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Examining the moderating role of cannabis use on the relationship between alcohol consumption and inflammation in individuals with alcohol use disorder

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Abstract

Inflammation appears to be a critical mechanism in the development of alcohol use disorder (AUD) and a consequence of chronic alcohol use. The potential anti-inflammatory properties of cannabis may modulate the proinflammatory effects of alcohol. This study sought to extend previous work investigating the relationship between alcohol consumption, cannabis use and circulating interleukin (IL)-6 levels in a sample with AUD. One hundred and thirty-three individuals with an AUD provided blood samples to assess IL-6 and answered questions regarding alcohol and cannabis use. An ordinary least squares multiple regression analysis was conducted to assess the effect of alcohol and cannabis use on IL-6. A moderation analysis examined cannabis use as a potential moderator of the relationship between alcohol use and circulating IL-6 levels. Alcohol use was predictive of higher log IL-6 levels (standardized $\beta = 0.16$, $p = 0.03$), while cannabis use was not predictive of log IL-6 levels ($p = 0.36$). Days of cannabis use moderated the relationship between alcohol use and IL-6 levels, such that the relationship between alcohol use and IL-6 levels was only significant in individuals with AUD without recent cannabis use. This study extends previous work to a clinical sample with an AUD and underscores the importance of considering cannabis use in studies on alcohol use and inflammation. This study also indicates the need for in-depth analyses on cannabinoids and inflammation and the interaction between cannabinoids and alcohol use on inflammation.

KEYWORDS
alcohol and cannabis co-use, IL-6, inflammation

1 | INTRODUCTION

Inflammation appears to be a critical mechanism in the development of alcohol use disorder (AUD) and a consequence of chronic alcohol use. Alcohol use modulates inflammation in part through its distinct effects on pattern recognition receptors (PRRs), which bind to pathogen-associated molecular patterns (PAMPs) to induce an innate immune response.[1] One such type of PRR involved in alcohol use is toll-like receptors (TLRs). Chronic alcohol use increases the sensitivity of TLRs, leading to increased expression of proinflammatory cytokines[1,2] and activated immune signalling.[3] TLR4 in particular is associated with alcohol use, as activation of the TLR4-mediated pathway by

alcohol is associated with the downstream production of proinflammatory cytokines.[2,3] In people with AUD, there are increased plasma levels of proinflammatory cytokines compared to controls, including interleukin (IL)-6, which is correlated with alcohol craving and alcohol consumption.[4–6]

One growing area of interest in the AUD field is individuals who drink and concurrently use cannabis. While alcohol use is largely associated with adverse changes in inflammatory signalling cascades, cannabis and its cannabinoid components may mitigate inflammation. There is substantial evidence that TLR4 signalling is also impacted by phytocannabinoids, such as cannabidiol (CBD) and cannabigerol (CBG), which are neuroprotective and reduce proinflammatory cytokine release.[7] Delta-9-tetrahydrocannabinol (THC), the main psychoactive phytocannabinoid, may not share these anti-inflammatory properties, as some evidence suggests that THC alone may not decrease proinflammatory cytokines.[8] Findings have been mixed regarding cannabis use and IL-6. In a nationally representative sample of adults in the United States, recent cannabis use was associated with lower levels of proinflammatory markers, including IL-6.[9] However, a recent meta-analysis found no significant effect of chronic cannabis use on IL-6 levels.[10] Given the increasing prevalence of co-use, the associations between alcohol, cannabis and inflammation warrant investigation.

The potential anti-inflammatory properties of cannabis may modulate the proinflammatory effects of alcohol.[11,12] There is evidence that the positive association between alcohol use and IL-6 levels is stronger in individuals who drink regularly but do not use cannabis, compared with individuals who co-use alcohol and cannabis.[13] Given this potential mitigation of the inflammatory consequences of alcohol consumption, cannabis (specifically CBD) has been proposed as a potential therapeutic agent for AUD.[14] To date, the vast majority of research on inflammatory signalling, AUD, cannabis use and their co-use is preclinical. The limited work conducted on human samples has been in non-clinical populations (regular drinkers without a diagnosed AUD[13]). As such, the adaptation of this line of work to human samples with clinical pathology is imperative.

This study sought to extend previous work investigating the relationship between alcohol consumption, cannabis use and circulating IL-6 levels in a sample with AUD. We hypothesized that alcohol use would be positively associated with peripheral inflammation, whereas cannabis use would be negatively associated with peripheral inflammation. Further, we hypothesized that alcohol and cannabis use would interact, such that cannabis use would mitigate the inflammatory effect of alcohol drinking.

2 | METHODS

This was a secondary analysis of data from two randomized controlled trials of ibudilast for AUD (Grodin, Bujarski[15]: NCT03489850; Ray et al., under review: NCT03594435). Both trials were approved by the Institutional Review Board of the University of California, Los Angeles. All participants provided written, informed consent.

2.1 | Participants

One hundred and fifty-four participants between the ages of 18 and 65 were recruited and enrolled in the parent study trials; 149 participants provided usable blood samples for analyses. Of those, 133 participants reported either no recent cannabis use ($n = 72$) or recent cannabis use ($n = 61$). The remaining 16 participants had reported cannabis use inconsistently or reported no past-month cannabis use but endorsed past 6-month cannabis use and were excluded. Eligible participants were non-treatment-seeking for AUD (Study 1; NCT03489850; $n = 50$) or treatment-seeking for AUD (Study 2; NCT03594435; $n = 99$), met criteria for a current DSM-5 (Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition) diagnosis of AUD and drank >14 (males) or >7 drinks/week (females) in the 30 days prior to screening. Exclusion criteria were a past-year DSM-5 diagnosis of substance use disorder (excluding alcohol or nicotine), a lifetime diagnosis of psychotic disorder (schizophrenia or bipolar disorder), clinically significant alcohol withdrawal or the use of medications or medical conditions that would interfere with safe study participation. Women of childbearing age had to be practising effective contraception and could not be pregnant or nursing.

2.2 | Procedure

Data for this secondary analysis were obtained from the in-person screening visit (clinical assessments) and the randomization visit (peripheral markers of inflammation) prior to any medication administration. At each visit, participants were required to have a breath alcohol concentration of 0.00 g/dL and test negative on a urine toxicology screen for all drugs of misuse (except cannabis).

2.3 | Assessments

The Structured Clinical Interview for DSM-5 (SCID-5) was used to assess for AUD and other diagnostic exclusionary criteria.[16] The Clinical Institute Withdrawal Assessment for Alcohol Scale—Revised (CIWA-Ar) was used to assess withdrawal symptoms.[17] Alcohol use assessments included the Alcohol Use Disorder Identification Test (AUDIT[18]) and the Alcohol Dependence Scale (ADS[19]), both assessed over the past 12 months. Cannabis use severity over the past 6 months was assessed with the Cannabis Use Disorder Identification Test (CUDIT[20]). Smoking status and severity of nicotine dependence were assessed through the Fagerstrom Test for Nicotine Dependence (FTND[21]).

Participants completed the 30-day Timeline Followback (TLFB) interview to capture recent alcohol, cigarette and cannabis use.[22] Alcohol use was converted to standard drinks, and drinks per drinking day (DPDD) were calculated from the interview data. Given the difficulty in quantifying the amount of cannabis used due to strain differences, methods of use and the limited knowledge of THC content in cannabis products,[23,24] cannabis use was only assessed via a frequency measure (i.e., days of cannabis use).

2.4 | Peripheral inflammation

Blood samples were collected by venipuncture into ethylenediaminetetraacetic acid (EDTA) tubes, placed on ice, centrifuged for plasma acquisition and stored at −80°C for batch testing. Plasma levels of IL-6 were evaluated using the Meso Scale Discovery MULTI-SPOT Assay System (Rockville, MD, USA). Plasma samples were assayed in duplicate on a custom 5-plex from the Proinflammatory Panel 1 Human Kit. Briefly, blood samples were processed at 4°C into plasma aliquots. Plasma aliquots were stored at −80°C until assayed in a single batch per study. Assays were performed according to the manufacturer's protocol. Electrochemiluminescence (ECL) signals were measured on the MESO QuickPlex SQ 120 instrument (Rockville, MD, USA), and the DISCOVERY WORKBENCH software (Rockville, MD, USA) was used to generate a four-parameter logistic fit curve. For Study 1, the mean intra-assay coefficient of variation (CV) for IL-6 was 2.5%, and the mean inter-assay CV was 7.8%. For Study 1, 3.5% of the samples ($n = 5$) were below the level of detection (0.2 pg/mL) and were assigned a value of 0.2 pg/mL. For Study 2, the mean intra-assay CV for IL-6 was 2.1%, and the mean inter-assay CV was 2.7%. For Study 2, 5.05% of the samples ($n = 5$) were below the level of detection (0.2 pg/mL) and were assigned a value of 0.2 pg/mL.

2.5 | Data analysis

As expected, IL-6 levels were not normally distributed (skewness = 6.16, kurtosis = 45.87); therefore, the data were log-transformed (log-transformed skewness = 1.05, kurtosis = 2.24). An ordinary least squares multiple regression analysis was conducted. Log-transformed IL-6 levels were regressed on age, sex, body mass index (BMI), AUD severity, smoking status, study, TLFB cannabis use days and TLFB DPDD. Age, sex, BMI and smoking status were included as covariates, as these variables impact levels of circulating cytokines.[25,26] Study type was included as an additional covariate given the combination of datasets from two studies recruiting different AUD samples (non-treatment-seekers vs. treatment-seekers).

A planned moderation analysis examined days of cannabis use as a moderator of the relationship between alcohol use and circulating IL-6 levels. Log-transformed IL-6 levels were regressed on age, sex, BMI, AUD severity, smoking status, study, DPDD, cannabis use days and the interaction between DPDD and cannabis use days. Alcohol and cannabis use variables were mean centred for the moderation analysis in order to aid in the interpretation of the results.

3 | RESULTS

3.1 | Sample characteristics

Table 1 presents the means, standard deviations and range for demographic and clinical variables for the total sample, separated by the presence or absence of recent cannabis use. On average, participants met criteria for moderate AUD. Participants drank frequently and drank at binge drinking levels on drinking days. Recent cannabis use ranged from 0 to 30 days in the overall sample. There were no differences between individuals with and without recent cannabis use in terms of recent drinking or the majority of clinical characteristics. Individuals with AUD and recent cannabis use were younger, had higher ADS scores and, as expected, had higher scores on the CUDIT compared to individuals with AUD and no recent use.

3.2 | Alcohol use and cannabis use on IL-6

The predictors accounted for 33.0% of the variance in log IL-6 levels ($F[7,137] = 9.97$, $p < 0.001$). There was a significant effect of DPDD on log IL-6 ($\beta = 0.16$, $t = 2.19$, $p = 0.03$), such that log IL-6 levels increased as the number of drinks consumed on a drinking day increased. There was no significant effect of cannabis use on log IL-6 levels ($\beta = -0.07$, $t = -0.92$, $p = 0.36$). Of the covariates, BMI, age and study type were significant (see Figure 1A,B and Table 2).

3.3 | Moderation analyses

The predictors accounted for 35.1% of the variance in log IL-6 levels ($F[7,126] = 8.88$, $p < 0.001$). There was a significant interaction between DPDD and cannabis use days on log IL-6 levels ($\beta = 0.28$, $t = 2.22$, $p = 0.03$; Table 2). To probe this interaction, partial correlations were examined in individuals who recently used cannabis ($n = 61$) and individuals who did not recently use cannabis ($n = 72$). In individuals who did not report recent cannabis use, there was a significant correlation between DPDD and log IL-6 levels ($r = 0.30$, $p = 0.02$). However, there was not a significant partial correlation between log IL-6 levels and DPDD in individuals who used cannabis within the past 30 days ($r = 0.07$, $p = 0.60$; see Figure 1C,D).

4 | DISCUSSION

This study sought to examine the relationship between alcohol use, cannabis use and inflammation in individuals with AUD. This study replicated the findings of Karoly et al.[13] and extended this work into a clinical sample with AUD. Specifically, we found that across all individuals with AUD, a greater number of drinks per drinking day was predictive of higher log IL-6 levels. This relationship was moderated by recent (past 30 days) cannabis use, such that in individuals with AUD without recent cannabis use, drinks per drinking day predicted log IL-6 levels, whereas in individuals with AUD and recent cannabis use (i.e., individuals with recent co-use), there was no predictive relationship between drinking and log IL-6 levels.

Consistent with the original hypothesis, we found that alcohol use was predictive of log IL-6 levels in individuals with AUD. This is in line with previous work in the AUD field, where serum concentrations of IL-6 have been identified as one of the most consistently elevated

TABLE 1 Demographic and clinical characteristics of participants.

Characteristic	All mean ± SD ($n = 133$)	All range	AUD-only mean ± SD ($n = 72$)	AUD + recent cannabis use mean ± SD ($n = 61$)
Age[a]	40.14 ± 11.83	19–62	43.034 ± 11.35	36.74 ± 11.58
Sex (M/F)	81/52		44/28	37/24
BMI	27.55 ± 5.56	15.34–31.49	28.27 ± 6.02	26.71 ± 4.88
Race				
American Indian/Alaska Native	3		1	2
Asian	4		3	1
Black/African American	32		18	14
White	68		31	37
Pacific Islander	2		2	0
Mixed	14		10	4
Unknown	10		7	3
Cigarette smoker (Y/N)	76/57		44/28	32/29
AUD symptoms	6.14 ± 2.24	2–11	5.96 ± 2.19	6.36 ± 2.30
Drinking days (30 days)	21.68 ± 7.22	4–30	21.49 ± 7.25	21.92 ± 7.24
Heavy drinking days (30 days)	12.70 ± 9.34	0–30	13.21 ± 9.76	12.10 ± 8.86
Total drinks (30 days)	147.08 ± 110.89	26.27–532.42	151.08 ± 115.60	142.36 ± 105.81
Drinks per drinking day (30 days)	7.03 ± 5.14	1.98–42.24	7.49 ± 5.93	6.49 ± 4.01
CIWA-Ar score	0.56 ± 1.36	0–6	0.68 ± 1.77	0.44 ± 0.77
AUDIT score	18.77 ± 7.18	6–37	18.40 ± 7.77	19.21 ± 6.45
ADS score[a]	14.26 ± 6.92	1–33	13.14 ± 7.02	15.59 ± 6.62
PACS score	13.43 ± 6.33	0–30	13.10 ± 6.44	13.82 ± 6.21
Cannabis use days[a]	6.11 ± 10.01	0–30	0 ± 0	13.31 ± 11.08
CUDIT score[a]	8.05 ± 5.74	0–23	2.76 ± 3.58	8.79 ± 5.71
IL-6 (mg/L)	0.79 ± 0.89	0.16–7.70	0.90 ± 1.02	0.65 ± 0.71

Note: Alcohol consumption and cannabis use day variables are derived from the 30-day retrospective Timeline Followback interview.
Abbreviations: ADS, Alcohol Dependence Scale; AUD, alcohol use disorder; AUDIT, Alcohol Use Disorder Identification Test; BMI, body mass index; CIWA-Ar, Clinical Institute Withdrawal Assessment for Alcohol Scale—Revised; CUDIT, Cannabis Use Disorder Identification Test; IL-6, interleukin-6; PACS, Penn Alcohol Craving Scale.
[a]Significant difference between individuals with AUD without recent cannabis use and those with AUD and recent cannabis use.

cytokines in individuals with AUD relative to controls.[5] Moreover, IL-6 expression has been shown to be upregulated in monocytes from individuals with AUD,[27] and peripheral IL-6 levels are increased after acute oral and intravenous alcohol consumption in individuals with AUD.[28,29] Preclinical studies have identified upregulated IL-6 signalling pathways in the brains of alcohol-consuming rodents.[30] Importantly, while there is robust preclinical evidence of an inflammatory hypothesis of AUD,[31] the present study provides supportive evidence in a clinical sample.

Cannabis use moderated the relationship between alcohol use and log IL-6 levels, such that cannabis use mitigated the association between alcohol use and increases in peripheral inflammation. Importantly, individuals with AUD and no recent cannabis use did not differ from those with recent cannabis use on AUD severity or recent drinking, indicating that the lack of relationship between alcohol use and peripheral inflammation in those who recently used cannabis was not due to less drinking or less AUD phenomenology. This finding is in line with evidence that IL-6 levels are attenuated in individuals who regularly use both alcohol and cannabis[13] and that cannabis alone reduces IL-6 levels.[9] Specific cannabinoids comprising cannabis may confer these reductions in IL-6 levels. Preclinical evidence suggests that THC, cannabidivarin (CBDV), cannabielsoin and dehydrocannabielsoin may reduce lipopolysaccharide (LPS)-induced IL-6 levels.[32–34] Evidence from human monocytes indicates that CBD inhibits LPS- and TLR-induced IL-6 levels.[35,36] Moreover, CBD and CBG may increase levels of the anti-inflammatory cytokine IL-10 while additionally attenuating levels of proinflammatory cytokines.[37]

Cannabinoids may exert these anti-inflammatory properties by modulating TLR signalling, including TLR4.[7] It is thought that cannabinoids attenuate the myeloid differentiation primary response 88 (MyD88)-dependent pathway to decrease nuclear factor-κB (NF-κB) activity, resulting in a decrease in cytokine production and cell growth through TLR2 and TLR4.[38] TLR4 is the primary TLR associated with alcohol use, its downstream increases in production of proinflammatory cytokines[2,3] and the subsequent increase in severity

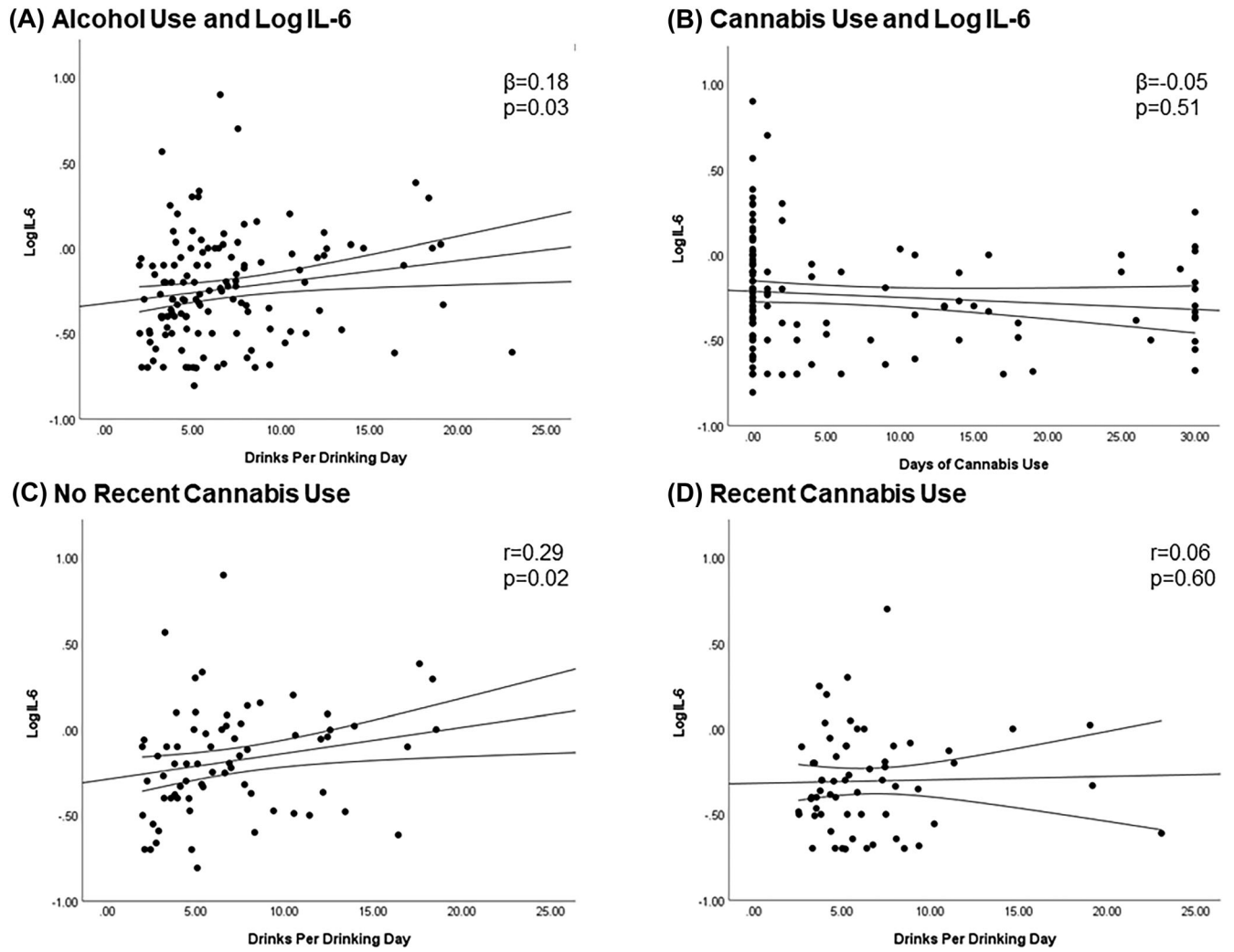

FIGURE 1 Relationship between alcohol use and cannabis use on interleukin (IL)-6. (A) Log IL-6 levels increased as the number of drinks per drinking day increased. (B) There was no significant effect of days of cannabis use on log IL-6 levels. (C) In individuals with alcohol use disorder (AUD) and no recent cannabis use, log IL-6 levels increased as the number of drinks per drinking day increased. (D) In individuals with AUD and recent cannabis use, there was no significant relationship between log IL-6 levels and the number of drinks per drinking day.

of alcohol problems,[4,6] alcohol craving and alcohol consumption.[6,31] Proinflammatory TLR signalling in turn leads to the activation of innate immune cells that may regulate the endocannabinoid system, upregulating receptor expression and endocannabinoid production.[38] Thus, the relationship between TLR signalling, cannabis and alcohol is complex and multifaceted. The present study suggests that the inflammatory properties of cannabis may moderate the proinflammatory effects of alcohol, effectively cancelling out the association between alcohol use and IL-6. Therefore, findings from this study indicate that the endocannabinoid system may serve as a therapeutic treatment target for AUD.[11] Specifically, cannabinoids with anti-inflammatory properties and without psychoactive effects (such as CBD) may be promising candidates to treat AUD.[14] However, while there may be a beneficial effect of cannabis and alcohol co-use in terms of inflammation, there are a number of negative consequences associated with the co-use of alcohol and cannabis, including increases in alcohol consumption and negative consequences such as more harm to relationships, finances and health.[39,40] Relatedly, this study excluded participants with a current cannabis use disorder (CUD). It is unknown if chronic cannabis use would similarly moderate the proinflammatory effects of alcohol or if it would potentiate these effects.

A primary limitation of the study is that no information was gathered on the type of cannabis product used or on the quantity of use. Without this information, it cannot be established what cannabinoids contributed to the moderating effect of cannabis use on the relationship between alcohol use and peripheral inflammation, nor can it provide evidence regarding a necessary amount of cannabis use to show a moderating effect. Additionally, the study sample was not a heavy cannabis use sample. Individuals with AUD who co-used cannabis reported ~13 days of cannabis use, on average, over the month prior to the study. While this pattern of use is similar to previous work in this area,[13] rates are lower than seen in heavy-use samples.[41] Relatedly, due to sample size concerns, all individuals who reported recent

TABLE 2 Main effect and interaction of alcohol consumption and cannabis use on interleukin-6 levels.

	Unstandardized β	Std. error	Standardized β	t	p
Main effect model					
Age	**0.01**	**0.002**	**0.41**	**4.70**	**<0.001**
Sex	0.04	0.05	0.06	0.82	0.41
BMI	**0.02**	**0.004**	**0.39**	**5.13**	**<0.001**
Smoking status	0.06	0.05	0.09	1.31	0.19
AUD severity	−0.005	0.04	−0.01	−0.12	0.91
Study type	**−0.21**	**0.06**	**−0.32**	**−3.44**	**<0.001**
Cannabis days	−0.002	0.002	−0.05	−0.67	0.51
DPDD	**0.01**	**0.005**	**0.18**	**2.26**	**0.03**
Moderation model					
Age	**0.01**	**0.002**	**0.41**	**4.79**	**<0.001**
Sex	0.04	0.05	0.07	0.88	0.38
BMI	**0.02**	**0.004**	**0.38**	**5.06**	**<0.001**
Smoking status	0.05	0.05	0.09	1.21	0.23
AUD severity	−0.003	0.04	−0.007	−0.08	0.94
Study type	**−0.21**	**0.06**	**−0.33**	**−3.56**	**<0.001**
Cannabis days (mean centred)	−0.008	0.005	−0.19	−1.43	0.16
DPDD (mean centred)	**0.04**	**0.02**	**0.33**	**2.15**	**0.04**
Cannabis days × DPDD	**−0.003**	**0.002**	**−0.28**	**2.22**	**0.03**

Note: Variables that were significant in each model are presented in bold typeface.
Abbreviations: AUD, alcohol use disorder; BMI, body mass index; DPDD, drinks per drinking day.

cannabis use were placed into the AUD and recent cannabis use group. This selection resulted in a heterogenous group of individuals who use cannabis, with cannabis use rates ranging from 1 to 30 days and CUDIT scores ranging from 1 to 23. Thus, the AUD + recent cannabis use group included individuals who used cannabis lightly to individuals who used cannabis frequently at potentially hazardous levels. Future studies should exclusively recruit individuals with AUD who use cannabis heavily to investigate the effect of heavy cannabis and alcohol use on inflammation. A secondary limitation of the study is that only peripheral cytokine-level inflammation was measured. Chronic alcohol consumption may result in sustained neuroinflammation, as detected in the post-mortem brains of humans with AUD. As such, measuring the impact of alcohol and cannabis co-use on neuroinflammation in addition to peripheral inflammation should be accomplished in the future. Moreover, only the proinflammatory cytokine IL-6 was measured in the present study to focus our findings on extending previous work[13] in a sample with AUD. It is recommended that future studies on inflammation and co-use incorporate measurements of anti-inflammatory cytokines (IL-10) as well as additional proinflammatory cytokines (tumour necrosis factor-α [TNF-α], IL-1β and IL-8). Doing so will provide additional insights into the inflammatory mechanisms of cannabis. A final limitation is that this study was a secondary analysis across two studies, which included non-treatment-seeking and treatment-seeking participants with a range of severity of AUD. As study type was a significant covariate in the analyses, it is recommended that future studies continue to consider treatment-seeking status.

In conclusion, this study found that heavier alcohol use was predictive of elevated circulating IL-6 levels in individuals with AUD and that cannabis use interacted with alcohol use to attenuate the relationship between alcohol consumption and increases in peripheral inflammation. This study extends previous work[13] to a clinical sample with an AUD and underscores the importance of considering cannabis use in studies on alcohol use and inflammation. This study also indicates the need for in-depth analyses on cannabinoids (e.g., THC, CBD and CBG) and inflammation and the interaction between cannabinoids and alcohol use on inflammation. Overall, this study provides clinical support for the inflammatory hypothesis of AUD and highlights the importance of considering and including co-use samples in AUD research.

AUTHOR CONTRIBUTIONS

Erica N. Grodin: Conceptualization; methodology; formal analysis; investigation; writing—original draft; visualization; funding acquisition. **Kaitlin R. McManus:** Writing—original draft; writing—review and editing. **Lara A. Ray:** Conceptualization; writing—review and editing; supervision; funding acquisition.

CONFLICT OF INTEREST STATEMENT

The authors have no conflicts of interest to disclose.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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