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An evening of alcohol consumption negatively impacts next-day immune fitness in both hangover-sensitive drinkers and hangover-resistant drinkers

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ABSTRACT

Background: Survey research found poorer baseline immune fitness for self-reported hangover-sensitive drinkers compared to hangover-resistant drinkers. However, up to now a limited number of clinical studies revealed mixed results regarding the relationship between the concentrations of biomarkers of systemic inflammation in blood or saliva with hangover severity, and could not differentiate between hangover-sensitive drinkers and hangover-resistant drinkers. The aim of this study was to assess immune fitness and saliva biomarkers of systemic inflammation at multiple timepoints following an alcohol day and alcohol-free control day. Methods: The study had a semi-naturalistic design. In the evening before the test days, participants were not supervised. They could drink ad libitum drinking on the alcohol test day and refrained from drinking alcohol on the control day. Activities and behaviors on the alcohol and control day were reported the follow morning. On both test days, from 09:30 to 15:30, hourly assessments of immune fitness (single-item scale) and overall hangover severity (single-item scale) were made and saliva samples were collected for biomarker assessments. Results: N = 14 hangover-resistant drinkers and n = 15 hangover-sensitive drinkers participated in the study. The amount of alcohol consumed on the alcohol day did not significantly differ between the hangover-resistant group (mean (SD) of 13.5 (7.9) alcoholic drinks) and the hangover-sensitive group (mean (SD) of 12.4 (4.4) alcoholic drinks). All hangover-sensitive drinkers reported having a hangover following the alcohol day (overall hangover severity score 6.1 (on a 0–10 scale) at 09:30, gradually decreasing to 3.3 at 15:30), whereas the hangover-resistant drinkers reported no hangover. On the control day, immune fitness of the hangover-sensitive group was significantly poorer than the hangover-resistant group. On the alcohol day, both groups showed a significant reduction in immune fitness. The effect was evident throughout the day, but significantly more pronounced in the hangover-sensitive group than the hangover-resistant group. No significant differences between the groups were found at any time point on the two test days for saliva concentrations of Interleukin (IL)-1β, IL-6, IL-8, and tumor necrosis factor (TNF)-α. Conclusions: Whereas hangover-sensitive drinkers reported a hangover following an alcohol day and hangover-resistant drinkers did not, both groups reported significantly reduced immune fitness throughout the day. However, the reduction in immune fitness among hangover-sensitive drinkers was significantly more pronounced in comparison to the hangover-resistant group.

1. Introduction

Immune fitness has been defined as the body's capacity to respond to health challenges (such as infections) by activating an appropriate immune response, which is essential to maintain health, prevent and resolve disease, and improve quality of life (Verster, Kraneveld, &

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Garssen, 2023). Reduced immune fitness has a significant negative impact on the economy in terms of absenteeism (not going to work), presenteeism (going to work while experiencing reduced immune fitness), and reduced productivity. A recent study estimated the associated costs for the Dutch economy in 2019 at 10.7 billion euro, with a reduction in productivity of 22.8 % on days working when experiencing reduced immune fitness (Sips, Severeijns, Kraneveld, Garssen, & Verster, 2023). Health and lifestyle factors can have a negative or positive impact on immune fitness, including but not limited to sleep (Ibarra-Coronado et al., 2015), physical activity status (Shephard & Shek, 1994), body mass index (Kiani et al., 2022), and alcohol consumption (Merlo et al., 2021). In the current study, we investigated next-day immune fitness after an evening of alcohol consumption.

The concept and assessment methods of immune fitness are described elsewhere in detail (Verster et al., 2023). The perception to what extent one is capable of preventing and resolving disease through adequate immune functioning is vital in health and disease. Reduced immune fitness is an important sign for an individual to take action in terms of visiting a physician or adjusting lifestyle (e.g., adopting a healthier lifestyle, daily diet, exercise). Although related concepts, immune fitness is not a synonym of general health or well-being. Instead, immune fitness is a prerequisite of general health and well-being. However, as there are several other factors determine general health and well-being, correlations with immune fitness are usually only modest (Verster et al., 2023). The concept of (reduced) immune fitness is well-known to the general public. Similar to anxiety and depression, (reduced) immune fitness can be assessed only via self-report (Verster et al., 2023). The assessments of immune fitness are often made with single-item rating scales, ranging from 0 (very poor) to 10 (excellent). Depending of the level of understanding of the participants of a study, a description of immune fitness can be added. There are no biomarkers that assess immune *fitness*. However, biomarkers of systemic inflammation can provide information on general functioning of the immune *system*. However, these biomarkers may not be as sensitive as self-reported assessments of immune fitness. For example, if a single item rating of immune fitness reduces over time from score 9 to score 6 this clearly indicates reduced immune fitness. However, general health and well-being are still in the 'healthy range' (scores > 6) and no changes may be seen on biomarkers, as this 'healthy range' is not associated with systemic inflammation. Taken together, immune fitness is an important perquisite of health and well-being, and reduced immune fitness increases the susceptibility to disease. As such, it has also been hypothesized that immune fitness may be related to the susceptibility of having alcohol hangovers (Van de Loo et al., 2020).

The day following an evening of alcohol consumption, a considerable number of individuals experience an alcohol hangover. The alcohol hangover is defined as the combination of negative mental and physical symptoms which can be experienced after a single episode of alcohol consumption, starting when blood alcohol concentration (BAC) approaches zero (Van Schrojenstein, Mackus, van de Loo, & Verster, 2016; Verster, Scholey, van de Loo, Benson, & Stock, 2020). Typical hangover symptoms include fatigue, headache, and nausea (Penning, McKinney, & Verster, 2012; Lawick, van Pabst, Devenney, & Verster, 2019; Van Schrojenstein, Mackus, van de Loo, & Verster, 2017), and these can have a significant negative impact on mood (McKinney, 2010), cognitive and psychomotor functioning (Gunn, Mackus, Griffin, Munafò, & Adams, 2018; Kruisselbrink, 2019), and daily activities such as driving a car (Alford et al., 2020; Verster et al., 2014; Verster, van der Maarel, McKinney, Olivier, & de Haan, 2014), or riding a bicycle (Hartung et al., 2015). Thus, the alcohol hangover state is not limited to feeling unwell, but comprises a variety of signs and symptoms including fatigue, sleepiness, apathy, concentration problems, headache, nausea, regret, heart pounding, heart racing, vomiting, shivering, clumsiness, weakness, dizziness, sweating, stomach pain, confusion, sensitivity to light, sensitivity to sound, thirst, anxiety, depression, and reduced appetite (Mackus et al., 2023). Several common symptoms of the alcohol hangover are unrelated to feeling unwell (e.g., reduced appetite). Often, overall hangover severity was assessed via a single item rating scale, ranging from 0 (absent) to 10 (extreme) (Verster, van de Loo, Benson, Scholey, & Stock, 2020). This global (single item) assessment is preferred, as it incorporates the drinker' evaluation of the overall presence, severity, and impact of symptoms of the overall hangover (Verster et al., 2020).

Although the alcohol hangover can occur after consuming any amount of alcohol (Verster et al., 2020) and at any age (Verster et al., 2021), there is a considerable number of drinkers (~10 to 20 %) that report not to experience hangovers, even after consuming large quantities of alcohol (Howland, Rohsenow, & Edwards, 2008; Kruisselbrink, Bervoets, de Klerk, van de Loo, & Verster, 2017; Verster, de Klerk, Bervoets, & Kruisselbrink, 2013). Up to now, it is unclear why this minority of drinkers seem to be hangover-resistant. Research into the pathology of the alcohol hangover revealed that hangovers are less severe in drinkers with a faster ethanol metabolism (Mackus et al., 20200a; Mackus et al., 2020b), and that the inflammatory response to alcohol consumption is involved in eliciting hangovers (Van de Loo et al., 2020). More research into the pathology of the alcohol hangover could provide an explanation as to why these hangover-resistant drinkers do not experience hangovers.

Research on the pathology of alcohol hangover is limited (Palmer et al., 2019; Tipple, Benson, & Scholey, 2017), and studies comparing hangover-sensitive drinkers with hangover-resistant drinkers are scarce (Hogewoning et al., 2016; Van de Loo et al., 2018; Van de Loo et al., 2021). Hogewoning et al. (2016) conducted a naturalistic study comprising an alcohol day and alcohol-free control day. Hangovers were reported by the hangover-sensitive group, including a variety of symptoms, whereas the hangover-resistant group reported no hangovers. In the latter group, if any, reported symptoms following the alcohol day were limited to mild sleepiness, tiredness, concentration problems, clumsiness, and thirst. Between the alcohol day and test day, both groups reported poorer sleep quality, but in contrast to the hangover-sensitive group, no significant effects on mood were reported by hangover-resistant group. Survey research (Van de Loo et al., 2018) revealed that hangover-sensitive drinkers reported significantly poorer general immune fitness than hangover-resistant drinkers. Only three studies investigated biomarkers of systemic inflammation in relation to next-day hangovers. In a first controlled study, Kim et al. (2003) found significant correlations between blood concentrations of Interleukin (IL)-12 and interferon (IFN)-γ and hangover severity. In this study only hangover-sensitive drinkers of Asian descent were included. A second controlled study in Asian, hangover-sensitive drinkers, also assessed cytokines concentration in the blood the morning after alcohol consumption (Van de Loo et al., 2020). Significant positive correlations were found between blood concentrations of IL-6, tumor necrosis factor-alpha (TNF-α) and c-reactive protein (CRP). Both of these studies did not include hangover-resistant drinkers.

These two groups were included in a third study (Van de Loo et al., 2021). This study had a naturalistic study design (meaning, drinking amounts, venues, and activities were uncontrolled), saliva samples for the determination of cytokine concentrations were collected at 09:00 am and hangover severity was assessed at the same time. While the hangover-sensitive group reported having a hangover and the hangover-resistant group did not, no significant differences between the two groups were found for saliva cytokine concentrations. Also, saliva cytokine concentrations did not significantly correlate with hangover severity. There are several possible explanations for the differences between the findings of the three studies. Most notably are the differences of assessments in blood (Kim et al., 2003; Van de Loo et al., 2020) or saliva (Van de Loo et al., 2021), differences in the study design and associated differences in alcohol intake, i.e. controlled trials (Kim et al., 2003; Van de Loo et al., 2020) vs a naturalistic study design (Van de Loo et al., 2021), differences in the assessment method of overall hangover severity, i.e. a single item (Van de Loo et al., 2021) vs composite

symptom score (Kim et al., 2003; Van de Loo et al., 2020), and different study populations, i.e. Asian descent (Kim et al., 2003; Van de Loo et al., 2020) vs Dutch students (Van de Loo et al., 2021).

Of note, the studies assessed biomarkers of systemic inflammation and hangover severity in the morning only. However, hangover symptoms are reported throughout the day and may differ in intensity at different timepoints (Verster et al., 2018). Similarly, cytokine concentrations vary during the day (Nakao, 2014). Therefore, the aim of this study was to compare assessments of immune fitness and saliva biomarker concentrations of hangover-sensitive drinkers and hangover-resistant drinkers throughout the day, assessed at hourly intervals between 09:30 and 15:30. Previous research has shown that biomarkers of systemic inflammation such as CRP and cytokines can be reliably assessed in saliva (Ouellet-Morin, Danese, Williams, & Arseneault, 2011; Riis et al., 2014). Multiple sample collections of saliva are less burdensome and non-invasive for participants compared to multiple blood samplings, and therefore, saliva sampling is generally preferred by study participants above blood sampling (Dhima, Salinas, Wermers, Weaver, & Koka, 2013). Given the fact that this study has seven biomarker assessments throughout the day on two test days (alcohol day and an alcohol-free control day), it was decided to collect saliva instead of blood. It was hypothesized that (a) at the control day immune fitness was poorer for the hangover-sensitive group compared to the hangover-resistant group, and (b) that following the alcohol day a significant reduction in immune fitness and increase in biomarkers of systemic inflammation would be found for the hangover-sensitive group, but not for the hangover-resistant group.

2. Materials and methods

This was a semi-naturalistic study (Verster et al., 2019) including a training day and two test days. The evening and night of the test days were uncontrolled and followed a naturalistic study design. No alcohol was consumed prior to the control day. On the alcohol day, participants consumed alcohol freely, with no restrictions on beverage types or quantity consumed. On both test days, participants were free to choose venues to spend the evening and activities. The next-day assessments followed a strictly controlled experimental study design, with hourly assessments. The study was approved by the University of Groningen Psychology Ethics Committee Ethics Committee (approval number: ppo-015–002, approval date: 3 September 2015). Before the start of the study, written, informed con-sent was obtained from all participants.

2.1. Screening procedures and compliance

To be included, participants had to consume alcohol and be in the age range of 18 to 30 years old. They had to be healthy (i.e., no physical or mental disease), non-smoker, not using illicit or medicinal drugs (except contraception), and not have received recent vaccinations. During test days, participants were not allowed to take any treatments to prevent or relieve hangover symptoms, medication that may have an impact on immune functioning, such as acetaminophen, aspirin, and non-steroidal anti-inflammatory drugs (NSAIDs). Participants were excluded if they reported acute inflammation (infections, common cold, severe acne, flu), allergic reactions (asthma and food allergy), autoimmune diseases (rheumatoid arthritis, multiple sclerosis, diabetes type II), inflammatory bowel disease (Crohn's disease, ulcerative colitis, irritable bowel syndrome), or other conditions that may have an impact on cytokine concentrations (e.g., chronic fatigue syndrome and fibromyalgia). The study physician verified their health status, and sex, age, weight, and height were recorded. At each study visit, a urine drug screen (AlfaScientic Designs Inc, Poway, CA, USA) was performed to determine possible illicit drug use (including amphetamines (including 3,4-Methylenedioxymethamphetamine, MDMA), barbiturates, cannabinoids, benzo-diazepines, cocaine, and opiates). In addition, possible pregnancy of female participants was checked with a urine β-human chorionic gonadotropin (HCG) pregnancy test. None of the participants were excluded based on these tests. At the start of each visit, a breath alcohol test was performed (Alcotest 7410 Breath Alcoholmeter, Dräger, Hoogvliet, The Netherlands). On the control day, this enabled establishing the absence of recent alcohol consumption, whereas following the alcohol day the possible presence of residual alcohol could be determined.

Participants were thoroughly screened to determine whether they should be allocated to the hangover-sensitive group or the hangover-resistant group. To this extent they were interviewed about their usual drinking behavior (quantity and frequency), and whether they ever experienced alcohol hangovers or not. Participants were included only if they consumed an amount of alcohol on typical drinking occasions that resulted in an estimated peak BAC of at least 0.08 %. The estimated BAC was estimated using a modified Widmark formula (Terpstra, Benson, Verster, & Scholey, 2020; Watson, Watson, & Batt, 1981), based on the self-reported typical drinking time and the amount of alcohol consumed. The formula takes into account sex and body weight. If participants passed the estimated BAC criterion they were, based on whether or not they claim to experience hangovers, allocated to either the hangover-sensitive group or the hangover-resistant group.

2.2. Test day procedures and assessments

On the control day, no alcohol consumption was allowed. The alcohol day was scheduled the day after the participants expected to consume alcohol. Both test days were planned approximately one week apart. However, participants were free to decide whether or not they consumed alcohol, and test day dates could be rescheduled if drinking behavior deviated from the original planning. The test days started at 09:00 at Utrecht University. Participants consumed a standardized breakfast (consisting of a currant bun and a glass of milk or water), and at 12:00 received the same for lunch. The consumption of caffeinated beverages was not allowed on test days. Assessments of immune fitness and hangover severity, and the collection of saliva samples were done hourly on both test days, from 09:30 to 15:30. Overall hangover severity was assessed using a single-item scale ("Please rate your overall hangover severity") ranging from 0 (absent) to 10 (extreme) (Verster et al., 2020). Immune fitness was assessed with a single-item scale ("Please rate your immune fitness") ranging from 0 (very poor) to 10 (excellent) (Van Schrojenstein Lantman et al., 2017; Verster et al., 2023). Saliva samples were collected by the passive drool method in 2-mL polypropylene cryovials, using SalivaBio's Saliva Collection Aid (Salimetrics, State College, PA, USA). The samples were stored at −80 °C.

2.3. Biomarkers of systemic inflammation

Saliva biomarkers of systemic inflammation were determined by multiplex immunoassays (customized Bio-Plex® Multiplex Immunoassay System, BioRad Laboratories, Veenendaal, The Netherlands). All incubations were conducted at room temperature, according to standardized procedures described elsewhere [31]. Assessments (in pg/mL saliva) were made for IL-1β, IL-2, IL-4, IL-5, IL-6, IL-8, IL-10, granulocyte–macrophage colony-stimulating factor (GM-CSF), interferon-gamma (IFN-γ) and tumor necrosis factor-alpha (TNF-α). Each multiplex plate had a unique lower limit of detection (LOD). For biomarker concentrations below LOD, half the LOD value was used for the respective assessment [31]. If more than 25 % of the biomarker assessments were below the LOD value, the results for that biomarker were considered unreliable and excluded from the analyses (Van de Loo et al., 2021). Given this, reliable data for the analyses was obtained for IL-1β, IL-6, IL-8, and TNF-α.

2.4. Statistical analysis

Statistical analyses were performed using IBM Statistical Package for

the Social Sciences (SPSS), version 29. The analyses compared outcomes of the hangover-sensitive group and the hangover-resistant group. Biomarker data were not normally distributed, and therefore nonparametric tests were used for statistical analyses. Multiple timepoint assessments of the groups were compared with the Independent-Samples Kruskal-Wallis test. Differences (at the same timepoint) between the hangover-resistant group and hangover-sensitive group, after Bonferroni's correction for multiple comparisons, were considered significant if $p < 0.0071$. Data from the control day and the hangover day were compared within groups with the Related-samples Friedman's Two-Way Analysis of Variance by Ranks Test. Differences (at the same timepoint) between the control day and the alcohol day, after Bonferroni's correction for multiple comparisons, were considered significant if $p < 0.0071$. Finally, for the hangover-sensitive group, difference scores (Δ, alcohol day — control day) of biomarker concentrations and immune fitness were correlated with overall hangover severity on the alcohol day, using nonparametric Spearman's rho correlations. The correlations, after Bonferroni's correction for multiple comparisons, were considered significant if $p < 0.0071$. Given the relatively small sample size, a confirmatory boot-strapping analysis (10.000 samples, bias-corrected and accelerated) was also conducted (Efron & Tibshirani, 1993; Efron, 1979). Correlations were significant if the bias-corrected and accelerated 95 % confidence interval (BCa 95 %CI) lies entirely above or below zero. In a similar way, correlations between difference scores (Δ, alcohol day — control day) of immune fitness and biomarker concentrations were computed. Additionally, Bayesian analyses (JASP software, version 0.17.1) were conducted. The provided Bayes Factor (BF_{10}) is more in favor of the alternative hypothesis (H1, the groups differ) if its values is larger than 1, and is more in favor of the null hypothesis (H0, the groups do not differ) if its value is smaller than 1. The level of evidence that the alternative hypothesis is true (i.e., the groups truly differ from each other) can be inferred from the BF_{10}, with larger values providing incrementally more evidence. Jeffreys (1961) suggested that a $BF_{10} < 1$ corresponds to no evidence for the alternative hypothesis, a BF_{10} between 1 and 3 corresponds to anecdotal evidence, a BF_{10} between 3 and 10 corresponds to moderate evidence, and a BF_{10} greater than 10 corresponds to strong evidence that the alternative hypothesis is true. Likewise, for correlational analysis, if BF_{10} values are greater than 1 this is more in favor of the alternative hypothesis (H1, there is a significant correlation).

3. Results

N = 29 healthy volunteers participated in the study. Of them, 14 were hangover-resistant and 15 were hangover-sensitive. Participants had a mean (SD) age of 21.1 (2.0) years old and a mean (SD) weight of 72.9 (10.6) kg. These demographics did not significantly differ between the groups. There were also no significant differences between the male/female ratio of the hangover-sensitive group (7/8) and the hangover-resistant group (8/6). On the alcohol day, no significant difference ($p = 0.847$; $BF_{10} = 0.362$) was found in the amount of alcohol consumed by the hangover-resistant group (mean (SD) of 13.5 (7.9) alcoholic drinks) and the hangover-sensitive group (mean (SD) of 12.4 (4.4) alcoholic drinks). Also, the corresponding estimated BAC of the hangover-resistant group and hangover-sensitive group did not significantly differ from each other (mean (SD) estimated BAC of 0.21 % (0.1) and 0.20 % (0.1), respectively, $p = 0.533$; $BF_{10} = 0.367$).

3.1. Overall hangover severity

On the control day, all hangover severity assessments were zero. The hangover severity assessments on the alcohol day are summarized in Table 1. At all timepoints, overall hangover severity ratings of the hangover-sensitive group were significantly higher than those of the hangover-resistant group. Severity scores were highest at 09:30 and then gradually decreased during the day. However, the considerable severity score of 3.2 (out of 10) at 15:30 shows that hangovers were still present in the after-noon.

Table 1
Overall hangover severity.

Time	Hangover-resistant group	Hangover-sensitive group	*p*-value
09:30	0.9 (1.3)	6.1 (2.1)	<0.001*
10:30	0.7 (1.2)	5.9 (1.8)	<0.001*
11:30	0.4 (0.8)	5.1 (2.0)	<0.001*
12:30	0.4 (0.9)	4.5 (1.8)	<0.001*
13:30	0.4 (0.8)	4.3 (2.7)	<0.001*
14:30	0.2 (0.4)	3.7 (2.3)	<0.001*
15:30	0.3 (0.6)	3.2 (2.3)	<0.001*

Mean and standard deviation (SD) are shown. Significant differences (at the same timepoint) between the hangover-resistant group and hangover-sensitive group ($p < 0.0071$, after Bonferroni's correction for multiple comparisons) are indicated by *.

3.2. Immune fitness

Immune fitness ratings are summarized in Table 2 and Fig. 1. For the hangover-resistant group, significant reductions in immune fitness were found on the alcohol day compared to the control day, except for the assessments at 12:30 and 15:30. Bayesian (Wilcoxon-signed-rank) analyses provided at least anecdotal evidence that a reduction in immune fitness was also more likely than the null hypothesis of no differences for those two time points (all $BF_{10} > 1$). The average ratings on both test days were at least 7.5 (out of 10), indicating an adequate immune fitness. In contrast, for the hangover-sensitive group, the immune fitness ratings remained below 6 on the alcohol day, indicating reduced immune fitness. For all timepoints, the immune fitness ratings on the alcohol day were significantly lower than those on the control day. On both the alcohol day and the control day, the immune fitness ratings of the hangover-sensitive group were significantly poorer than those of the hangover-resistant group. For the only data point where this was not the case, Bayesian (Mann-Whitney) analyses provided moderate evidence that a lower immune fitness of the hangover-sensitive group was more likely than the null hypothesis of no differences between the groups ($BF_{10} > 3$).

3.3. Biomarker assessments

The results of the biomarker assessments are summarized in Tables 3–6. On both the test days, on none the timepoints there was a significant difference between the hangover-sensitive group and the hangover-resistant group. For the hangover-sensitive group, the saliva concentration IL-6 at 09:30 on the alcohol day was significantly higher than the concentration at the same timepoint on the control day. No significant differences were found for the other timepoints. For both groups, for IL-1β, IL-8, and TNF-α, no significant differences between the control day and hangover day were found.

3.4. Correlations of immune fitness and biomarkers with hangover severity

For the hangover-sensitive group, correlations between change scores (Δ, alcohol day – control day) of immune fitness, the biomarkers of systemic inflammation, and overall hangover severity on the alcohol day are summarized in Table 7. The assessments were not conducted for the hangover-sensitive group, as they reported no hangover. High correlations were found between Δ immune fitness and overall hangover severity, which were statistically significant at 10:30, 11:30 and 12:30. After bootstrapping (10.000 samples, bias-corrected and accelerated), these correlations remained significant, and also the correlation between immune fitness and overall hangover severity at 09:30 (BCa 95 % CI: lower = −0.934, upper = −0.060) and 13:30 (BCa 95 % CI: lower =

Table 2
Immune fitness.

Group	Hangover-resistant group			Hangover-sensitive group			R vs S group, *p*-value	
Time	Control day	Alcohol day	*p*-value	Control day	Alcohol day	*p*-value	Control day	Alcohol day
09:30	8.8 (0.8)	7.5 (1.7)	<0.001 *	7.5 (1.5)	4.9 (2.1)	<0.001 *	0.006 ‡	0.003 ‡
10:30	8.8 (0.9)	7.8 (1.3)	<0.001 *	7.7 (1.3)	5.0 (1.9)	<0.001 *	0.008	<0.001 ‡
11:30	8.9 (0.8)	8.0 (1.3)	<0.001 *	7.5 (1.2)	5.3 (1.7)	0.002 *	0.002 ‡	<0.001 ‡
12:30	8.8 (0.9)	8.3 (1.0)	0.020	7.5 (1.2)	5.3 (1.8)	<0.001 *	0.006 ‡	<0.001 ‡
13:30	8.9 (0.8)	8.0 (1.4)	<0.001 *	7.8 (1.3)	5.6 (2.0)	<0.001 *	0.006 ‡	0.002 ‡
14:30	9.0 (0.8)	8.2 (1.1)	<0.001 *	7.7 (1.2)	5.4 (2.0)	<0.001 *	0.002 ‡	<0.001 ‡
15:30	8.9 (0.9)	8.4 (1.1)	0.130	7.6 (1.3)	5.6 (2.0)	<0.001 *	0.006 ‡	<0.001 ‡

Mean and standard deviation (SD) are shown. Significant differences (at the same timepoint) between the control day and the alcohol day ($p < 0.0071$, after Bonferroni's correction for multiple comparisons) are indicated by *. Significant differences (at the same timepoint) between the hangover-resistant group and hangover-sensitive group ($p < 0.0071$, after Bonferroni's correction for multiple comparisons) are indicated by ‡. Abbreviations: R = hangover-resistant group, S = hangover-sensitive group.

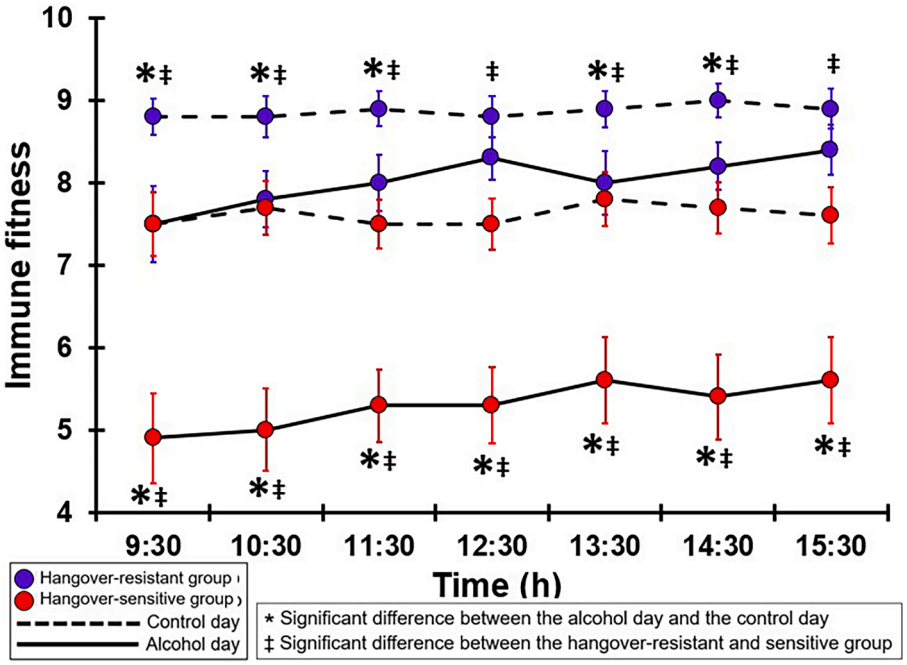

Fig. 1. Immune fitness. Mean and standard error (SE) are shown. Significant differences (at the same timepoint) between the control day and the alcohol day ($p < 0.0071$, after Bonferroni's correction for multiple comparisons) are indicated by *. Significant differences (at the same timepoint) between the hangover-resistant group and hangover-sensitive group ($p < 0.0071$, after Bonferroni's correction for multiple comparisons) are indicated by ‡.

−0.831, upper = −0.025) were significant. Except for IL-1β at 12:30, none of the correlations between biomarker assessments and overall hangover severity were significant. After bootstrapping, the correlation between overall hangover severity and IL-1β at 12:30 remained significant, and at the same timepoint the correlation between overall hangover severity and IL-6 was also significant (BCa 95 % CI: lower = 0.091, upper = 0.779).

3.5. Correlations between immune fitness and biomarkers of systemic inflammation

The results thus far revealed significant relationships between immune fitness and hangover severity in hangover-sensitive drinkers, and significantly differentiated between the hangover-sensitive group and the hangover-resistant group. It is of interest to evaluate to what extent changes in immune fitness are related to changes in saliva biomarkers of systemic inflammation per se. To this extent, Spearman's correlations between change scores (Δ, alcohol day – control day) of immune fitness and the biomarkers of systemic inflammation were computed for each timepoint. The analysis was conducted for the full sample (N = 29) and the results are summarized in Table 8. A significant correlation was found between immune fitness and IL-1β at 12:30. After bootstrapping, at 11:30 a significant correlation was found between immune fitness and IL-8 (BCa 95 % CI: lower = −0.753, upper = −0.021), and the correlation between immune fitness and IL-1β remained significant (BCa 95 % CI: low-er = −0.720, upper = −0.142).

For the hangover sensitive group only (data not shown), no significant Spearman's correlations were found at any timepoint. Also after bootstrapping, none of the correlations were statistically significant. For the hangover resistant group only (data not shown), the analyses revealed no significant correlations between immune fitness and the biomarkers of inflammation, except after bootstrapping between immune fitness and IL-8 at 11:30 (BCa 95 % CI: lower = −0.962, upper = −0.200).

4. Discussion

This study revealed that both hangover-sensitive drinkers and hangover-resistant drinkers report significantly reduced immune fitness the day after an evening of alcohol consumption. However, there are marked differences between the groups. At the control day, immune fitness of the hangover-sensitive group was significantly poorer than

Table 3
IL-1β.

Group	Hangover-resistant group			Hangover-sensitive group			R vs S group *p*-value & BF_{10}	
Time	Control day	Alcohol day	*p*-value BF_{10} value	Control day	Alcohol day	*p*-value BF_{10} value	Control day	Alcohol day
09:30	226.0 (430.1)	129.5 (177.1)	$p = 0.619$ $BF_{10} = 0.292$	77.7 (104.4)	93.3 (108.5)	$p = 0.371$ $BF_{10} = 0.468$	$p = 0.315$ $BF_{10} = 0.592$	$p = 0.861$ $BF_{10} = 0.334$
10:30	72.0 (85.8)	74.2 (94.4)	$p = 0.651$ $BF_{10} = 0.271$	91.6 (117.0)	92.4 (185.1)	$p = 0.793$ $BF_{10} = 0.265$	$p = 0.616$ $BF_{10} = 0.384$	$p = 0.861$ $BF_{10} = 0.359$
11:30	43.1 (62.7)	47.2 (54.9)	$p = 0.557$ $BF_{10} = 0.339$	71.2 (162.8)	33.6 (38.5)	$p = 0.647$ $BF_{10} = 0.300$	$p = 0.694$ $BF_{10} = 0.410$	$p = 0.337$ $BF_{10} = 0.446$
12:30	101.2 (96.1)	45.7 (43.3)	$p = 0.047$ $BF_{10} = 6.490$	53.4 (75.8)	35.1 (26.6)	$p = 0.239$ $BF_{10} = 0.296$	$p = 0.070$ $BF_{10} = 0.813$	$p = 0.727$ $BF_{10} = 0.410$
13:30	43.5 (49.7)	38.1 (42.5)	$p = 0.498$ $BF_{10} = 0.345$	20.6 (16.2)	21.3 (14.5)	$p = 0.471$ $BF_{10} = 0.313$	$p = 0.138$ $BF_{10} = 0.872$	$p = 0.337$ $BF_{10} = 0.482$
14:30	48.2 (35.1)	39.7 (34.2)	$p = 0.155$ $BF_{10} = 0.473$	55.6 (58.8)	74.2 (179.4)	$p = 0.371$ $BF_{10} = 0.421$	$p = 0.861$ $BF_{10} = 0.372$	$p = 0.600$ $BF_{10} = 0.417$
15:30	86.9 (110.6)	135.1 (208.2)	$p = 0.946$ $BF_{10} = 0.279$	60.8 (69.3)	62.0 (97.7)	$p = 0.485$ $BF_{10} = 0.266$	$p = 0.513$ $BF_{10} = 0.426$	$p = 0.556$ $BF_{10} = 0.391$

Mean and standard deviation (SD) (in pg/mL saliva) are shown. No significant differences (at the same timepoint) between the control day and the alcohol day ($p < 0.0071$, after Bonferroni's correction for multiple comparisons) were found. No significant differences (at the same timepoint) between the hangover-resistant group and hangover-sensitive group ($p < 0.0071$, after Bonferroni's correction for multiple comparisons) were found. Abbreviations: R = hangover-resistant group, S = hangover-sensitive group, BF_{10} = Bayesian Factor.

Table 4
IL-6.

Group	Hangover-resistant group			Hangover-sensitive group			R vs S group *p*-value & BF_{10} value	
Time	Control day	Alcohol day	*p*-value BF_{10} value	Control day	Alcohol day	*p*-value BF_{10} value	Control day	Alcohol day
09:30	10.7 (10.2)	18.8 (31.8)	$p = 0.701$ $BF_{10} = 0.373$	5.5 (4.9)	12.0 (10.3)	$p = 0.005$ * $BF_{10} = 9.022$	$p = 0.144$ $BF_{10} = 0.829$	$p = 0.711$ $BF_{10} = 0.365$
10:30	7.6 (7.5)	10.8 (10.9)	$p = 0.109$ $BF_{10} = 0.504$	4.8 (3.6)	7.1 (7.2)	$p = 0.116$ $BF_{10} = 0.723$	$p = 0.419$ $BF_{10} = 0.462$	$p = 0.315$ $BF_{10} = 0.505$
11:30	4.8 (3.6)	12.7 (20.3)	$p = 0.044$ $BF_{10} = 15.432$	5.9 (8.9)	6.2 (5.6)	$p = 0.663$ $BF_{10} = 0.297$	$p = 0.861$ $BF_{10} = 0.350$	$p = 0.471$ $BF_{10} = 0.397$
12:30	14.2 (26.8)	14.7 (23.0)	$p = 0.718$ $BF_{10} = 0.329$	4.8 (3.4)	6.2 (4.3)	$p = 0.844$ $BF_{10} = 0.463$	$p = 0.150$ $BF_{10} = 0,729$	$p = 0.407$ $BF_{10} = 0.452$
13:30	5.9 (4.9)	13.7 (17.6)	$p = 0.082$ $BF_{10} = 2.701$	3.1 (2.5)	5.9 (5.6)	$p = 0.067$ $BF_{10} = 1.370$	$p = 0.047$ $BF_{10} = 1.326$	$p = 0.138$ $BF_{10} = 0.824$
14:30	5.2 (3.7)	12.3 (20.2)	$p = 0.484$ $BF_{10} = 0.775$	5.3 (4.5)	7.9 (6.4)	$p = 0.326$ $BF_{10} = 0.679$	$p = 0.827$ $BF_{10} = 0.363$	$p = 0.896$ $BF_{10} = 0.354$
15:30	7.5 (11.1)	20.5 (34.7)	$p = 0.061$ $BF_{10} = 9.349$	5.4 (3.8)	8.2 (9.8)	$p = 0.315$ $BF_{10} = 0.429$	$p = 0.793$ $BF_{10} = 0.360$	$p = 0.541$ $BF_{10} = 0.426$

Mean and standard deviation (SD) (in pg/mL saliva) are shown. Significant differences (at the same timepoint) between the control day and the alcohol day ($p < 0.0071$, after Bonferroni's correction for multiple comparisons) are indicated by *. No significant differences (at the same timepoint) between the hangover-resistant group and hangover-sensitive group ($p < 0.0071$, after Bonferroni's correction for multiple comparisons) were found. Abbreviations: R = hangover-resistant group, S = hangover-sensitive group, BF_{10} = Bayesian Factor.

that reported by the hangover-resistant group. This finding is in line with a previous survey report (Van de Loo et al., 2018). Following the alcohol day, immune fitness scores of the hangover-resistant group are significantly reduced, but remain well in the range of scores that can be considered as having an adequate immune fitness (scores between 6 and 10). All hangover-resistant drinkers reported to have no hangover. In contrast, in the hangover-sensitive group all participants reported to having an alcohol hangover. Their immune fitness scores dropped from adequate immune fitness scores around 7.5 on the control day to scores that were well below 6 following the alcohol day, indicating reduced immune fitness throughout the post-alcohol consuming test day. The findings were not supported by the hourly saliva assessments of biomarkers of systemic inflammation. No significant difference at any timepoint of test day was observed between the two groups. These findings were confirmed by Bayesian analysis showing a $BF_{10} < 1$ (no evidence for the alternative hypothesis that the groups differ) for almost all comparisons. This is in line with a previous study assessing saliva biomarkers at 09:30 (Van de Loo et al., 2021), that also failed to find differences in biomarker concentrations between hangover-sensitive drinkers and hangover-resistant drinkers.

Overall hangover severity correlated significantly with reductions in immune fitness (hangover day – control day) in the morning assessments up to 12:30, but correlations with biomarkers of systemic inflammation were usually not significant. This is in line with findings of a previous study that assessed these biomarkers in saliva (Van de Loo et al., 2021), but in contrast to two studies that assessed the biomarkers in blood and did find a significant correlation between overall hangover severity and some of the cytokines assessed (Kim et al., 2003; Van de Loo et al., 2020). Bayesian analyses confirmed the significant correlation between immune fitness and hangover severity. For some timepoints, there was also moderate to strong evidence ($BF_{10} > 3$) for significant correlations between hangover severity and saliva concentrations of Il-6, IL-8, TNF-α, and in particular IL-1β. Thus, the relationship between immune fitness and hangover severity was most pronounced. Changes in biomarkers of systemic inflammation were related to hangover severity, but less consistent over time.

Table 5
IL-8.

Group	Hangover-resistant group			Hangover-sensitive group			R vs S group *p*-value & BF_{10} value	
Time	Control day	Alcohol day	*p*-value BF_{10} value	Control day	Alcohol day	*p*-value BF_{10} value	Control day	Alcohol day
09:30	1722.5 (2899.6)	835.6 (879.5)	$p = 0.074$ $BF_{10} = 0.651$	972.9 (1188.0)	839.1 (883.3)	$p = 0.810$ $BF_{10} = 0.288$	$p = 0.336$ $BF_{10} = 0.480$	$p = 0.861$ $BF_{10} = 0.365$
10:30	719.1 (783.8)	909.5 (912.8)	$p = 0.769$ $BF_{10} = 0.299$	601.1 (730.1)	505.1 (568.0)	$p = 0.631$ $BF_{10} = 0.288$	$p = 0.570$ $BF_{10} = 0.337$	$p = 0.457$ $BF_{10} = 0.395$
11:30	580.0 (747.0)	349.3 (548.6)	$p = 0.378$ $BF_{10} = 0.487$	300.5 (525.3)	152.7 (232.0)	$p = 0.023$ $BF_{10} = 16.705$	$p = 0.600$ $BF_{10} = 0.378$	$p = 0.116$ $BF_{10} = 0.728$
12:30	799.1 (819.4)	564.6 (686.8)	$p = 0.078$ $BF_{10} = 0.395$	512.2 (715.1)	291.5 (318.9)	$p = 0.407$ $BF_{10} = 0.599$	$p = 0.471$ $BF_{10} = 0.392$	$p = 0.315$ $BF_{10} = 0.519$
13:30	522.0 (719.2)	274.9 (305.4)	$p = 0.259$ $BF_{10} = 0.672$	144.8 (111.1)	126.9 (126.5)	$p = 0.407$ $BF_{10} = 0.294$	$p = 0.213$ $BF_{10} = 0.528$	$p = 0.162$ $BF_{10} = 0.660$
14:30	802.6 (888.5)	469.2 (571.5)	$p = 0.206$ $BF_{10} = 1.457$	516.6 (744.3)	302.8 (529.1)	$p = 0.163$ $BF_{10} = 1.844$	$p = 0.930$ $BF_{10} = 0.361$	$p = 0.305$ $BF_{10} = 0.475$
15:30	1009.6 (1151.3)	1010.8 (1237.1)	$p = 0.456$ $BF_{10} = 0.365$	527.9 (598.8)	445.6 (710.4)	$p = 0.556$ $BF_{10} = 0.328$	$p = 0.256$ $BF_{10} = 0.662$	$p = 0.190$ $BF_{10} = 0.560$

Mean and standard deviation (SD) (in pg/mL saliva) are shown. No significant differences (at the same timepoint) between the control day and the alcohol day ($p < 0.0071$, after Bonferroni's correction for multiple comparisons) were found. No significant differences (at the same timepoint) between the hangover-resistant group and hangover-sensitive group ($p < 0.0071$, after Bonferroni's correction for multiple comparisons) were found. Abbreviations: R = hangover-resistant group, S = hangover-sensitive group, BF_{10} = Bayesian Factor.

Table 6
TNF-α.

Group	Hangover-resistant group			Hangover-sensitive group			R vs S group *p*-value & BF_{10} value	
Time	Control day	Alcohol day	*p*-value BF_{10} value	Control day	Alcohol day	*p*-value BF_{10} value	Control day	Alcohol day
09:30	49.7 (35.4)	64.9 (63.2)	–	38.8 (36.2)	41.4 (42.6)	$p = 0.616$ $BF_{10} = 0.271$	$p = 0.407$ $BF_{10} = 0.453$	$p = 0.238$ $BF_{10} = 0.628$
10:30	49.1 (67.5)	41.6 (22.9)	–	36.2 (43.5)	32.7 (40.3)	$p = 0.879$ $BF_{10} = 0.274$	$p = 0.371$ $BF_{10} = 0.489$	$p = 0.060$ $BF_{10} = 1.102$
11:30	27.4 (24.0)	31.5 (21.4)	–	27.6 (30.7)	35.3 (43.6)	$p = 0.793$ $BF_{10} = 0.379$	$p = 0.646$ $BF_{10} = 0.375$	$p = 0.458$ $BF_{10} = 0.422$
12:30	41.2 (43.7)	37.0 (33.8)	–	24.1 (29.8)	20.6 (14.7)	$p = 0.527$ $BF_{10} = 0.324$	$p = 0.156$ $BF_{10} = 0.668$	$p = 0.359$ $BF_{10} = 0.579$
13:30	23.9 (19.4)	41.6 (33.4)	–	11.9 (16.9)	22.5 (18.1)	$p = 0.085$ $BF_{10} = 2.307$	$p = 0.030$ $BF_{10} = 1.295$	$p = 0.044$ $BF_{10} = 1.908$
14:30	28.0 (20.1)	36.0 (36.0)	–	25.7 (25.1)	39.1 (43.7)	$p = 0.743$ $BF_{10} = 0.378$	$p = 0.407$ $BF_{10} = 0.429$	$p = 0.896$ $BF_{10} = 0.366$
15:30	50.2 (72.6)	59.1 (71.6)	–	26.6 (28.9)	39.6 (31.2)	$p = 0.025$ $BF_{10} = 0.553$	$p = 0.348$ $BF_{10} = 0.450$	$p = 0.983$ $BF_{10} = 0.379$

Mean and standard deviation (SD) (in pg/mL saliva) are shown. No significant differences (at the same timepoint) were found between the control day and the alcohol day ($p < 0.0071$, after Bonferroni's correction for multiple comparisons). For the hangover-resistant group, the main effect of control vs alcohol day was not significant ($p = 0.143$). Therefore, no paired comparisons were conducted (indicated by –). No significant differences (at the same timepoint) between the hangover-resistant group and hangover-sensitive group ($p < 0.0071$, after Bonferroni's correction for multiple comparisons) were found. Abbreviations: R = hangover-resistant group, S = hangover-sensitive group, BR_{10} = Bayesian Factor.

The correlations between changes in immune fitness and biomarkers of systemic inflammation were usually poor and not significant. This was supported by the Bayesian analyses. The latter was expected given the fact that the immune system is very complex and dynamic with numerous substances interacting with each other, which then together determine the level of immune fitness, homeostasis, or an inflammatory response. It would therefore be very unlikely that any single biomarker of systemic inflammation would strongly correlate with an overall global assessment of immune fitness (Verster et al., 2023). The latter may also explain why correlations between immune fitness and hangover severity are more strong and consistent compared to the correlations between hangover severity and biomarker concentrations.

The strength of this study is the fact that assessments were made throughout the day. The importance of this design is underlined by the findings of the study. For some variables, the assessments show considerable variability between the timepoints. For example, high hangover severity scores were reported in the morning which gradually decreased during the day. However, for other variables such as immune fitness, the poorer scores following the alcohol day remain fairly consistent throughout the test day. Another strength of the article is that the traditional statistical methods were supported by Bayesian analyses. The study is limited by the relatively small sample size. This was taken into account with some of the correlational analyses by conducting confirmatory bootstrapping analysis. In addition, the participants were healthy, young adults, between 18 and 30 years old. It is therefore unclear to what extent the findings can be generalized towards older age groups, or those with underlying conditions or illness. The current sample size also did not allow for statistical evaluation to determine possible sex differences. Therefore, it is recommended that future research replicates this study with a more diverse and larger population sample. Participants were recruited as being hangover-sensitive or hangover-resistant, and at screening they were thoroughly interviewed

Table 7
Correlations of changes in immune fitness and biomarker assessments with overall hangover severity.

Time	Δ immune fitness	Δ IL-1β	Δ IL-6	Δ IL-8	Δ TNF-α
09:30	r = −0.567 p = 0.027 BF_{10} = 12.049	r = −0.098 p = 0.729 BF_{10} = 0.321	r = −0.337 p = 0.219 BF_{10} = 0.647	r = −0.197 p = 0.481 BF_{10} = 0.746	r = −0.373 p = 0.171 BF_{10} = 1.235
10:30	r = −0.683 p = 0.005 * BF_{10} = 7.340	r = −0.067 p = 0.811 BF_{10} = 5.901	r = −0.120 p = 0.669 BF_{10} = 0.974	r = 0.184 p = 0.512 BF_{10} = 0.347	r = −0.120 p = 0.669 BF_{10} = 0.318
11:30	r = −0.707 p = 0.003 * BF_{10} = 1.799	r = 0.331 p = 0.259 BF_{10} = 4.020	r = 0.293 p = 0.289 BF_{10} = 3.280	r = 0.499 p = 0.058 BF_{10} = 4.900	r = 0.340 p = 0.214 BF_{10} = 0.544
12:30	r = −0.683 p = 0.005 * BF_{10} = 3.419	r = −0.770 p < 0.001 * BF_{10} = 25.661	r = 0.515 p = 0.050 BF_{10} = 1.031	r = 0.516 p = 0.049 BF_{10} = 1.267	r = 0.541 p = 0.037 BF_{10} = 5.916
13:30	r = −0.509 p = 0.053 BF_{10} = 1.014	r = −0.060 p = 0.823 BF_{10} = 0.326	r = −0.024 p = 0.993 BF_{10} = 0.359	r = 0.096 p = 0.733 BF_{10} = 0.354	r = 0.432 p = 0.108 BF_{10} = 0.975
14:30	r = −0.413 p = 0.126 BF_{10} = 0.893	r = −0.271 p = 0.329 BF_{10} = 0.944	r = −0.260 p = 0.350 BF_{10} = 0.506	r = −0.123 p = 0.664 BF_{10} = 0.410	r = −0.130 p = 0.645 BF_{10} = 0.321
15:30	r = −0.553 p = 0.040 BF_{10} = 2.943	r = −0.413 p = 0.216 BF_{10} = 0.682	r = −0.337 p = 0.219 BF_{10} = 1.718	r = −0.239 p = 0.390 BF_{10} = 0.595	r = −0.308 p = 0.264 BF_{10} = 0.930

Only data of the hangover-sensitive group was included in the analysis. Spearman's rho, corresponding p-value and BF_{10} value are shown. Significant correlations (at the same timepoint) with overall hangover severity ($p < 0.0071$, after Bonferroni's correction for multiple comparisons) are indicated by *. Abbreviations: IL = interleukin, TNF = tumor necrosis factor, BF_{10} = Bayesian Factor.

Table 8
Correlations between changes in immune fitness and biomarkers of systemic inflammation.

Time	Δ IL-1β	Δ IL-6	Δ IL-8	Δ TNF-α
09:30	r = −0.163 p = 0.399 BF_{10} = 0.326	r = −0.101 p = 0.602 BF_{10} = 0.357	r = −0.305 p = 0.107 BF_{10} = 0.378	r = 0.220 p = 0.251 BF_{10} = 1.055
10:30	r = −0.084 p = 0.663 BF_{10} = 0.531	r = −0.020 p = 0.919 BF_{10} = 0.320	r = −0.278 p = 0.145 BF_{10} = 0.333	r = 0.089 p = 0.646 BF_{10} = 0.406
11:30	r = −0.087 p = 0.672 BF_{10} = 0.698	r = −0.047 p = 0.809 BF_{10} = 1.253	r = −0.454 p = 0.013 ‡ BF_{10} = 0.864	r = −0.091 p = 0.637 BF_{10} = 0.532
12:30	r = −0.490 p = 0.007 *‡ BF_{10} = 0.700	r = −0.141 p = 0.466 BF_{10} = 0.471	r = −0.218 p = 0.257 BF_{10} = 0.614	r = −0.064 p = 0.741 BF_{10} = 0.542
13:30	r = −0.153 p = 0.428 BF_{10} = 0.491	r = −0.037 p = 0.847 BF_{10} = 1.970	r = −0.201 p = 0.295 BF_{10} = 0.433	r = 0.097 p = 0.616 BF_{10} = 0.839
14:30	r = −0.130 p = 0.503 BF_{10} = 0.318	r = −0.187 p = 0.330 BF_{10} = 0.419	r = 0.063 p = 0.744 BF_{10} = 0.404	r = 0.064 p = 0.743 BF_{10} = 0.404
15:30	r = 0.001 p = 0.994 BF_{10} = 0.339	r = 0.321 p = 0.095 BF_{10} = 0.366	r = −0.036 p = 0.855 BF_{10} = 0.329	r = 0.076 p = 0.699 BF_{10} = 0.603

Spearman's rho, corresponding p-value and BF_{10} value are shown. Significant Spearman's correlations (at the same timepoint) with immune fitness ($p < 0.0071$, after Bonferroni's correction for multiple comparisons) are indicated by *. Significance after bootstrapping is indicated by ‡. Abbreviations: IL = interleukin, TNF = tumor necrosis factor, BF_{10} = Bayesian Factor.

to confirm this status. Therefore, beliefs and expectations about their hangover sensitivity could potentially have biased their ratings on the day after drinking, since participants were aware of the study group they had been assigned to.

Taken together, this study provides further evidence that immune fitness is reduced the day after an evening of alcohol consumption, and that reduced immune fitness is significantly related to the severity of the alcohol hangover. In line with previous research (Van de Loo et al., 2018), at baseline (i.e., the control day) hangover-resistant drinkers report a better immune fitness than hangover-sensitive drinkers. Future research should continue to investigate the role of the immune system and immune fitness in the pathology of the alcohol hangover.

5. Institutional review board statement

The study was conducted in accordance with the Declaration of Helsinki, and approved by the University of Groningen Psychology Ethics Committee (approval number: ppo-015-002, approval date: 3 September 2015).

6. Informed consent statement

Informed consent was obtained from all subjects involved in the study.

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CRediT authorship contribution statement

Agnese Merlo: Conceptualization, Writing – original draft, Writing – review & editing. **Marlou Mackus:** Conceptualization, Writing – review & editing. **Aurora J.A.E. van de Loo:** Conceptualization, Writing – review & editing, Investigation, Methodology. **Renier H.P. van Neer:** Investigation, Writing – review & editing. **Sterre A. Vermeulen:** Investigation, Writing – review & editing. **Suzan S. Thijssen:** Formal analysis, Writing – review & editing. **Karen Knipping:** Methodology, Writing – review & editing. **Gillian Bruce:** Conceptualization, Writing – review & editing. **Johan Garssen:** Conceptualization, Writing – review & editing. **Joris C. Verster:** Conceptualization, Methodology, Formal analysis, Writing – original draft, Writing – review & editing.

Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Johan Garssen is a part-time employee of Nutricia Research and received research grants from Nutricia research foundation, Top Institute Pharma, Top Institute Food and Nutrition, GSK, STW, NWO, Friesland Campina, CCC, Raak-Pro, and EU. Over the past 3 years, Joris Verster has acted as a consultant/advisor for Eisai, KNMP, Red Bull, Sen-Jam Pharmaceutical, and Toast! The other authors declare no conflicts of interest.

Data availability

The data are available upon request from the corresponding author.

References

Alford, C., Broom, C., Carver, H., Johnson, S. J., Reece, R., Lands, S., & Verster, J. C. (2020). The impact of alcohol hangover on simulated driving performance during a 'commute to work' – zero and residual alcohol effects compared. *Journal of Clinical Medicine, 9*, 1435.

Dhima, M., Salinas, T. J., Wermers, R. A., Weaver, A. L., & Koka, S. (2013). Preference changes of adult outpatients for giving saliva, urine and blood for clinical testing after actual sample collection. *Journal of Prosthodontic Research, 57*, 51–56.

Efron, B. (1979). Bootstrap methods: Another look at the jackknife. *The Annals of Statistics, 7*, 1–26.

Efron, B., & Tibshirani, R. (1993). *An introduction to the bootstrap*. Boca Raton, FL, USA: Chapman & Hall/CRC.
Gunn, C., Mackus, M., Griffin, C., Munafò, M. R., & Adams, S. (2018). A systematic review of the next-day effects of heavy alcohol consumption on cognitive performance. *Addiction, 113*, 2182–2193.
Hartung, B., Schwender, H., Mindiashvili, N., Ritz-Timme, S., Malczyk, A., & Daldrup, T. (2015). The effect of alcohol hangover on the ability to ride a bicycle. *International Journal of Legal Medicine, 129*, 751–758.
Hogewoning, A., van de Loo, A. J. A. E., Mackus, M., Raasveld, S. J., De Zeeuw, R., Bosma, E. R., … Verster, J. C. (2016). Characteristics of social drinkers with and without a hangover after heavy alcohol consumption. *Substance Abuse and Rehabilitation, 7*, 161–167.
Howland, J., Rohsenow, D. J., & Edwards, E. M. (2008). Are some drinkers resistant to hangover? A literature review. *Current Drug Abuse Reviews, 1*, 42–46.
Ibarra-Coronado, E. G., Pantaleón-Martínez, A. M., Velazquéz-Moctezuma, J., Prospéro-García, O., Méndez-Díaz, M., Pérez-Tapia, M., … Morales-Montor, J. (2015). The bidirectional relationship between sleep and immunity against infections. *Journal of Immunology Research, 2015*, Article 678164.
Jeffreys, H. (1961). *Theory of probability* (3rd ed.). Oxford: Oxford University Press.
Kiani, P., Mulder, K. E. W., Balikji, J., Kraneveld, A. D., Garssen, J., & Verster, J. C. (2022). Pandemic preparedness: Maintaining adequate immune fitness by attaining a normal, healthy bodyweight. *Journal of Clinical Medicine, 11*, 3933.
Kim, D. J., Kim, W., Yoon, S. J., Choi, B. M., Kim, J. S., Go, H. J., … Jeong, J. (2003). Effects of alcohol hangover on cytokine production in healthy subjects. *Alcohol, 31*, 167–170.
Kruisselbrink, L. D. (2019). The neurocognitive effects of alcohol hangover: Patterns of impairment/nonimpairment within the neurocognitive domain of the Diagnostic and Statistical Manual of Mental Disorders. In V. R. Preedy (Ed.), *Neuroscience of alcohol: Mechanisms and treatment* (5th ed., pp. 391–402). Cambridge, MA, USA: Academic Press.
Kruisselbrink, L. D., Bervoets, A. C., de Klerk, S., van de Loo, A. J. A. E., & Verster, J. C. (2017). Hangover resistance in a Canadian uni-versity student population. *Addictive Behaviors Reports, 5*, 14–18.
Lawick, V., van Pabst, A. E., Devenney, L. E., & Verster, J. C. (2019). Sex differences in the presence and severity of alcohol hangover symptoms. *Journal of Clinical Medicine, 8*, 867.
Mackus, M., van de Loo, A. J. E. A., Garssen, J., Kraneveld, A. D., Scholey, A. D., & Verster, J. C. (2020a). The role of alcohol metabolism in the pathology of alcohol hangover. *Journal of Clinical Medicine, 9*, 3421.
Mackus, M., van de Loo, A. J. E. A., Garssen, J., Kraneveld, A. D., Scholey, A., & Verster, J. C. (2020b). The association between ethanol elimination rate and hangover severity. *International Journal of Environmental Research and Public Health, 17*, E4324.
Mackus, M., Van de Loo, A. J. A. E., Van Neer, R. H. P., Vermeulen, S. A., Terpstra, C., Brookhuis, K. A., … Verster, J. C. (2023). Differences in next-day adverse effects and impact on mood of an evening of heavy alcohol consumption between hangover-sensitive drinkers and hangover-resistant drinkers. *Journal of Clinical Medicine, 12*, 2090.
McKinney, A. (2010). A review of the next day effects of alcohol on subjective mood ratings. *Current Drug Abuse Reviews, 3*, 88–91.
Merlo, A., Severeijns, N. R., Benson, S., Scholey, A., Garssen, J., Bruce, G., & Verster, J. C. (2021). Mood and changes in alcohol consumption in young adults during COVID-19 lockdown: A model explaining associations with perceived immune fitness and experiencing COVID-19 symptoms. *International Journal of Environmental Research and Public Health, 18*, 10028.
Nakao, A. (2014). Temporal regulation of cytokines by the circadian clock. *Journal of Immunology Research, 2014*, Article 614529.
Ouellet-Morin, I., Danese, A., Williams, B., & Arseneault, L. (2011). Validation of a high-sensitivity assay for C-reactive protein in human saliva. *Brain, Behavior, and Immunity, 25*, 640–646.
Palmer, E., Tyacke, R., Sastre, M., Lingford-Hughes, A., Nutt, D., & Ward, R. J. (2019). Alcohol hangover: Underlying biochemical, inflammatory and neurochemical mechanisms. *Alcohol and Alcoholism, 54*, 196–203.
Penning, R., McKinney, A., & Verster, J. C. (2012). Alcohol hangover symptoms and their contribution to overall hangover severity. *Alcohol and Alcoholism, 47*, 248–252.
Riis, J. L., Out, D., Dorn, L. D., Beal, S. J., Denson, L. A., Pabst, S., … Granger, D. A. (2014). Salivary cytokines in healthy adolescent girls: Intercorrelations, stability, and associations with serum cytokines, age, and pubertal stage. *Developmental Psychobiology, 56*, 797–811.
Shephard, R. J., & Shek, P. N. (1994). Potential impact of physical activity and sport on the immune system–a brief review. *British Journal of Sports Medicine, 28*, 247–255.
Sips, A. S. M., Severeijns, N. R., Kraneveld, A. D., Garssen, J., & Verster, J. C. (2023). Reduced immune fitness: Related absenteeism from work, presenteeism, and estimated costs for the Dutch economy. *International Journal of Environmental Research and Public Health, 20*, 1761.
Terpstra, C., Benson, S., Verster, J. C., & Scholey, A. (2020). Prevalence of hangover resistance according to two methods for calculating estimated blood alcohol concentration (eBAC). *Journal of Clinical Medicine, 9*, 2923.
Tipple, C., Benson, S., & Scholey, A. (2017). A review of the physiological factors associated with an alcohol-induced hangover. *Current Drug Abuse Reviews, 9*, 93–98.
Van de Loo, A. J. A. E., Mackus, M., Kwon, O., Krishnakumar, I., Garssen, J., Kraneveld, A. D., … Verster, J. C. (2020). The inflammatory response to alcohol consumption and its role in the pathology of alcohol hangover. *Journal of Clinical Medicine, 9*, 2081.
Van de Loo, A. J. A. E., Mackus, M., van Schrojenstein Lantman, M., Kraneveld, A. D., Garssen, J., Scholey, A., & Verster, J. C. (2018). Susceptibility to alcohol hangovers: The association with self-reported immune status. *International Journal of Environmental Research and Public Health, 15*, 1286.
Van de Loo, A. J. A. E., Raasveld, S. J., Hogewoning, A., de Zeeuw, R., Bosma, E. R., Bouwmeester, N. H., … Verster, J. C. (2021). Immune responses after heavy alcohol consumption: Cytokine concentrations in hangover-sensitive and hangover-resistant drinkers. *Healthcare, 9*, 395.
Van Schrojenstein Lantman, M., Otten, L. S., Mackus, M., de Kruijff, D., van de Loo, A. J. A. E., Kraneveld, A. D., … Verster, J. C. (2017). Mental resilience, perceived immune functioning, and health. *Journal of Multidisciplinary Healthcare, 10*, 107–112.
Van Schrojenstein, L. M., Mackus, M., van de Loo, A. J. A. E., & Verster, J. C. (2016). Development of a definition for the alcohol hangover: Consumer descriptions and expert consensus. *Current Drug Abuse Reviews, 9*, 148–154.
Van Schrojenstein, L. M., Mackus, M., van de Loo, A. J. A. E., & Verster, J. C. (2017). The impact of alcohol hangover symptoms on cognitive and physical functioning, and mood. *Human Psychopharmacology: Clinical and Experimental, 32*, e2623.
Verster, J. C., Bervoets, A. C., de Klerk, S., Vreman, R. A., Olivier, B., Roth, T., & Brookhuis, K. A. (2014). Effects of alcohol hangover on simulated highway driving performance. *Psychopharmacol., 231*, 2999–3008.
Verster, J. C., de Klerk, S., Bervoets, A. C., & Kruisselbrink, D. (2013). Can Hangover Immunity be Really Claimed? *Current Drug Abuse Reviews, 6*, 253–254.
Verster, J. C., Kraneveld, A. D., & Garssen, J. (2023). The assessment of immune fitness. *Journal of Clinical Medicine, 12*, 22.
Verster, J. C., Kruisselbrink, L. D., Slot, K. A., Anogeianaki, A., Adams, S., Alford, C., … Stock, A.-K. (2020). on behalf of the Alcohol Hangover Research Group. Sensitivity to experiencing alcohol hangovers: Reconsideration of the 0.11 % blood alcohol concentration (BAC) threshold for having a hangover. *Journal of Clinical Medicine, 9*, 179.
Verster, J. C., Scholey, A., van de Loo, A. J. A. E., Benson, S., & Stock, A.-K. (2020). Updating the definition of the alcohol hangover. *Journal of Clinical Medicine, 9*, 823.
Verster, J. C., Severeijns, N. R., Sips, A. S. M., Saeed, H. M., Benson, S., Scholey, A., & Bruce, G. (2021). Alcohol hangover across the lifespan: Impact of sex and age. *Alcohol and Alcoholism, 56*, 589–598.
Verster, J. C., van de Loo, A. J. A. E., Adams, S., Stock, A.-K., Benson, S., Alford, C., … Bruce, G. (2019). Advantages and limitations of naturalistic study designs and their implementation in alcohol hangover research. *Journal of Clinical Medicine, 8*, 2160.
Verster, J. C., van de Loo, A. J. A. E., Benson, S., Scholey, A., & Stock, A.-K. (2020). The assessment of overall hangover severity. *Journal of Clinical Medicine, 9*, 786.
Verster, J. C., van der Maarel, M., McKinney, A., Olivier, B., & de Haan, L. (2014). Driving during alcohol hangover among Dutch professional truck drivers. *Traffic Injury Prev., 15*, 434–438.
Verster, J. C., van Schrojenstein Lantman, M., Mackus, M., van de Loo, A. J. A. E., Garssen, J., & Scholey, A. (2018). Differences in the temporal typology of alcohol hangover. *Alcoholism, Clinical and Experimental Research, 2*, 691–697.
Watson, P. E., Watson, I. D., & Batt, R. D. (1981). Prediction of blood alcohol concentrations in human subjects. Updating the Widmark Equation. *Journal of Studies on Alcohol, 42*, 547–556.