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Peripheral proinflammatory markers are upregulated in abstinent alcohol-dependent patients but are not affected by cognitive bias modification: Preliminary findings

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ABSTRACT

Background: Inflammatory pathways are known to be negatively affected in patients with alcohol use disorder (AUD). Cognitive bias modification (CBM), an emerging behavioral treatment that involves the ‘re-training’ of cognitive biases using computerized tasks, has been reported to reduce alcohol craving and relapse rates. The aim of this study was to compare peripheral concentrations of the proinflammatory biomarkers IL-18, IL-6, IL-1 β , TNF- α and CRP in AUD patients versus controls and to identify whether CBM treatment affected these biomarkers in AUD patients.

Methods: This 3-week double-blind randomized controlled study tested 36 male abstinent AUD patients receiving CBM or placebo-training, who were also compared to 18 male healthy controls. The approach avoidance task (AAT) was used to test the AUD patients before and after training. CBM training took place over 6 sessions, using a joystick-based approach-avoidance task. Blood samples were collected after the pre- and post-AAT test sessions for the AUD groups, and during an outpatient appointment with the controls.

Results: AUD patients, versus controls, presented with significantly higher plasma levels of TNF- α ($P < 0.0001$) and CRP ($P = 0.0031$). No changes in the CBM versus placebo groups were noted in IL-18, TNF- α and CRP concentrations following pre-post change or within group pretest- posttest analysis. IL-6 and IL-1 β levels fell under the lower detection limit, thus were not included in the final analyses.

Conclusions: This study confirms that the inflammatory system is altered in AUD. This was the first study that investigated whether CBM training affected proinflammatory markers in AUD patients.

1. Introduction

Millions of people worldwide suffer from alcohol use disorder (AUD). Several treatments have been developed over the years to help AUD individuals, ranging from medications to behavioral treatments (Pace and Uebelacker, 2018). CBT is currently used in clinical settings and has shown efficacy in reducing excessive alcohol drinking or relapse rates (Carroll and Kiluk, 2017). An emerging tool that is gaining

increased interest in the past decade is cognitive bias modification (CBM), which aims to target and modify automatic behavioral biases. CBM, a behavioral intervention that can be combined with the standard first-line treatment cognitive behavioral therapy (CBT), has been gaining recognition as a potential low-threshold and user-friendly group of adjunct interventions for treating alcohol and substance use disorders (Wiers et al., 2011). An advantage of CBM compared to standard CBT is its flexibility, easy accessibility as well as high cost

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effectiveness since patients do not require meeting up with a therapist.

AUD is associated with cognitive biases that include a higher inclination to approach, than avoid, alcohol-related stimuli in behavioral tasks such as the computerized implicit indirect AAT (Wiers et al., 2015a, b; Wiers et al., 2015). AUD patients also tend to pull the joystick faster than they push alcohol pictorial cues (as opposed to soft drink pictorial cues), an approach bias that has been linked to both alcohol craving and brain reactivity to alcohol cues in the amygdala and medial prefrontal cortex (Wiers et al., 2015a, 2014). The AAT-based CBM training of AUD patients is an implicit process that directs the individual how to avoid alcohol cues in the AAT by pushing them away. When compared to placebo or no training, CBM training has shown to lower alcohol craving and relapse rates (Eberl et al., 2013; Wiers et al., 2015b, 2011; Wiers et al., 2015, 2010), and to reduce brain activation in the amygdala during passive viewing of alcohol cues, associated with reduced alcohol craving (Wiers et al., 2015b) and medial prefrontal cortex brain activation on the AAT, associated with decreases in the behavioral alcohol approach bias scores (Wiers et al., 2015a).

As behavioral interventions, both CBM and CBT utilize a set of behavioral intervention elements that aim to alter negative thoughts and behavior patterns to treat mental health disorders, including addiction, albeit using different approaches. CBT aims to target conscious reflective processes whereas CBM aims to target unconscious processes (Wiers et al., 2016).

A recent Bayesian meta-analysis of individual patient data from 14 studies, investigating the clinical effectiveness of CBM in alcohol and smoking addiction, shows promise in this technique yet also the need for additional information on its effectiveness in AUD (Boffo et al., 2019). Additionally, there are no studies investigating the possible alterations of physiological mechanisms following CBM interventions. In the case of CBT, a relationship with inflammation has been suggested, with the proinflammatory markers Interleukin (IL)-6, Tumor Necrosis Factor alpha (TNF- α), and C Reactive Protein (CRP) being the most commonly analyzed (Lopresti, 2017). This poses the hypothesis that modifying inflammatory pathways plays a role in the mechanisms contributing to the benefits of CBT for treatment of mental health disorders such as depression (Lopresti, 2017). For instance, it has been reported that following CBT, major depressive disorder (MDD) patients showed an improvement in depressive symptoms together with a notable decrease in the proinflammatory marker levels, albeit the mechanism taking place is yet unknown (Gazal et al., 2013; Moreira et al., 2015). Furthermore, elevated proinflammatory cytokine concentrations have also been associated with the pathophysiology of anxiety and AUD (Crews and Vetreno, 2014; Felger, 2018; Renna et al., 2018). Changes in the innate immune system play an important role in AUD-related comorbidities, which can induce mood disorders (Kelley and Dantzer, 2011). Studies have shown that the immune system is activated in the brains of individuals with AUD, with chronic alcohol exposure resulting in an increase in different proinflammatory markers (Crews et al., 2015; Kelley and Dantzer, 2011). Acknowledging that acute neuroinflammation may not always be harmful, chronic neuroinflammation has a negative effect on the brain and may lead to different disorders and complications (DiSabato et al., 2016). Additionally, despite these observations, the CBT-inflammation link is somewhat debatable, with other studies showing no significant alterations in blood inflammatory levels such as patient populations with depression, anxiety (Memon et al., 2017), or with coronary heart disease (Olsson et al., 2018). Therefore, to date, it remains unclear whether inflammatory pathways may play a role as predictors and/or biomarkers of efficacy of cognitive interventions in patients with mental health disorders (Lopresti, 2017).

It is unknown whether a relationship exists between inflammatory pathways and the more novel behavioral intervention CBM in AUD individuals. There are several reasons for addressing this question, which was the overall aim of this study, specifically: 1) albeit not without inconsistencies, the literature on MDD and anxiety briefly summarized above support work on the potential link between certain

behavioral interventions and inflammatory pathways in other brain disorders beyond mental health (Felger, 2018; Lopresti, 2017; Moreira et al., 2015; Renna et al., 2018); 2) chronic alcohol use executes well-known proinflammatory effects when compared to healthy controls (Bell et al., 2017; Cannon et al., 2016; Crews and Vetreno, 2014; Imhof et al., 2001; Kawaratani et al., 2013; Neuman et al., 2015); and 3) there is growing evidence suggesting a role of peripheral and central inflammatory pathways in the development and maintenance of AUD (Crews and Vetreno, 2014; Vetreno and Crews, 2014). Relevant to the two latter points, heavy alcohol drinkers were reported to have significantly elevated levels of the peripheral proinflammatory markers (Bell et al., 2017; Crews and Vetreno, 2014; Imhof et al., 2001; Kawaratani et al., 2013; Neuman et al., 2015) when compared to non-drinkers.

The goal of this study was to conduct a preliminary investigation to assess: 1) potential differences in proinflammatory (CRP, TNF- α , IL-6, IL-18, IL-1 β) biomarkers in AUD patients compared to healthy controls; and 2) within AUD individuals, the potential effects of the Approach Avoidance Task (AAT)-based CBM, on these specific peripheral proinflammatory biomarkers, which were selected due to their known elevated levels in AUD individuals (Bell et al., 2017; Crews and Vetreno, 2014; Imhof et al., 2001; Kawaratani et al., 2013; Kiefer et al., 2002; Neuman et al., 2015; Szabo and Saha, 2015).

2. Materials and methods

2.1. Study design and participants

This investigation is a secondary analysis of two studies performed by Wiers and colleagues (Wiers et al., 2015a, 2014). Briefly, the two parent studies employed a double-blind, randomized design testing CBM versus a placebo-training control condition in AUD patients, and healthy controls. The AUD sample was recruited from the Salus Clinic in Lindow, Germany while the healthy controls were recruited via online advertisements from the same region. Eligibility for this study required AUD and healthy control patients to be abstinent from psychoactive medication and other drugs for a minimum of 6 months prior to participation, as determined by self-report with recent non-use confirmed by urine drug screening. Exclusion criteria included history of neurological disorders, DSM-IV axis I psychiatric disorders other than alcohol dependence, and > 4 months abstinence from alcohol prior participation. Smokers were abstinent from tobacco for at least 90 min prior to sessions. All participants were financially compensated for participation.

The plasma samples of 18 male healthy controls and 36 male AUD patients, the latter of which 19 underwent CBM training and 17 underwent placebo training, were analyzed in this study. AUD patients were randomly assigned to receive CBM or placebo training. All groups were matched for age and Body Mass Index (BMI), since BMI and obesity have been associated with inflammation (Park et al., 2010). The data for healthy controls and AUD patients are summarized in Table 1. The number of smokers versus non-smokers for the different groups was as follows: CBM (15 vs 4), placebo (15 vs 2), and healthy controls (6 vs 12), respectively. For additional information on the study participants and design, one can refer to the parent studies (Wiers et al., 2015a, 2014).

The parent studies were approved by the Ethical Committee of the Charité- Universitätsmedizin Berlin and participants were enrolled after providing written informed consent. The secondary analysis, described here, used fully de-identified data and samples from the parent studies, and transferred to the National Institutes of Health (NIH) after review and determination (# 13,344) of exclusion from Institutional Review Board review, as determined by the NIH Office of Human Subjects Research Protections.

Table 1
Demographic and clinical data of male healthy controls and male AUD patients in the CBM and placebo training group.

Characteristic	Healthy controls (n = 18)		AUD Patients (n = 36)			
	Mean	SD	CBM (n = 19)		Placebo (n = 17)	
			Mean	SD	Mean	SD
Age (years)	43.0	9.08	44.8	6.44	41.7	7.93
BMI	24.6	2.33	27.1	4.76	26.3	5.21
Length of abstinence (days)			71	121.5	61	48.2
Alcohol Dependence Scale			19	8.1	14	4.5

AUD = alcohol use disorder; CBM = cognitive bias modification; SD = standard deviation; BMI = body mass index.

2.2. Approach-avoidance task (AAT) at pretest and Posttest

The AAT was used to measure approach bias before and after training. The patient was required to respond to the format of the cue (portrait or landscape; i.e. irrelevant feature task) by pulling or pushing pictures with a joystick within 2 s, which resulted in the decrease or increase of cue size, respectively. Reliability scores of the irrelevant feature AAT have been reported as acceptable (Cousijn et al., 2011; Rinck and Becker, 2007). In both pre- and post-tests, patients performed 20 practice trials before undergoing 80 test trials (20:20 alcohol push:pull; 20:20 soft drink push:pull) that were presented over 2 blocks. A counterbalanced approach was used for picture format to response assignment (Wiers et al., 2015b).

2.3. Cognitive Bias modification (CBM) training

This was a modified version of the AAT, where each training session consisted of 400 trials (equal alcohol and soft drink cues) lasting around 15 min. Both experimental CBM (90:10 alcohol push:pull; 10:90 soft drink push:pull) and placebo (50:50 push:pull for both drink types) groups underwent 6 training sessions over a 3-week period (i.e. 2 training sessions per week), which was found to be the optimal number of sessions for clinical efficacy of CBM (Eberl et al., 2014). Training involved the use of 20 cues (10:10 alcohol:soft drink). Additional information on test assessment has been detailed in the parent studies (Wiers et al., 2015a, 2014). All AUD participants included in this study performed the required 6 training sessions.

2.4. Blood collection and assay analysis

Fasting ethylenediaminetetraacetic acid (EDTA) blood samples were taken directly after the pre- and post-AAT test sessions for the AUD groups, and during an outpatient appointment with the healthy control group. Blood collection was performed around the same time of the day in order to limit any confounding circadian changes in proinflammatory marker levels. Blood samples were centrifuged, and plasma aliquots were collected and stored at -80 °C. Plasma levels of IL-1β, IL-6, IL-18,

TNF-α, and CRP were quantified by ProteinSimple Simple Plex Ella Immunoassay per manufacturer instructions (ProteinSimple, Wallingford, CT) (Aldo et al., 2016). Plasma samples were thawed on wet ice, agitated using a vortex mixer, and centrifuged again at 3000 g for 10 min at 4 °C to separate out lipid content from the plasma. Samples were diluted 1:2 for IL-18 and TNF-α, and 1:2000 for CRP. Fifty μL of diluted sample was loaded into the Ella Simple Plex Immunoassay cartridge. The cartridge was run using provided ProteinSimple Ella software and equipment per manufacturer instructions (Aldo et al., 2016). Investigators performing the assay analysis were blinded to the study group the participants were in.

2.5. Statistical analysis

Baseline cytokine values were compared between the AUD and the healthy subjects using the Wilcoxon rank-sum test. To assess the effect of CBM on the AUD patients' proinflammatory status, the Wilcoxon rank-sum test was used to compare the change from pretest to posttest between the CBM and the placebo groups. Furthermore, the Wilcoxon signed rank test was performed to evaluate the pre-post change for each group separately. Nonparametric tests were used considering the small sample size and non-normal distributions of the data. The level of significance of 0.05 was chosen for this exploratory study. Effect sizes (ES) between CBM and the placebo training were calculated using the method proposed by Acion and colleagues (Acion et al., 2006). This ES measures the chance of a treated subject having better response than a placebo subject, with 0.5 indicating equally effective groups. Analyses were performed in SAS 9.3 (SAS Institute, Cary NC), whereas graphs were produced using GraphPad Prism 8.0 software (GraphPad, San Diego CA).

3. Results

There were significantly higher plasma levels of TNF-α ($P < 0.0001$) and CRP ($P = 0.0031$) in the AUD patient group when compared to the healthy controls' cohort, whereas no significant changes were observed in IL-18 levels ($P = 0.56$) (Table 2; Fig. 1A-C).

Table 2
Median and interquartile intervals of plasma IL-18, TNF-α, and CRP levels of healthy controls versus AUD patients and of AUD patients pre- and post-intervention.

	Healthy controls	AUD (total)	p-Value	CBM			Placebo		
				Pre-test	Post-test	p-Value	Pre-test	Post-test	p-Value
IL-18 (pg/ml)	316.3 (232.7-384.1)	361.0 (260.2-425.7)	0.56	340.9 (213.9-451.3)	303.9 (145.4-396.8)	0.94	380.2 (274.5-435.0)	398.2 (303.9-419.5)	0.73
TNF-α (pg/ml)	4.9 (3.8-5.8)	7.4 (5.8-9.5)	< 0.0001	6.9 (5.7-8.8)	7.4 (6.4-9.8)	0.30	9.0 (6.8-10.3)	7.3 (6.1-8.5)	0.13
CRP (mg/ml)	1.1 (0.9-1.9)	3.5 (2.0-8.9)	0.0031	3.4 (2.4-9.7)	4.9 (1.6-8.2)	0.83	2.4 (1.4-11.6)	4.4 (0.6-9.2)	0.97

All P values were > 0.05.

CBM = cognitive bias modification; AUD = alcohol use disorder; IL-18 = interleukin 18; TNF-α = tumor necrosis factor alpha; CRP = C-reactive protein.

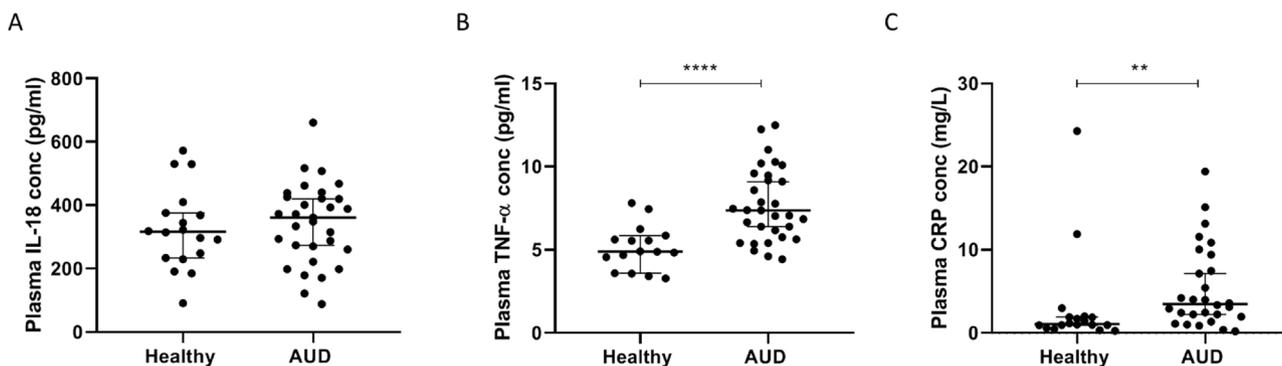


Fig. 1. Plasma concentrations of pro-inflammatory markers in the healthy control group and AUD patient group. A) Plasma IL-18 concentrations in pg/ml (Healthy controls: n = 18; AUD group: n = 31); B) Plasma TNF- α concentrations in pg/ml (Healthy controls: n = 16; AUD group: n = 31); C) Plasma CRP concentrations in mg/L (Healthy controls: n = 18; AUD group: n = 28). Individual data points of participants were plotted with the calculated median with 95% confidence interval (CI).

** indicates $P < 0.01$, **** indicates $P < 0.0001$ (Wilcoxon ranked sum test).

AUD = alcohol use disorder; IL-18 = interleukin 18; TNF- α = tumor necrosis factor alpha; CRP = C-reactive protein.

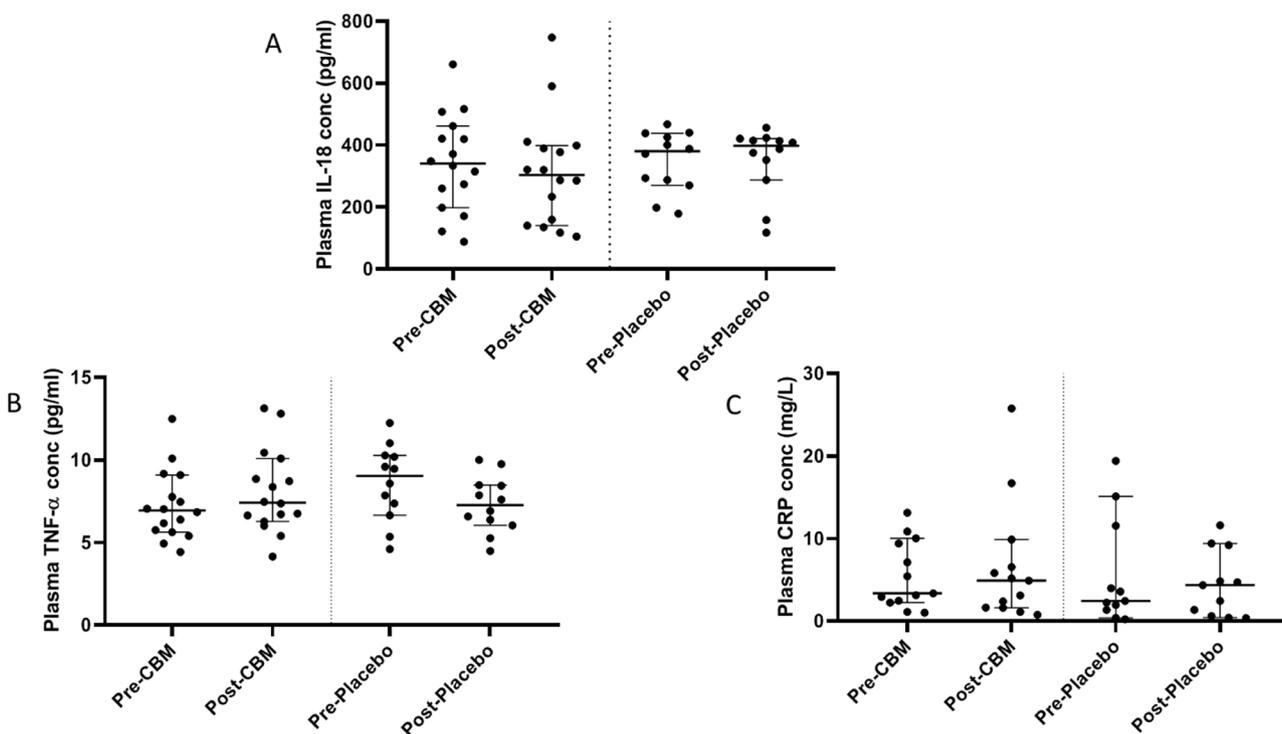


Fig. 2. Plasma concentrations of pro-inflammatory markers before and after CBM and Placebo treatment in AUD participants. A) Plasma IL-18 concentrations in pg/ml (Pre-CBM: n = 16; Post-CBM: n = 16; Pre-placebo: n = 12; Post-placebo: n = 12); B) Plasma TNF- α concentrations in pg/ml (Pre-CBM: n = 16; Post-CBM: n = 16; Pre-placebo: n = 12; Post-placebo: n = 12); C) Plasma CRP concentrations in mg/L (Pre-CBM: n = 13; Post-CBM: n = 13; Pre-placebo: n = 11; Post-placebo: n = 11). Individual data points of participants were plotted with the calculated median with 95% confidence interval (CI).

CBM = cognitive bias modification; AUD = alcohol use disorder; IL-18 = interleukin 18; TNF- α = tumor necrosis factor alpha; CRP = C-reactive protein.

In certain instances, the proinflammatory marker analyzed was lower (IL-18, TNF- α) or higher (CRP) than the detectable threshold, resulting in a different number of participants analyzed per graph. We performed an additional analysis where values that were lower (LLQ) or higher (ULQ) than the detection limit were considered as $\frac{1}{2}$ value, though no overall change of the results was noted (*data not shown*). We were unable to determine the plasma level concentrations of IL-1 β and IL-6 in any group since they fell below LLQ, therefore these two cytokines were not included in the final analyses.

Next, we analyzed whether there were any differences in the peripheral concentrations of the proinflammatory markers tested here in the CBM versus placebo groups. There were no significant differences in the CBM versus placebo pre-post change in either IL-18 ($P = 0.87$),

TNF- α ($P = 0.10$), or CRP ($P = 1.00$) (Fig. 2A–C). Additionally, within-group pretest-posttest analysis following either CBM or placebo treatment did not result in any significant changes in IL-18 (CBM $P = 0.94$; Placebo $P = 0.73$), TNF- α (CBM $P = 0.30$; Placebo $P = 0.13$), or CRP (CBM $P = 0.83$; Placebo $P = 0.97$) (Table 2; Fig. 2A–C). The effect sizes between CBM and placebo for these three cytokines are 0.52, 0.69, and 0.50, respectively.

4. Discussion

To the best of our knowledge, this is the first investigation of whether a cognitive behavior approach, namely CBM-training, as compared to matched placebo training, results in changes in peripheral

proinflammatory biomarkers in AUD patients. We started by assessing whether there were any differences in the levels of selected proinflammatory markers between AUD patients and healthy controls. Unfortunately, we were unable to obtain the plasma level concentrations of IL-1 β and IL-6 in any group since they fell below LLQ. Consistent to published literature (Bell et al., 2017; Crews and Vetreno, 2014; Imhof et al., 2001; Kawaratani et al., 2013), the AUD group had higher blood concentrations of CRP and TNF- α as compared to the healthy controls. The elevated proinflammatory marker level difference was expected and consistent with the knowledge that chronic alcohol initiates the inflammatory cascade, the consequences of which hold important clinical value. Alcohol-induced inflammation, a process initiated from the gut as a result of it being the first line of contact, is arguably one of the root causes of a list of organ dysfunctions and disorders such as certain cancers, as well as chronic liver and neurological diseases (Bishehsari et al., 2017; Vetreno and Crews, 2014). Increased CRP levels are widely used inflammatory biomarkers, such as to detect an increased risk of coronary events (Bell et al., 2017; Kosmidou et al., 2019), chronic inflammatory diseases, including rheumatoid arthritis (Urman et al., 2018), and recently a suggested biomarker for numerous adult solid tumors (Shrotiya et al., 2018). Similarly, elevated TNF- α has been connected to several neurological and inflammatory disorders (Crews and Vetreno, 2014; Thilagar et al., 2018), whereas the proinflammatory cytokine IL-18 has been associated with heart disease (O'Brien et al., 2014), cancer, autoimmunity, and inflammatory-mediated conditions (Esmailbeig and Ghaderi, 2017). Despite the median value of IL-18 being lower in the healthy controls than the AUD group, we did not observe any significant differences, suggesting that the magnitude of the difference for IL-18 between AUD individuals and controls is weaker compared to CRP and TNF- α .

Subsequently, we investigated whether there were any significant alterations in peripheral proinflammatory marker levels following CBM and placebo training. We had anticipated that the reported efficacy of CBM-trained patients would be in part due to a reduction in the selected proinflammatory marker levels, however plasma concentrations of IL-18, TNF- α , and CRP in the AUD patient group following CBM-based therapy did not result in any changes when compared to pre-test values or to the placebo treatment. Previously published data involving the same set of participants showed that CBM training led to a decrease in alcohol cue-induced amygdala activity when compared to the placebo group (Wiers et al., 2015b). Despite this clear effect on neural cue reactivity and significantly reduced craving, together with a correlation on behavioral effects on arousal ratings following CBM training, the interaction effect of group by time on approach bias fell short of significance (Eberl et al., 2013; Wiers et al., 2011, 2010). The authors addressed this lack of effect to be likely due to the small patient sample size, the reasoning of which may be extended to this present study. It should also be taken into account that not all AUD patients suffer from alcohol-associated pathologies, and those that do have varying degrees of disease severity depending on multiple factors, including individual traits, as well as external modifiable factors (Bishehsari et al., 2017), therefore resulting in a high heterogeneity of the patients' phenotypes and high variability on peripheral biomarkers, including cytokines.

It is important to note that this study was not specifically tailored for this investigation since this is a secondary analysis. As a result, there are additional limitations that may have affected the results obtained, as well as identified factors that may lead to a more comprehensive assessment in recognizing whether CBM-led training aids in the normalization of the altered proinflammatory axis. For example, in a recent study, male AUD patients were found to have significantly lower plasma cytokine concentrations, including TNF- α , 4 weeks following alcohol abstinence when compared to baseline levels, yet remained high enough to be significantly different from the healthy control group (Yen et al., 2017). This observation is in line to what we have encountered between total AUD patients and healthy controls,

considering that the AUD cohort of this study was abstinent for a longer period than the study by Yen and colleagues (Yen et al., 2017). In this study, we did not have a proper baseline value since the participating AUD patients did not initiate their abstinence to alcohol on the first day of the study. Thus, for future studies it would be ideal to assess whether CBM training has an effect on the inflammatory axis in AUD subjects that have not been abstinent from alcohol prior study initiation.

The length of abstinence prior to participation may have also affected the blood concentrations of the selected proinflammatory markers, as both AUD patient groups had a mean duration of abstinence of more than a month. This abstinence may also explain why we did not observe a significant change between IL-18 concentrations of the healthy control group and AUD patient group. The brief three-week CBM intervention could also be questioned, since the longer (seven weeks) CBT performed in MDD patients resulted in significant reductions in cytokine serum levels (Moreira et al., 2015). CBM targets implicit biases related to alcohol use (Wiers et al., 2015a) while CBT uses explicit processes to alter distorted cognitions, challenge longstanding beliefs using behavioral experiments etc., in an effort to support more functional patterns of thought and behavior across many life contexts (David and Szentagotai, 2006). It may be that CBT's effects on the immune system that were reported by Moreira and colleagues (Moreira et al., 2015) are due to these explicit modifications to thoughts and behavior having a pervasive impact across a patient's life, whereas CBM interventions may be more specific to implicit alcohol-related behavior only. An interesting factor in the study by Moreira and colleagues was that the participating patients were under no current psychological, psychiatric, or psychoactive substances; thus, no therapies directed at proinflammatory alterations at the start of the protocol. This present study also required that patients were free of psychoactive medication and other drugs, however, as previously discussed, being abstinent from alcohol for days/weeks prior to study commencement may have affected the pathology of alcohol-induced inflammation.

Another limitation to this study, was that we were unable to control for participants that were also smokers. Different studies have shown the link between cigarette smoking and the modulation of inflammatory processes (Lee et al., 2012; Strzelak et al., 2018), and we cannot exclude that part of the elevated inflammatory markers observed in this study may be also due to the participant's smoking habits.

An alternative explanation for the results of this study is that any changes in cytokine levels were masked due to all AUD patients being analyzed as one homogenous group. A recent study by Hanak and colleagues indicated notable differences in pro-inflammatory status following a 21-day alcohol detoxification exercise when a heterogeneous group of alcoholic patients were subdivided into sub-groups according to a classification named Lesch typology (Hanak et al., 2017). Briefly, the Lesch typology is divided into 4 groups, according to the origin of craving: (1) craving caused by alcohol, (2) craving caused by stress, (3) craving caused by mood, and (4) craving caused by compulsion. Beyond that specific classification, therapeutic approaches to enhance relapse prevention in AUD patients, tailored according to specific sub-types (Leggio et al., 2009) and psychotherapeutic approaches such as CBM-based therapies, may be effective depending on the heterogenic subgroup. It is also important to assert that the parent study (Wiers et al., 2015a) assessed craving as a clinical outcome measure and was not powered to detect between-group differences in relapse that were found by Wiers et al., 2011 and Eberl et al., 2013 (Eberl et al., 2013; Wiers et al., 2011).

The study was performed in male alcohol-dependent patients only to minimize confounding factors [e.g. previous studies have shown gender effects in neurobiological reactivity to alcohol stimuli (Seo et al., 2011) and effects of CBM on relapse (Eberl et al., 2013; Wiers et al., 2011)]. This is of course a significant limitation that leads to the lack of generalizability of results to females. Future studies are necessary to test whether CBM has comparable effects in female patients.

5. Conclusions

To summarize, we found that, despite being abstinent from alcohol prior to study commencement, both peripheral levels of TNF- α and CRP of AUD patients were still significantly higher than the healthy control group. Following chronic and excessive alcohol drinking, it may take weeks or even months for numerous physiological processes to return to the non-drinking basal state. No changes in IL-18, TNF- α , or CRP plasma concentrations were present following CBM treatment, however addressing the aforementioned limitations as well as analyzing a wider array of inflammatory markers may lead to a more conclusive assessment of the role of inflammatory pathways in CBM and associated psychotherapies. To conclude, there is a rapidly growing interest in modified versions of cognitive therapy due to its efficacy in the field of alcohol misuse. Identifying the underlying mechanisms of such therapies aid in the improvement of such techniques. More in-depth studies on the neuroimmunological and pathophysiological alterations, such psychological therapies, are crucial, especially for the personalized treatment of AUD patients.

Contributors

LL was responsible for the conceptualization of this analysis. JP, CEW and LL developed the rationale and design of the analyses. FB and LL provided research funding for this work. SLD and GRM processed the blood samples and generated the results presented in this analysis. JP and XL performed the statistical analysis. CEW and LL assisted with data analysis. All authors contributed to the interpretation of findings. JP wrote the first draft of the manuscript. All authors critically reviewed content and approved the final version for publication.

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Declaration of Competing Interest

No conflict declared.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:<https://doi.org/10.1016/j.drugalcdep.2019.107553>.

References

- Acion, L., Peterson, J.J., Temple, S., Arndt, S., 2006. Probabilistic index: an intuitive non-parametric approach to measuring the size of treatment effects. *Stat. Med.* 25, 591–602.
- Aldo, P., Marusov, G., Svancara, D., David, J., Mor, G., 2016. Simple Plex : a novel multi-analyte, automated microfluidic immunoassay platform for the detection of human and mouse cytokines and chemokines. *Am. J. Reprod. Immunol.* 75, 678–693.
- Bell, S., Mehta, G., Moore, K., Britton, A., 2017. Ten-year alcohol consumption typologies and trajectories of C-reactive protein, interleukin-6 and interleukin-1 receptor antagonist over the following 12 years: a prospective cohort study. *J. Intern. Med.* 281, 75–85.
- Bishehsari, F., Magno, E., Swanson, G., Desai, V., Voigt, R.M., Forsyth, C.B., Keshavarzian, A., 2017. Alcohol and gut-derived inflammation. *Alcohol Res.* 38, 163–171.
- Boffo, M., Zerhouni, O., Gronau, Q.F., van Beek, R.J.J., Nikolaou, K., Marsman, M., Wiers, R.W., 2019. Cognitive bias modification for behavior change in alcohol and smoking addiction: bayesian meta-analysis of individual participant data. *Neuropsychol. Rev.* 29, 52–78.
- Cannon, A.R., Morris, N.L., Hammer, A.M., Curtis, B., Remick, D.G., Yeligar, S.M., Poole, L., Burnham, E.L., Wyatt, T.A., Molina, P.E., So-Armah, K., Cisneros, T., Wang, G., Lang, C.H., Mandrekar, P., Kovacs, E.J., Choudhry, M.A., 2016. Alcohol and inflammatory responses: highlights of the 2015 Alcohol and Immunology Research Interest Group (AIRIG) meeting. *Alcohol* 54, 73–77.
- Carroll, K.M., Kiluk, B.D., 2017. Cognitive behavioral interventions for alcohol and drug use disorders: through the stage model and back again. *Psychol. Addict. Behav.* 31, 847–861.
- Cousijn, J., Goudriaan, A.E., Wiers, R.W., 2011. Reaching out towards cannabis: approach-bias in heavy cannabis users predicts changes in cannabis use. *Addiction* 106, 1667–1674.
- Crews, F.T., Sarkar, D.K., Qin, L., Zou, J., Boyadjieva, N., Vetreno, R.P., 2015. Neuroimmune function and the consequences of alcohol exposure. *Alcohol Res.* 37 (331–341), 344–351.
- Crews, F.T., Vetreno, R.P., 2014. Neuroimmune basis of alcoholic brain damage. *Int. Rev. Neurobiol.* 118, 315–357.
- David, D., Szentagotai, A., 2006. Cognitions in cognitive-behavioral psychotherapies; toward an integrative model. *Clin. Psychol. Rev.* 26, 284–298.
- DiSabato, D.J., Quan, N., Godbout, J.P., 2016. Neuroinflammation: the devil is in the details. *J. Neurochem.* 139, 136–153.
- Eberl, C., Wiers, R.W., Pawelczack, S., Rinck, M., Becker, E.S., Lindenmeyer, J., 2013. Approach bias modification in alcohol dependence: do clinical effects replicate and for whom does it work best? *Dev. Cogn. Neurosci.* 4, 38–51.
- Eberl, C., Wiers, R.W., Pawelczack, S., Rinck, M., Becker, E.S., Lindenmeyer, J., 2014. Implementation of approach bias re-training in alcoholism-how many sessions are needed? *Alcohol. Clin. Exp. Res.* 38, 587–594.
- Esmailbeig, M., Ghaderi, A., 2017. Interleukin-18: a regulator of cancer and autoimmune diseases. *Eur. Cytokine Netw.* 28, 127–140.
- Felger, J.C., 2018. Role of inflammation in depression and treatment implications. *Handb. Exp. Pharmacol.* 250, 255–286.
- Gazal, M., Souza, L.D., Fucolo, B.A., Wiener, C.D., Silva, R.A., Pinheiro, R.T., Jansen, K., Ghislene, G., Oses, J.P., Kaster, M.P., 2013. The impact of cognitive behavioral therapy on IL-6 levels in unmedicated women experiencing the first episode of depression: a pilot study. *Psychiatry Res.* 209, 742–745.
- Hanak, C., Benoit, J., Fabry, L., Hein, M., Verbanck, P., de Witte, P., Walter, H., Dexter, D.T., Ward, R.J., 2017. Changes in pro-inflammatory markers in detoxifying chronic alcohol abusers, divided by lesch typology, reflect cognitive dysfunction. *Alcohol Alcohol.* 52, 529–534.
- Imhof, A., Froehlich, M., Brenner, H., Boeing, H., Pepys, M.B., Koenig, W., 2001. Effect of alcohol consumption on systemic markers of inflammation. *Lancet* 357, 763–767.
- Kawaratan, H., Tsujimoto, T., Douhara, A., Takaya, H., Moriya, K., Namisaki, T., Noguchi, R., Yoshiji, H., Fujimoto, M., Fukui, H., 2013. The effect of inflammatory cytokines in alcoholic liver disease. *Mediators Inflamm.* 2013, 495156.
- Kelley, K.W., Dantzer, R., 2011. Alcoholism and inflammation: neuroimmunology of behavioral and mood disorders. *Brain Behav. Immun.* 25, S13–20.
- Kiefer, F., Jahn, H., Schick, M., Wiedemann, K., 2002. Alcohol intake, tumour necrosis factor-alpha, leptin and craving: factors of a possibly vicious circle? *Alcohol Alcohol.* 37, 401–404.
- Kosmidou, I., Redfors, B., Chen, S., Crowley, A., Lembo, N.J., Karpaliotis, D., Brown 3rd, W.M., Maupas, E., Durrleman, N., Shah, A., Reardon, M.J., Dressler, O., Ben-Yehuda, O., Kappetein, A.P., Sabik 3rd, J.F., Serruys, P.W., Stone, G.W., 2019. C-reactive protein and prognosis after percutaneous coronary intervention and bypass graft surgery for left main coronary artery disease: analysis from the EXCEL trial. *Am. Heart J.* 210, 49–57.
- Lee, J., Taneja, V., Vassallo, R., 2012. Cigarette smoking and inflammation: cellular and molecular mechanisms. *J. Dent. Res.* 91, 142–149.
- Leggio, L., Kenna, G.A., Fenton, M., Bonenfant, E., Swift, R.M., 2009. Typologies of alcohol dependence. From Jellinek to genetics and beyond. *Neuropsychol. Rev.* 19, 115–129.
- Lopresti, A.L., 2017. Cognitive behaviour therapy and inflammation: a systematic review of its relationship and the potential implications for the treatment of depression. *Aust. N. Z. J. Psychiatry* 51, 565–582.
- Memon, A.A., Sundquist, K., Ahmad, A., Wang, X., Hedelius, A., Sundquist, J., 2017. Role of IL-8, CRP and epidermal growth factor in depression and anxiety patients treated with mindfulness-based therapy or cognitive behavioral therapy in primary health care. *Psychiatry Res.* 254, 311–316.
- Moreira, F.P., Cardoso Tde, A., Mondin, T.C., Souza, L.D., Silva, R., Jansen, K., Oses, J.P.,

- Wiener, C.D., 2015. The effect of proinflammatory cytokines in cognitive behavioral therapy. *J. Neuroimmunol.* 285, 143–146.
- Neuman, M.G., Maor, Y., Nanau, R.M., Melzer, E., Mell, H., Opris, M., Cohen, L., Malnick, S., 2015. Alcoholic liver disease: role of cytokines. *Biomolecules* 5, 2023–2034.
- O'Brien, L.C., Mezzaroma, E., Van Tassel, B.W., Marchetti, C., Carbone, S., Abbate, A., Toldo, S., 2014. Interleukin-18 as a therapeutic target in acute myocardial infarction and heart failure. *Mol. Med.* 20, 221–229.
- Olsson, E.M.G., Norlund, F., Pingel, R., Burell, G., Gulliksson, M., Larsson, A., Karlsson, B., Svardsudd, K., Held, C., 2018. The effect of group-based cognitive behavioral therapy on inflammatory biomarkers in patients with coronary heart disease—results from the SUPRIM-trial. *Ups. J. Med. Sci.* 123, 167–173.
- Pace, C.A., Uebelacker, L.A., 2018. Addressing unhealthy substance use in primary care. *Med. Clin. North Am.* 102, 567–586.
- Park, E.J., Lee, J.H., Yu, G.Y., He, G., Ali, S.R., Holzer, R.G., Osterreicher, C.H., Takahashi, H., Karin, M., 2010. Dietary and genetic obesity promote liver inflammation and tumorigenesis by enhancing IL-6 and TNF expression. *Cell* 140, 197–208.
- Renna, M.E., O'Toole, M.S., Spaeth, P.E., Lekander, M., Mennin, D.S., 2018. The association between anxiety, traumatic stress, and obsessive-compulsive disorders and chronic inflammation: a systematic review and meta-analysis. *Depress. Anxiety* 35, 1081–1094.
- Rinck, M., Becker, E.S., 2007. Approach and avoidance in fear of spiders. *J. Behav. Ther. Exp. Psychiatry* 38, 105–120.
- Seo, D., Jia, Z., Lacadie, C.M., Tsou, K.A., Bergquist, K., Sinha, R., 2011. Sex differences in neural responses to stress and alcohol context cues. *Hum. Brain Mapp.* 32, 1998–2013.
- Shrotriya, S., Walsh, D., Nowacki, A.S., Lorton, C., Aktas, A., Hullihen, B., Benanni-Baiti, N., Hauser, K., Ayvaz, S., Estfan, B., 2018. Serum C-reactive protein is an important and powerful prognostic biomarker in most adult solid tumors. *PLoS One* 13, e0202555.
- Strzelak, A., Ratajczak, A., Adamiec, A., Feleszko, W., 2018. Tobacco smoke induces and alters immune responses in the lung triggering inflammation, allergy, asthma and other lung diseases: a mechanistic review. *Int. J. Environ. Res. Public Health* 15, 1033.
- Szabo, G., Saha, B., 2015. Alcohol's effect on host defense. *Alcohol Res.* 37, 159–170.
- Thilagar, S., Theyagarajan, R., Sudhakar, U., Suresh, S., Saketharaman, P., Ahamed, N., 2018. Comparison of serum tumor necrosis factor-alpha levels in rheumatoid arthritis individuals with and without chronic periodontitis: a biochemical study. *J. Indian Soc. Periodontol.* 22, 116–121.
- Urman, A., Taklalsingh, N., Sorrento, C., McFarlane, I.M., 2018. Inflammation beyond the joints: rheumatoid arthritis and cardiovascular disease. *Scifed. J. Cardiol.* 2.
- Vetreno, R.P., Crews, F.T., 2014. Current hypotheses on the mechanisms of alcoholism. *Handb. Clin. Neurol.* 125, 477–497.
- Wiers, C.E., Ludwig, V.U., Gladwin, T.E., Park, S.Q., Heinz, A., Wiers, R.W., Rinck, M., Lindenmeyer, J., Walter, H., Bermpohl, F., 2015a. Effects of cognitive bias modification training on neural signatures of alcohol approach tendencies in male alcohol-dependent patients. *Addict. Biol.* 20, 990–999.
- Wiers, C.E., Stelzel, C., Gladwin, T.E., Park, S.Q., Pawelczack, S., Gawron, C.K., Stuke, H., Heinz, A., Wiers, R.W., Rinck, M., Lindenmeyer, J., Walter, H., Bermpohl, F., 2015b. Effects of cognitive bias modification training on neural alcohol cue reactivity in alcohol dependence. *Am. J. Psychiatry* 172, 335–343.
- Wiers, C.E., Stelzel, C., Park, S.Q., Gawron, C.K., Ludwig, V.U., Gutwinski, S., Heinz, A., Lindenmeyer, J., Wiers, R.W., Walter, H., Bermpohl, F., 2014. Neural correlates of alcohol-approach bias in alcohol addiction: the spirit is willing but the flesh is weak for spirits. *Neuropsychopharmacology* 39 (3), 688–697.
- Wiers, R.W., Becker, D., Holland, R.W., Moggi, F., Lejuez, C.W., 2016. Cognitive motivational processes underlying addiction treatment. In: Kopetz, C.E.L., Lejuez, C.W. (Eds.), *Addictions: A Social Psychological Perspective*. Routledge, Abingdon, pp. 201–236.
- Wiers, R.W., Eberl, C., Rinck, M., Becker, E.S., Lindenmeyer, J., 2011. Retraining automatic action tendencies changes alcoholic patients' approach bias for alcohol and improves treatment outcome. *Psychol. Sci.* 22, 490–497.
- Wiers, R.W., Houben, K., Fadardi, J.S., van Beek, P., Rhemtulla, M., Cox, W.M., 2015. Alcohol cognitive bias modification training for problem drinkers over the web. *Addict. Behav.* 40, 21–26.
- Wiers, R.W., Rinck, M., Kordts, R., Houben, K., Strack, F., 2010. Retraining automatic action-tendencies to approach alcohol in hazardous drinkers. *Addiction* 105, 279–287.
- Yen, C.H., Ho, P.S., Yeh, Y.W., Liang, C.S., Kuo, S.C., Huang, C.C., Chen, C.Y., Shih, M.C., Ma, K.H., Sung, Y.F., Lu, R.B., Huang, S.Y., 2017. Differential cytokine levels between early withdrawal and remission states in patients with alcohol dependence. *Psychoneuroendocrinology* 76, 183–191.