

Effects of binge alcohol consumption on sleep and inflammation in healthy volunteers

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Abstract

Objective: Alcohol is a hypnotic that modifies immune function, specifically the cytokines interferon gamma (IFN- γ) and interleukin 2 (IL-2). We evaluated the association between unscheduled napping and acute alcohol-induced augmentation of IFN- γ and IL-2 expression.

Methods: In this prospective, observational pilot study, volunteers completed questionnaires on sleep quality, alcohol use, and hangover characteristics. Actigraph recordings began three nights before and continued for four nights after study initiation. Napping was recorded by actigraphy and self-reporting. A weight-based dose of 100-proof vodka was consumed, and the blood alcohol content (BAC) and phytohemagglutinin-M stimulated cytokine level were measured before and 20 minutes, 2 hours, and 5 hours after binge consumption.

Results: Ten healthy volunteers participated (mean age, 34.4 ± 2.3 years; mean body mass index, 23.9 ± 4.6 kg/m²; 60% female). The mean 20-minute BAC was 137.7 ± 40.7 mg/dL. Seven participants took an unscheduled nap. The ex vivo IFN- γ and IL-2 levels significantly increased at all time points after binge consumption in the nappers, but not in the non-nappers.

Conclusion: Augmented IFN- γ and IL-2 levels are associated with unscheduled napping after binge alcohol consumption. Further studies are needed to clarify the associations among alcohol consumption, sleep disruption, and inflammatory mediators.

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Introduction

Sleep is important for cognitive and physical restoration as well as memory consolidation. Alcohol is a commonly used hypnotic and is often used as self-medication for symptoms of insomnia.¹ However, despite its known somnogenic properties, alcohol causes severe disruptions in sleep architecture including a reduced percentage of total sleep time spent in rapid eye movement (REM) sleep, increased time spent in slow-wave sleep, and increased wake after sleep onset.² Additionally, the total sleep time is reduced under the effects of alcohol.³ Healthy subjects under the effect of high doses (≥ 1 g/kg) of acute alcohol consumption have been shown to have a reduction in the total night REM sleep percentage compared with similar healthy volunteer subjects.² Sleep onset latency is reduced in subjects who ingest alcohol; however, the time to the first REM sleep period (REM onset latency) increases with high doses (>4 standard drinks) of acute alcohol ingestion immediately prior to bedtime when compared with afternoon alcohol ingestion.² Healthy subjects who acutely consume two or more standard drinks of alcohol close to bedtime have been shown to have a truncated sleep time and increasingly disrupted sleep in the second half of the night because rebound wakefulness often coincides with a return of the blood alcohol level to zero; this results in fragmented sleep with heightened arousals and less REM sleep.^{2,4}

Recent studies have shown a relationship between alcohol exposure and physiological inflammatory responses to challenges

such as trauma, infection, injury, or toxins.⁵⁻⁸ Alcohol intoxication most notably affects innate immunity, which is responsible in part for antimicrobial defenses and inflammation.⁹ The effects of alcohol on cytokines have been studied in animal models; binge drinking suppresses proinflammatory cytokines [i.e., tumor necrosis factor- α (TNF- α), Interleukin (IL)-1, and IL-6] as well as select cytokines and chemokines that normally would be produced in response to endotoxin lipopolysaccharides (i.e., IL-1, IL-6, IL-9, and IL-12).^{6,10-12} Although alcohol consumption is a recognized risk factor for sepsis, prolonged mechanical ventilation, and increased mortality, the cellular mechanisms are not clearly defined.¹³

Proinflammatory cytokines are also associated with sleep architecture disturbances. Studies of immune function involving normal physiological sleep have shown a peak in immune cells' response to immune challenges during the night, suggesting that sleep strengthens the immune response.^{14,15} Infection and other inflammatory challenges have been observed in animal studies to increase total amount of non-REM (NREM) sleep, specifically slow-wave sleep. Although the most widely studied cytokines in relationship to sleep are IL-1 β and TNF- α , numerous studies have shown associations between lesser studied cytokines and sleep promotion or inhibition.¹⁶⁻¹⁸ These disturbances in sleep architecture are associated with changes in specific cytokine levels by either promotion (IL-1, IL-2, IL-6, IL-8, and IL-18) or inhibition

(IL-4, IL-10, IL-13, and TNF- β) of NREM sleep.¹⁹

This pilot study was performed to characterize the correlations among alcohol ingestion, cytokines, and sleep. Specifically, we focused on the association of binge alcohol consumption, napping, and expression of ex vivo stimulated proinflammatory cytokines [interferon (IFN)- γ and IL-2]. We tested our hypothesis that binge drinking alters the innate immune response and results in disrupted sleep behavior.

Methods

Study design and setting

This pilot study was performed to investigate the innate immune response after binge drinking. Binge alcohol consumption was defined according to the National Institute of Alcoholism and Alcohol Abuse.²⁰ Healthy adults with a history of low to moderate alcohol consumption volunteered for the study. The subjects arrived after fasting overnight and abstaining from alcohol, illicit drugs, sleep aids, and caffeine for the previous 24 hours. They were observed at the General Clinical Research Center (GCRC) at the University of Maryland Medical Center in a private room with adequate light and an available bed and bathroom. The study participants were encouraged to engage in relaxing activities such as listening to music, watching television, and reading. They were discharged from the GCRC when their breathalyzer alcohol content (BrAC) was $\leq 0.03\%$. Hangover symptom assessments were completed by the subjects the following morning. Actigraph recording, along with other survey tools and sleep logs, were continued in the subjects' usual home environment without any lifestyle modifications.

Volunteer selection

All volunteers in this study were low to moderate drinkers with normal liver function and no history of alcohol use disorder. Volunteers were recruited on campus at the University of Maryland Medical Center by word of mouth. The inclusion criteria were a healthy clinical status, age of ≥ 21 years, use of reliable birth control, ability to tolerate five (four if female) or more standard drinks in the past (a standard drink was defined as 12 ounces of beer, 5 ounces of wine, or 1.5 ounces of 80-proof distilled spirits), and current nonsmoker. Volunteers were excluded if they had a personal or family history of alcohol or other substance abuse, had been diagnosed with a mental health disorder, had a recent infection, or had used specific medications (anticoagulants, corticosteroids, aspirin, or nonsteroidal anti-inflammatory drugs) in the preceding 5 days. The subjects were screened for alcohol use disorder by completing the Short Michigan Alcohol Screening Test. Drinking habits were characterized using the three questions in the National Health Interview Survey 2010 on alcohol consumption. Serum tests for liver function were obtained to screen for hepatic impairment. Additionally, the subjects completed Pittsburgh Sleep Quality Index (PSQI) questionnaires to assess baseline sleep quality and disturbances during the 1-month time period leading up to study, and sleep quality was scored as follows: 1 = very good, 2 = fairly good, 3 = fairly bad, and 4 = very bad. Three nights prior to the study session date, the subjects continuously wore an actigraph (MicroMini Motionlogger Actigraph; Ambulatory Monitoring Inc., Ardsley, NY, USA). The volunteers were also instructed to concurrently keep a sleep diary, noting the hours when they were asleep either at night or during the day. This study was approved by the Institutional Review Board of the University of Maryland

Baltimore. Written informed consent was obtained from all volunteers.

Data collection and analysis

Participants were enrolled and studied from July to September 2012. All subjects were required to have a baseline breathalyzer reading of 0.00 g/dL and negative urine pregnancy test, when appropriate. Starting at approximately 9:00 AM and continuing at 20-minute intervals, the subjects consumed 100-proof vodka mixed with a palatable chilled sugar-free flavored seltzer beverage in a 1:4 ratio. The alcohol dose was calculated based on the subject's actual body weight and sex (0.89 g/kg for men and 0.81 g/kg for women) to reach a target BrAC of $>0.1\%$. After a 20-minute absorption period, the BrAC level was measured using a BACtrack[®] S80 Select Breathalyzer (KHN Solutions LLC, San Francisco, CA, USA; DOT/NHTSA approved and FDA 501(k) cleared; sensor accuracy $\pm 0.005\%$ BAC @ 0.050% BAC) and was repeated every 30 minutes. A binge intoxication level was achieved once the BrAC reached $\geq 0.1\%$; this occurred in all subjects at 20 minutes post-binge consumption. The serum blood alcohol content (BAC) was measured and heparinized whole blood collected for ex vivo stimulation prior to and 20 minutes, 2 hours, and 5 hours after binge consumption.

Cytokines were analyzed from RPMI 1640 medium and lipopolysaccharide (LPS) *Escherichia coli* 0111B4 obtained from Sigma-Aldrich Chemie (Steinheim, Germany). Sterile phytohemagglutinin (PHA) solution was obtained from GIBCO/Invitrogen (Paisley, UK). Aliquots of 0.5 mL of blood diluted 1:1 with RPMI 1640 medium were placed into wells of a 24-well plate (Becton Dickinson, Sunnyvale, CA, USA). PHA (GIBCO/Invitrogen) at concentrations of 0.3% v/v,

1% v/v, and 3% v/v and LPS (Sigma-Aldrich Chemie) at final concentrations of 1 ng/mL, 10 ng/mL, and 100 ng/mL were used as stimuli in all cases. Cultures were incubated for 24 hours at 37°C in a humidified atmosphere containing 5% carbon dioxide. At the end of the incubation period, plasma supernatants were removed after centrifugation at 6000 rpm for 3 minutes. The supernatants were kept at -80°C until assayed. Cytokines were measured by enzyme linked immunosorbent assay at the University of Maryland Cytokine Core Laboratory.

The morning after the study date, the volunteers completed the nine-item Acute Hangover Scale questionnaire produced by Rohsenow et al.²⁰ with the Brown University Center for Alcohol and Addiction Studies. A repeat PSQI questionnaire was completed with the participants answering the questions based on the previous 24-hour period. The participants continued to wear the actigraph for four subsequent nights and maintain sleep logs following the study date. Actigraph measurements were recorded using zero crossing mode. Motion was continuously detected in 30-second epochs throughout the 7-day study period for each participant.

Data were analyzed in several ways. The actigraph data were processed using Action4 software (Ambulatory Monitoring Inc.), and hourly averages were computed from these readings. Wakefulness was defined as an actigraph hourly mean of ≥ 50 counts/minute, and sleeping was defined as a mean of <50 counts/minute. In addition to studying nighttime sleep, episodes of sleeping (hourly mean of <50 counts/minute) during daytime hours were investigated. Napping events were recorded for each subject; in those who napped, delays from the peak BAC to nap time as well as the duration of napping were recorded. The actigraph values were correlated with the sleep log data when

appropriate. The mean baseline sleep time by the actigraph was calculated as an average of the hours of sleep during the two nights prior to the study day. The Day 1 post-study sleep time was calculated using the number of hours asleep per actigraph criteria (“actigraphy”) and the number of hours that were self-reported on the follow-up PSQI filled out the morning after the study (“self-reported”).

The stimulated cytokine levels at the various time points were compared among and across the two groups of “nappers” and “non-nappers.” Nappers and non-nappers were assessed by repeated-measures analysis of variance or a paired-samples t-test. Results are expressed as mean \pm standard deviation unless otherwise noted. We applied the traditional definition of $p \leq 0.05$ for statistical significance.

Results

Ten healthy adults (six women, four men) participated in this study. Their mean age was 34.4 ± 2.3 years, and their mean body mass index was 23.9 ± 4.6 kg/m². The mean self-reported baseline sleep time was 7.1 ± 0.8 hours, and the baseline sleep

quality was self-reported as fairly good or very good for all participants (1.7 ± 0.7). No subjects reported pre-existing sleep disorders. No subjects took naps in the days prior to the study. The baseline sleep time of the cohort as measured by the actigraph was 8.0 ± 1.0 hours (Table 1).

The subjects in this study were followed for a total for 4 days after their initial encounter in the GCRC. During this period, the subjects did not nap more than once per day, and only the subjects designated as “nappers” in the final analysis actually napped. Naps occurred an average of 8.7 ± 1.5 hours post-binge alcohol consumption. The mean duration of the naps was 0.6 ± 0.3 hours. Seven of the 10 participants took an unscheduled nap. When comparing nappers with non-nappers, the mean peak BAC was 135.3 ± 40.7 versus 143.3 ± 48.9 mg/dL, respectively, with no significant difference. The nappers had a mean 8.3 ± 1.7 -hour delay to nap (time elapsed until nap) after binge drinking with a mean napping duration of 1.3 ± 0.5 hours. The night after binge drinking, both groups had an increased sleep time as measured by actigraphy (8.7 ± 1.8 hours in the napper group

Table 1. Baseline characteristics of study cohort.

	Total (n = 10)	Nappers (n = 7)	Non-nappers (n = 3)
Age, years	34.4 ± 2.3	34.1 ± 2.6	35.0 ± 1.7
Male sex	4 (40.0)	2 (28.6)	2 (66.7)
BMI, kg/m ²	23.9 ± 4.6	23.3 ± 6.4	25.5 ± 2.9
SMAST, mean score/13	0	0	0
Baseline sleep time, hours			
Actigraphy	8.0 ± 1.0	8.0 ± 0.8	8.1 ± 1.6
Self-reported	7.1 ± 0.8	7.3 ± 0.5	6.5 ± 1.3
Baseline sleep quality*	1.7 ± 0.7	1.4 ± 0.5	2.3 ± 0.6
Pre-existing sleep disorder**	0	0	0

Continuous data are presented as mean \pm standard deviation, and categorical data are presented as n (%).

*1 = very good, 2 = fairly good, 3 = fairly bad, 4 = very bad.

**0 = no, 1 = yes.

BMI, body mass index; SMAST, Short Michigan Alcohol Screening Test.

and 8.1 ± 0.9 hours in the non-napper group). When the self-reported and actigraph post-study sleep times were compared, the differences between the napper and non-napper groups were not statistically significant (Table 2, Figure 1).

An increase in release of the proinflammatory cytokines IFN- γ and IL-2 across all

time points ($p=0.008$ and $p=0.013$, respectively) occurred in the napper group only (Table 3). No difference in the stimulated release of IL-8 occurred in either group (Table 3). No significant difference in the severity of hangovers was shown between the groups (1.1 ± 0.6 versus 1.3 ± 1.8 , respectively) when completed

Table 2. Post-study sleep characteristics in nappers and non-nappers.

	Nappers (n = 7)	Non-nappers (n = 3)	p value
Peak BAC, mg/dL	135.3 ± 40.7	143.3 ± 48.9	0.79
Delay to nap, hours	8.3 ± 1.7	-	-
Duration of nap, hours	1.3 ± 0.5	-	-
Day 1 post-study sleep time, hours			
Actigraphy	8.7 ± 1.8	8.1 ± 0.9	0.61
Self-reported	7.5 ± 1.4	6.8 ± 0.8	0.45
Acute hangover scale score	1.1 ± 0.6	1.3 ± 1.8	0.79

Data are presented as mean \pm standard deviation and were analyzed using the t-test. BAC, blood alcohol content.

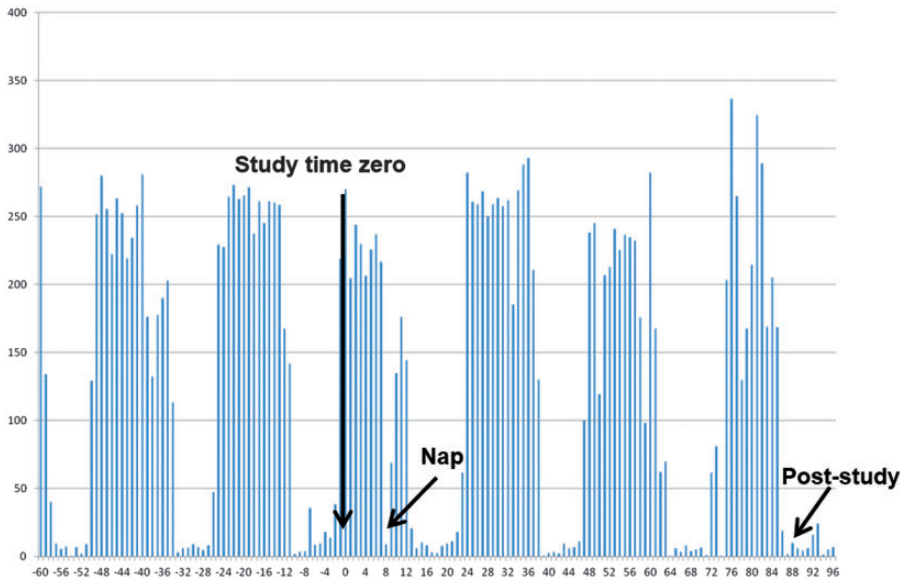


Figure 1. Representative 7-day actigraph of a napper. Hourly averages of the actigraph values (counts/minute) are shown on the Y axis in reference to binge drinking as time zero on the X axis. Negative X-axis values represent hours leading up to the study, and positive X-axis values represent hours after the study.

Table 3. Mean peak stimulated cytokine levels of non-nappers and nappers.

	Time ₁	Time ₂	Time ₃	Time ₄	p value
Non-nappers (n = 3)					
IFN- γ , pg/mL	5511 \pm 5114	5131 \pm 5854	3881 \pm 5193	4960 \pm 4103	0.672
IL-2, pg/mL	275 \pm 182	206 \pm 213	265 \pm 273	337 \pm 249	0.592
IL-8, pg/mL	26211 \pm 25856	32178 \pm 29213	32410 \pm 10336	33367 \pm 14457	0.832
Nappers (n = 7)					
IFN- γ , pg/mL	4615 \pm 5611	11541 \pm 10830	7281 \pm 5378	6806 \pm 4526	0.008
IL-2, pg/mL	154 \pm 89	242 \pm 154	238 \pm 137	289 \pm 158	0.013
IL-8, pg/mL	45413 \pm 31424	51169 \pm 32693	63227 \pm 25493	56653 \pm 42545	0.240

Data are presented as mean \pm standard deviation.

Time₁, before binge alcohol consumption.

Time₂, 20 minutes after binge alcohol consumption.

Time₃, 2 hours after binge alcohol consumption.

Time₄, 5 hours after binge alcohol consumption.

IFN, interferon; IL, interleukin.

approximately 24 hours after binge drinking (Table 2).

Discussion

Most subjects in this study had sleep architecture disruption after binge alcohol daytime consumption as evidenced by unscheduled naps. Naps occurred several hours post-intoxication when the BAC levels were decreasing. Only the nappers had an increase in proinflammatory cytokines (IFN- γ and IL-2) after ex vivo PHA stimulation from the pre- to post-binge consumption time points. The only difference in subjective sleep quality between the two groups was better baseline sleep quality reported by the nappers. Subjects who napped demonstrated a greater capacity for somnogenic cytokine release during ex vivo stimulation.

Our study, which prospectively assessed the association among binge drinking, stimulated cytokine levels, and sleep in healthy adults, was performed in an attempt to determine the mechanistic inflammatory underpinnings of the somnogenic properties of alcohol. Sleep has vast restorative functions and plays an important, although not

yet well-defined, role in inflammatory states. The two most studied somnogenic cytokines, IL-1 and TNF- α , have been found to have diurnal variation in their measured levels that correlate with the sleep-wake cycle in physiological sleep.^{8,16} Imeri and Opp²¹ described a better capacity to thermoregulate during NREM sleep, resulting in a more effective febrile and immune response during NREM sleep. Perras and Born²² found that the levels of somnogenic cytokines (IL-1, IL-6, and TNF- α) were increased during infection, resulting in an increased duration of sleep and disproportionately more NREM sleep. However, neither of these studies addressed the effects of binge alcohol consumption on inflammation and the resultant sleep architecture.

The acute effect of heavy alcohol drinking, when ingested close to bedtime, is typically a reduced sleep latency with paradoxical rebound wakefulness as the alcohol is metabolized. With a nap onset delay at approximately 8 hours in the present study, the naps took place as the BAC returned to baseline, not as the BAC increased or peaked. The timing of these naps in relation to binge drinking suggests possible causal or additive relationships

between the changes in the immune response and the somnogenic properties of alcohol.

Although a significant increase in IFN- γ and IL-2 was noted in nappers compared with no change in non-nappers, a limitation of this study is the small sample size, which may have resulted in a type II error with respect to IL-8 release between the two groups. Additionally, the use of the GCRC as the study site may have created an irregular environment with respect to the socially acceptable timing of alcohol consumption because the subjects were required to consume alcohol between 8:00 and 10:00 AM. Because alcohol is often ingested in the evening, the timing of its ingestion may have affected unscheduled napping and the post-study sleep time/quality; for example, the circadian rhythm could have affected the timing of napping because the nap occurrence coincided with the usual circadian dip in daytime wakefulness.²³ None of the participants took naps in the 2 days leading up to the study. Additionally, it is expected that the post-study nighttime sleep would have been more disrupted if the alcohol levels had been falling throughout the night rather than reaching zero BAC by the time the subjects went to sleep.

Interestingly, it is possible that increasing cytokine levels may result in increased sleep "pressure," or building of sleepiness, given the inherent somnogenic properties of these inflammatory substances.²⁴ TNF- α and IL-1 in particular are known as "sleep-regulating substances" because they have been shown to increase delta sleep in many vertebrate species, including humans.²⁵ Additionally, the administration of steroids and corticotropin-releasing hormone has been shown to inhibit TNF- α and IL-1 while simultaneously reducing NREM sleep,²⁶ demonstrating at least a possible association between sleep and the release

of TNF- α and IL-1 as sleep-regulating substances.

The lack of a statistically significant difference in hangover symptoms between the groups and the low severity of hangover symptoms may be attributable to the 24-hour lag time between binge drinking and the administration of the questionnaire. The participants may have reported increased hangover symptoms if this questionnaire had been completed shortly after they reached an undetectable BAC because this is when drinkers typically begin experiencing hangover symptoms (instead of 24 hours after drinking, which is when symptoms resolve).²⁷

Another potential limitation of this study is that sleep was studied using noninvasive actigraphy rather than polysomnography. The routine use of polysomnography in research studies is limited because its application may be labor-intensive, costly, and technically challenging to achieve and maintain. Compared with polysomnography, actigraphy is known to overestimate sleep and underestimate wake time.²⁸ Its greatest limitation in this study was the inability to characterize sleep architecture with respect to staging sleep. The capability of being able to subdivide sleep into NREM and REM categories would enhance the interpretation of this data, both when studying unscheduled naps and studying nocturnal sleep. This limitation may have resulted in an under-representation of sleep, especially because several subjects were noted to intermittently sleep while being observed in the GCRC (while BAC levels remained high). These observed naps did not typically correlate with naps as defined by actigraphic measurements. Inclusion of these observed naps could potentially alter the results and subsequent interpretation.

In conclusion, subjects who napped by actigraphic criteria following acute alcohol intoxication demonstrated a modified innate immune response as opposed to

non-nappers, who did not demonstrate such a response. Nappers had an increased capacity for cytokines specific for lymphocytes, specifically IFN- γ and IL-2, after *ex vivo* stimulation. Further research is needed to elucidate the relationships among binge alcohol intoxication, the innate immune response, and sleep (both nocturnal and unscheduled daytime napping).

Declaration of conflicting interest

The authors declare that there is no conflict of interest.

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