levels of IL-1 β compared to non-transfected ethanol-treated cells, indicating that alcohol modulates changes in cannabinoid receptors, thereby impacting inflammatory effects such as cytokine production (Agudelo et al., 2013). Thus, given the opposing effects of alcohol and cannabinoids on inflammatory signaling, and the role of cannabinoid receptors in alcohol-induced inflammatory signaling, cannabinoid receptors may serve as an important treatment target for alcohol use disorders (Nair et al., 2015).

Given that we cannot directly measure circulating inflammatory markers in the brain *in vivo*, the present study examined relationships between alcohol consumption, cannabis use, and peripheral levels of three primary pro-inflammatory cytokines that have been associated in prior work with both alcohol and cannabis use, IL-6, IL-8 and IL-1 β . We hypothesized that alcohol use would be positively associated with blood levels of these three cytokines. We further expected that the positive relationship between alcohol consumption and cytokine levels would be stronger among individuals with lower levels of cannabis use compared to individuals who reported regular cannabis consumption.

Materials and Methods

The study was approved by the local ethics committee and written informed consent was obtained from all participants.

Sample

A total of 66 male and female participants between the ages of 25–40 were recruited from the greater Boulder-Denver area using flyers and online advertisements (e.g., craigslist) seeking participants who endorsed current drinking. The age range was selected to minimize

the impact of development and aging on inflammatory markers (Jaspan et al., 2006). Interested individuals were asked screening questions via phone. In order to assess the continuum of alcohol use behaviors, we recruited individuals broadly who endorsed any level of drinking over the past 3 months. However, given that prior work in this area has primarily been done in subjects with AUD (e.g., Leclercq et al., 2012; Leclercq et al., 2014a), we oversampled heavy drinking individuals (e.g., those who reported binge drinking 5 times or more per month, with binge drinking defined as 5 or more drinks in one sitting for men, and 4 or more drinks for women) to increase power at the more severe end of the alcohol use continuum.

Exclusion criteria for the present study included: (1) Daily smoker and/or tobacco user; (i.e. smoking less than 4 days out of the week); (2) Serious medical illness or injury within past 6 months; (4) Ever received a diagnosis of bipolar disorder or a diagnosis of psychotic spectrum disorder and not currently under treatment with antipsychotic or anticonvulsant medications; (5) Ever received a diagnosis of a neurological disorder (e.g. epilepsy); and (6) Use of cocaine, methamphetamine, heroin or other illicit drugs (except cannabis) in the previous 60 days (assessed on the phone screen and TLFB interview).

Procedure

To ensure that participants were not currently under the influence of any substances while completing the study, they were asked ahead of time to refrain from drinking alcohol within 48 hours, smoking cannabis within 6 hours or smoking cigarettes within 2 hours of the session. At the start of this session, all participants were breathalyzed. Only participants with a breath alcohol level (BrAC) of zero were allowed to participate in the study.

Substance Use Measures—A demographics questionnaire was used to collect information on age, sex, marital status, SES, occupation, income, education, and race. All participants also completed the Timeline Follow-back (TLFB (Sobell and Sobell, 1992), which is an assessment method that obtains estimates of daily alcohol, cigarette, and other drug use. The TLFB has been shown to have good psychometric characteristics and can generate variables that provide a wide range of information about an individual's drinking (e.g., pattern, variability, and magnitude of drinking). This instrument requires subjects to recall from memory the number of drinks consumed for each day over the prior 90 days as well as their use of tobacco products and recreational drugs, including cannabis. To further characterize alcohol use disorder severity in our sample, we also administered the Alcohol Dependence Scale (ADS), which has been used widely and found to have excellent predictive value with respect to DSM diagnosis (Kivlahan, Sher, & Donovan, 1989), ($\alpha = .838$). The total score on the ADS ranges from 0 to 47, with scores of 9 or more suggesting that alcohol dependence is likely.

Collection of Blood Samples

Blood samples were collected by a certified phlebotomist. Up to 63 mL (4.26 tablespoons) of blood was collected from each subject to provide adequate samples of plasma for the immunoassays.

Circulating Cytokine Assays

Within 30 minutes of blood collection, plasma was separated from blood cells by centrifugation (1000×g, 15 minutes). Plasma was aliquoted and stored at -80°C until immunoassays were performed. Circulating plasma levels of IL-1 β , IL-6, and IL-8 were measured using a quantitative high-sensitivity sandwich enzyme immunoassay technique (Quantikine HS ELISA, R&D Systems), following the manufacturer's instructions. Prior to performing the assay, samples and reagents were brought to room temperature ($18-25^{\circ}\text{C}$) without additional heating. Plasma IL-1 β concentration was measured using IL-1 β /IL-1F2 Quantikine HS ELISA, IL-6 using Human IL-6 Quantikine HS ELISA, and IL-8 using Human CXCL8/IL-8 Quantikine HS ELISA. Plasma samples were undiluted, ran in duplicate, and measured using a microplate reader Elx800 (Biotek Inc, Winooski, VT) set to the wavelength suggested by the manufacturer.

Overview of Analyses

Selection of TLFB variables

Regarding cannabis consumption, the TLFB only assesses for frequency (e.g., days of cannabis use), not quantity of cannabis used per day. Given the difficulty of quantifying the amount of cannabis used by an individual per day due to different THC strain potencies, varying methods of consumption, and because individual users do not always know the exact amount consumed in a particular sitting (Gray et al., 2009), we selected the TLFB variable “total cannabis days” to use in the present analyses.

Regarding the TLFB alcohol consumption variable, given that binge drinking has been associated with increased inflammation in both animals and humans (Bala et al., 2014; Pascual et al., 2014), and in order to capture the full range of drinking behaviors in the sample, we selected “drinks per drinking day” (which was calculated by dividing the total number of drinks consumed by the total number of drinking days) as the most appropriate TLFB measure to use in the present set of analyses. We selected “drinks per drinking day” because total number of drinking days alone would fail to capture the variance in drinks consumed per day, and total number of drinks alone would fail to capture binge drinking patterns (i.e., it would not highlight the difference between an individual who, for example, consumes 7 drinks in one day or 1 drink per day for 7 days). Note that the TLFB variable “number of heavy drinking days” was also not ideal, as this variable fails to capture the variance in number of drinks consumed per heavy drinking day, as it simply counts all days in which the person consumed at least 5 drinks per day (or 4 for women) as a heavy drinking day. This is not a good measure of drinking in this sample, as the maximum number of drinks consumed on a heavy drinking day in these subjects is, on average, 8.34 (SD=6.46, see Table 1).

Calculation of Cytokine Results

Following the manufacturer's instructions, for each cytokine assay, duplicate optical density (O.D.) readings were averaged for each standard, control, and sample and the average zero standard O.D. reading was subtracted from each. A standard curve was created using Microsoft Excel (Version 15.35 for Macintosh) and plotting the mean absorbance for each

standard on the y-axis against the concentration on the x-axis. Concentrations were estimated in pg/mL based on the line of best fit through the points on the graph.

Regression Analyses

We conducted a series of ordinary least squares (OLS) multiple regression analyses using SPSS Version 24 (IBM, Armonk, NY) in which we regressed each circulating cytokine separately on gender, TLFB drinks per drinking day, and TLFB days of cannabis use. Regression was selected in part due to the fact that it does not assume normality of the dependent variable (Aiken & West, 1991). In all models reported below, residuals were approximately normally distributed¹ and homoscedasticity was established, as assessed by a visual inspection of a plot standardized residuals versus standardized predicted values for each model.

In all models, slope values are reported as standardized coefficients. Critical alpha was set at .05 for all analyses. Given the exploratory nature of this work, we did not correct for multiple tests. We also limited the number of covariates included in each model to increase our power to detect effects. Gender was included as a covariate in all models, given that the relationship between alcohol consumption and circulating cytokines may differ for males and females (e.g., Pai et al., 2006). Because individuals were excluded from the study if they smoked cigarettes more than 3 days per week, and because they were instructed not to smoke cigarettes within 2 hours of the study session, smoking data was not included as a covariate in primary regression analyses. However, given the link between smoking and increased inflammatory signaling (Van der Vaart et al., 2005), we included each smoking variable (TLFB total cigarettes and TLFB cigarettes per smoking day) separately in all models in which a significant result emerged, in order to ensure that the significant result was not due to cigarette consumption. In addition, given that individuals were screened out for recent illicit drug use, we did not include a drug-use covariate in primary regression models. However, because, as we have now clarified, there were 6 individuals who did report use of an illicit substance 1–2 times in the past 90-days, we conducted follow-up analyses using a binary covariate for the presence of an illicit drug-use episode in the past 90 days, to ensure that illicit drug use was not significantly impacting these results.

Moderation Analyses

Because TLFB drinks per drinking day and TLFB days of cannabis use both significantly predicted circulating cytokines (cannabis was significantly negatively associated with circulating IL-1 β , whereas drinks per drinking day was positively associated with IL-6) and given that days of cannabis use and drinks per drinking days were significantly positively correlated with each other ($r=.380$, $p<.001$), we examined days of cannabis use as a potential moderator of the relationship between drinking and circulating IL-6 and IL-1 β . We regressed circulating IL-6 and IL-1 β separately on TLFB drinks per drinking day, gender, TLFB days of cannabis use, and the TLFB drinks per drinking day by TLFB days of cannabis use interaction term. Note that both drinks per drinking day and days of cannabis

¹One slight outlier remains in plot of standardized residuals for IL-6 models. Removal of this value does not change the overall pattern of significant results. To maintain the 3 SD cutoff for removing outliers, we have allowed this point to remain in the analysis.

use were mean centered for all moderation models. Because moderation analyses were planned *a priori* (Shadish et al., 2002), and given the difficulty in detecting interactions in field studies (McClelland and Judd, 1993), critical alpha was maintained at .05 for all analyses.

Results

Sample Characteristics

Table 1 presents the means and standard deviations for various demographic and substance use variables. Note that on average, the subjects in this sample were not severely alcohol dependent (mean ADS score=6.17, SD=4.4). Although subjects were screened out for illicit drug use on the phone screen, 6 subjects reported illicit drug use once or twice during the past 90 days on the TLFB. Given that none of these individuals reported use in the past 90 days (i.e., none had used more than twice in the past 90 days) or had used within a week of the study appointment, we allowed these individuals to remain in the analysis in an effort to preserve sample size.

Circulating Cytokine Levels

Peripheral cytokines were quantified in each of the 66 participants via the Quantikine HS ELISA kits. The total number of individuals included in analyses for each cytokine were slightly lower than 66 due to removal of several outliers for each cytokine, defined as individuals whose data points were more than 3 standard deviations above or below the mean (Tabachnick & Fidell, 2001), as well as several subjects who had circulating cytokine concentrations that were below the level of detection of the assay. Specifically, for IL-6, 2 outliers were dropped; for IL-8, 1 outlier was removed and 1 individual was dropped for having a concentration that was too low to detect; for IL-1 β , 7 individuals were removed due to having concentrations that were too low to detect. Final n-values were IL-6, n=64 (mean=.85, SD=.45); IL-8, n=64 (mean=3.51, SD=1.58); and IL-1 β , n= 59 (mean=.27, SD=.3). Given the controversy regarding whether to drop or assign values to data points below the level of detection, it should be noted that all analyses were also performed replacing the 7 non-detectable IL-1 β values and 1 non-detectable IL-8 value with zero.

Note that cytokine data were non-normally distributed, even following removal of outliers (for IL-8, skewness=.917, kurtosis=.242, Shapiro-Wilks=.923, p =.001; for IL-6, skewness=1.522, kurtosis=2.772, Shapiro-Wilks=.874, p <.001; for IL-1 β , skewness=1.837, kurtosis=2.824, Shapiro-Wilks=.748, p <.001). Circulating IL-8 was correlated with IL-6 (r =.290, p =.021) and IL-1 β (r =.402, p =.002). IL-6 and IL-1 β were not significantly correlated (r =.153, p =.257).

Regression Results

In the model in which IL-6 was the criterion, the predictors accounted for 10.1% of the variance in cytokines, $F(3, 60)=2.254$, p =.091, and inspection of individual regression slopes revealed a significant main effect of TLFB drinks per drinking day, (b =.339 $t(63)=2.378$, p =.021, See Figure 1). In the model in which IL-1 β was the criterion, the predictors accounted for 25.1% of the variance in cytokines, $F(3, 55)=6.129$, p =.001. Inspection of individual

regression slopes revealed a significant main effect of days of cannabis use ($b = -.471$ $t(58) = -3.778$, $p < .001$) but no main effect of TLFB drinks per drinking day (see Figure 2). In the model in which IL-8 was the criterion, the predictors explained 5.8% of the variance in cytokines, $F(3, 60) = 1.234$, $p = .305$, and no main effects emerged. All significant results remained when 1) TLFB total number of cigarettes and TLFB cigarettes per smoking day were each separately included as covariates; 2) when a covariate for illicit drug use was added to models; 3) by replacing the 7 non-detectable IL-1 β values and 1 non-detectable IL-8 value with zero.

Tests of Moderation

In the model in which IL-6 was the criterion, predictors explained 26.8% of the variance, $F(4, 59) = 5.402$, $p = .001$. Inspection of individual regression slopes indicated a significant cannabis by drinks per drinking day interaction ($b = -.968$ $t(63) = -3.666$, $p = .001$). To further explore the nature of this interaction, we compared cannabis non-users ($n = 29$ subjects) to individuals who had used cannabis at least once over the period covered by the TLFB ($n = 29$ subjects). In the non-users, the partial correlation, covarying for gender was positive (partial $r = .724$, $p < .001$), but in the users, the partial correlation covarying for gender was not significant (partial $r = -.138$, $p = .452$). Figure 3 illustrates these group differences. All significant results remained even when TLFB total number of cigarettes and TLFB cigarettes per smoking day were each separately included as covariates.

In the model in which IL-1 β was the criterion, predictors explained 28.1% of the variance $F(4, 54) = 5.266$, $p = .001$. Inspection of individual regression slopes indicates no significant cannabis by drinks per drinking day interaction. Results of main effects and interaction models are presented in Table 2.

Discussion

This study examined the relationship between alcohol use, cannabis use and blood levels of three circulating cytokines (IL-6, IL-8, IL-1 β). We found significant positive associations between alcohol consumption and IL-6, and significant negative associations between days of cannabis use and IL-1 β . Most notably, we observed an alcohol by cannabis interaction predicting circulating IL-6, such that individuals who did not report cannabis use demonstrated a significant positive association between alcohol consumption and IL-6, whereas those who did use cannabis showed no association. We did not observe any significant relationships between alcohol or cannabis use and IL-8.

Our finding that drinks per drinking day was positively associated with circulating IL-6 is consistent with prior work demonstrating that circulating interleukins are correlated with AUD severity (Leclercq et al., 2014a). For circulating IL-1 β , we found significant negative effects of cannabis use, which is also consistent with the literature (Klein, 2005), but no significant relationship with alcohol use. This suggests that, at least in the present sample of less clinically severe (i.e., not necessarily alcohol dependent) drinkers, the impact of cannabis use on IL-1 β may be more robust than the impact of alcohol consumption. However, in more severe samples such as alcohol dependent inpatients, alcohol consumption has been associated with increased circulating IL-1 β (Leclercq et al., 2014a). Thus, the lack

of association between alcohol consumption and IL-1 β in the present study may be related to the relatively low clinical severity of alcohol use symptoms in the sample.

Counter to our hypotheses, circulating IL-8 was not associated with alcohol consumption or cannabis use in the present study. Increased plasma IL-8 has been previously shown in alcohol dependent patients (Hill et al., 1993), however the individuals in this study were suffering from alcoholic hepatitis, or liver inflammation, which occurs in some patients as a result of chronic heavy drinking (Lucey et al., 2009). Similarly, another study demonstrated increased IL-8 among alcohol dependent inpatients compared to healthy controls (Leclercq et al., 2014a). The subjects in the present study did not have significant chronicity of alcohol use, and none reported alcohol-related liver problems or prior inpatient hospitalization, which may explain our lack of findings for IL-8. Further, although there is some evidence for an indirect link between cannabinoid signaling and IL-8 (Mormina et al., 2006), the nature of this potential relationship is not well understood. These results suggest that compared to other cytokines, IL-8 may not play a primary role in inflammatory signaling in substance use disorders.

We also examined cannabis use as a moderator of the relationship between alcohol consumption and circulating cytokines, given that recent studies have suggested that cannabinoids impact immune system function and inflammation in the context of alcohol use (Erdozain & Callado, 2011; Klein, 2005; Nair et al., 2015), and because alcohol consumption and cannabis use are often associated, as they were in the present sample. In the moderation model, a significant interaction between cannabis use and drinks per drinking day emerged. The direction of this interaction is such that lower cannabis use was associated with a stronger relationship between alcohol consumption and circulating IL-6. Specifically, among individuals who did not consume cannabis during the 90-days immediately prior to the study, there was a strong positive relationship between alcohol consumption and IL-6, but in those who did consume cannabis, there was no relationship. This finding suggests that the pro-inflammatory effects of alcohol use were attenuated in those who use both alcohol and cannabis. These results are consistent with findings from human studies demonstrating that cannabis use is associated with reductions in IL-6 (Keen et al., 2014).

This cannabis by alcohol interaction suggests that more in-depth exploration of the mechanism(s) by which cannabinoids may exert anti-inflammatory properties, particularly in the context of heavy alcohol use, are warranted. To date, the influence of cannabis and alcohol together on inflammatory signaling in humans is unknown. However, cannabis is now legal for medicinal use in more than 20 states, and as many as 40% of medical cannabis patients may use medical cannabis to reduce their alcohol consumption (Reiman, 2009). Although it has been previously suggested that cannabis may exacerbate the risks of using alcohol, this study suggests that cannabinoids may also have the potential to reduce alcohol-related harm. The identification of cannabinoids or combinations of cannabinoids that have anti-inflammatory properties without psychoactive effects (e.g., CBD) may have treatment implications. In light of the present findings, and given the role of cannabinoid receptors in alcohol-induced inflammation (Agudelo et al., 2013), the findings that cannabinoids may mitigate the damaging effects of alcohol (Liput et al., 2013; Hamelink et al., 2005; Yang et

al., 2014), and the data suggesting that individuals are increasingly attempting self-treatment with cannabis (Reiman, 2009), the relationship between alcohol consumption, cannabis use and inflammation should be further explored.

Limitations and Future Directions

A primary limitation of the present study is that we did not directly measure neuroinflammation, and it is unclear to what extent peripheral measures of inflammation such as peripheral cytokines are associated with neuroinflammation. Notably, research has demonstrated an association between circulating cytokines including IL-6 and functional and structural brain abnormalities in major depressive disorder and Alzheimer's disease (Frodl & Amico, 2014), suggesting that peripheral inflammation may be a useful marker for neural changes associated with disease-states. However, to date, it is unclear whether peripheral cytokines are significantly associated with neuroinflammation in human subjects with AUDs. Further, although alcohol is likely to contribute to peripheral inflammation, given that alcohol dependence is associated with elevated plasma cytokine levels in humans (e.g., (Achur et al., 2010; Leclercq et al., 2012; Leclercq et al., 2014a), it is possible that alcohol's effect on peripheral inflammation does not play a primary role in inducing neuroinflammation. Future animal research directly measuring neuroinflammatory markers is necessary to better understand this relationship. However, although it is not currently possible to collect direct measures of neuroinflammation from human subjects, there may be additional peripheral markers that could help to explicate the relationship between alcohol use and inflammation. In particular, recent work has suggested that peripheral inflammation may play a role in the development and maintenance of AUDs through alcohol-induced gut-permeability and intestinal dysbiosis, which refers to the development pathogenic bacteria in the gut. Intestinal dysbiosis has been shown to not only exacerbate depression, anxiety and craving, but possibly contribute to the symptoms of alcohol addiction through interacting with the stress system, disrupting normal sleep processes and interfering with social interaction (de Timary et al., 2017). Not surprisingly, given that the gut is a major source of immune factors, considerable human research has already demonstrated the critical role of gut microbiota in influencing psychiatric disorders and promoting aberrant behavior (Dinan et al., 2015; Foster and Neufeld, 2013). Further, alcohol dependent subjects with dysbiosis have demonstrated greater severity of AUD symptoms compared to those without dysbiosis (Leclercq et al., 2014b). Thus, an important next step is to further examine the possible role of gut permeability and intestinal dysbiosis in alcohol-induced inflammation through incorporating gut microbiome sample collection into studies observing the relationship between alcohol use and inflammation.

Another limitation of the present study is that no diagnostic information was collected about current or past alcohol or other substance use disorders. The inclusion of such clinical information in future work would be useful, given that inflammatory changes are likely related to clinical severity and chronicity of substance use (e.g., Leclercq et al., 2014a). Similarly, we did not collect diagnostic information about other psychiatric disorders such as depression, anxiety and bipolar disorders. This information should also be included in future research, given the emerging link between psychiatric illness and altered inflammatory signaling (Reus et al., 2015). Another limitation of the present study is that subjects were

scheduled throughout the day, and blood collection times were not standardized across participants. Future studies should consider controlling for the time of day when blood collections were performed, given that cytokines and other immune mediators are influenced by circadian rhythms (Lange et al., 2010). Further, the present study did not administer urine-drug screens to verify self-reported cannabis use. Future studies should incorporate an objective biological measure of cannabis and other illicit drug use in order to corroborate self-report data. Also, the present study included some light cigarette smokers (e.g., individuals who reported smoking less than 3 days per week) and several individuals who had reported using illicit drugs other than cannabis 1–2 times in the past three months. Although this pattern of substance use serves to make the present results more generalizable across the heavy-drinking population, future studies would ideally recruit subjects with no cigarette or illicit drug use, in order to more specifically examine the relationship between alcohol use and inflammation.

In addition, the present study did not directly measure circulating LPS in the blood. The addition of such a measure, as done in previous human studies (Bala et al., 2014), may provide useful information about the relationship between alcohol consumption and gut barrier function, given that LPS is thought to be translocated from the gut in AUDs (Wang et al., 2010), and shed light on the association between circulating LPS and downstream mediators of inflammation, such as cytokines. Finally, the present study includes only cross-sectional data, which means that causal conclusions about the relationship between alcohol use and inflammation cannot be drawn. To test causal hypotheses, future longitudinal work should examine how changes in drinking patterns over time may impact inflammatory markers.

Summary and Conclusions

In light of previously demonstrated associations between alcohol, cannabis and inflammation, the present results can serve to inform the selection of inflammatory markers to test in future analyses (e.g., peripheral IL-6 appears to be associated with alcohol consumption, while IL-8 may be less critical), and indicate the need for further research to better understand the role of inflammatory signaling in the brain and periphery in cannabis and alcohol use disorders. Ultimately, convergence between human and animal studies of alcohol consumption and inflammatory signaling across brain and peripheral tissues would provide the most compelling evidence for better understanding the relationship between inflammation and alcohol use, including explicating the possible role of peripheral immune signaling. These results also highlight the need for future work exploring the potential role for cannabinoids such as THC and CBD as anti-inflammatory treatments in the context of alcohol use disorders. Overall, despite the preliminary nature of these results and the need for replication and corroboration across human and animal work, our findings provide promising initial data to inform future investigations, with the goal of ultimately leveraging knowledge of the role of inflammatory signaling in alcohol use disorders to develop more effective treatments focused on novel targets within the immune system.

Acknowledgments

This work was supported by the University of Colorado Boulder discretionary funds to KEH and by NIH/NIAAA R01 AA024632 to KEH. LCB is supported by NIH/NIDA K23DA033302.

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Figure 1. Scatterplot showing the correlation between TLFB drinks per drinking day and circulating IL-6 (pg/mL).

Figure 2. Scatterplot showing the correlation between TLFB cannabis days and circulating IL-1 β (pg/mL).

Figure 3. Comparison of the relationship between drinks per drinking day and circulating IL-6 (pg/mL) in subjects who had not used cannabis in the past 90 days (A) and individuals who had consumed cannabis in the past 90 days (B).

Table 1

Sample Demographics

Demographic Variable	Overall Sample (n=66)
	Mean (SD) or Number (%)
Age	30.08 (4.7)
Gender (male)	32 (48.5%)
Race	
<i>American Indian/Alaska Native</i>	1 (1.5%)
<i>Asian</i>	6 (9.1%)
<i>African American</i>	3 (4.5%)
<i>White</i>	54 (81.8%)
Note. TLFB=Timeline Followback; ADS=Alcohol Dependence Scale <i>Mixed</i>	2 (3.0%)
ADS total	6.17 (4.4)
Drinks/Drinking Day^a	3.48 (2.4)
Heavy Drinking Days^a	12.39 (18.4)
Total Drinks^a	130.08 (147.8)
Total Drinking Days^a	31.86 (23.1)
Maximum Drinks^a	8.35 (6.46)
Cannabis Days^a	23.61 (33.9)
Total Cigarettes^a	9.61 (37.5)
Cigarettes/Smoking Day^a	.3 (.9)

Note.

^a90-day Timeline Followback; ADS=Alcohol Dependence Scale

Table 2

Multivariate association of gender, cannabis use and alcohol consumption with circulating cytokines: main effects and interactions.

Main Effects Models	Circulating Cytokines: IL-6				Circulating Cytokines: IL-1B				Circulating Cytokines: IL-8			
	Estimate	(se)	Beta	p-value	Estimate	(se)	Beta	p-value	Estimate	(se)	Beta	p-value
Overall Model $F=2.3$, Adj $R^2=0.056$, $p=.091$					Overall Model $F=6.1$, Adj $R^2=0.210$, $p=0.001$				Overall Model $F=1.2$, Adj $R^2=0.011$, $p=0.305$			
Intercept	0.69	0.098		<.001	0.212	0.061		0.001	3.358	0.358		<.001
Gender	-0.034	0.129	-0.038	0.791	0.165	0.084	0.277	0.055	-0.057	0.46	-0.018	0.901
Cannabis Days	-0.002	0.002	-0.132	0.308	-0.004	0.001	-0.471	<.001	-0.01	0.006	-0.213	0.11
Drinks Per Drinking Day	0.063	0.027	0.339	0.021	0.021	0.017	0.176	0.207	0.118	0.096	0.179	0.223
Interaction Models												
Overall Model $F=5.4$, Adj $R^2=0.218$, $p=0.001$					Overall Model $F=5.3$, Adj $R^2=0.227$, $p=0.001$							
Intercept	1.103	.097		<.001	0.265	0.067		<.001				
Gender	-0.043	0.117	-0.048	0.717	0.16	0.083	0.269	0.06				
Cannabis Days	0.009	0.003	0.651	0.01	-0.001	0.002	-0.13	0.617				
Drinks Per Drinking Day	0.11	0.077	0.592	<.001	0.034	0.019	0.283	0.071				
Cannabis* Alcohol Interaction	-0.002	0.001	-0.968	0.001	-0.001	<.001	-0.417	0.139				

Note. Interaction models were not run for IL-8 given that significant main effects were not found.