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Combined and Isolated Effects of Alcohol Intake and One Night of Sleep Deprivation on Mood States, Hormonal and Inflammatory Responses in Healthy Male Adults: a Crossover Randomized Controlled Trial

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Abstract

Alcohol (ALC) intake and sleep deprivation (SDP) are conditions that cause changes in the physiological status. However, the relationship between biological markers and mood states is not entirely clear yet. The purpose of the study was to compare isolated and combined effects of ALC intake and SDP on hormonal and inflammatory responses and in changes in the state of mood. Also, we intended to explain possible changes in mood states through biochemical variations using multiple stepwise regression analysis. Ten healthy male were randomized in four situations: (1) placebo intake + normal sleep (PLA + SLE); (2) ALC intake + SLE; (3) PLA intake + SDP; (4) ALC intake + SDP. While subjects ingested ALC (1 g/kg of beer), PLA intake was a non-alcoholic beer. The subjects had one night of SLE or were subjected to SDP in the lab for 8 h. After each experimental condition, morning blood samples were taken for assessments of serum levels of glucose, cortisol, testosterone, epinephrine, interleukin-6 (IL-6), interleukin-10 (IL-10) and C-reactive protein (CRP). The subjects were also asked to fill in a Profile of Mood State questionnaire. The results showed that the glucose level was significant lower in ALC + SDP compared to the PLA + SLE condition. Total Mood Disturbance was lower in ALC + SDP and PLA + SDP compared to the PLA + SLE condition. Fatigue was higher under SDP conditions compared to PLA + SLE. Vigor was lower under the ALC + SDP condition compared to the PLA + SLE condition. Regression analysis showed that Total Mood Disturbance and fatigue under ALC + SDP were associated with changes in the cortisol levels. Our results showed that combined and isolated ALC intake and one night of SDP did not change the hormonal and inflammatory responses tested, and the combined effects caused a reduction in the glucose levels. Vigor, fatigue and Total Mood Disturbance were affected by each condition. Furthermore, Total Mood Disturbance and fatigue were possibly explained by changes in the cortisol levels in the combined condition.

Key Words: alcohol, hormones and inflammation, mood, sleep deprivation

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Introduction

Alcohol (ALC) intake and sleep deprivation (SDP) are conditions that cause changes in the physiological status (33). Heavy acute episodic or binge drinking, classified as the consumption of 60 g ALC in a single drinking episode (4), is very common in the world population, including physically active people and professional athletes (4, 40, 49), and heavy ALC consumption is also associated with significant physical, psychological and social harm (4). In addition, binge drinking is related to acute alterations in hormonal (18, 32, 46, 61) and inflammatory responses (20, 51). The most common episodes of acute SDP are from traveling (41, 43), anxiety disorder (6, 37) and social events (43). These conditions have been shown to compromise several neurobehavioral and cognitive domains (27). In addition, SDP is related to hormonal and inflammatory changes (13, 24). These modifications are related to vigor reduction and increased fatigue and tiredness (27, 54, 59). The main relationship between ALC and SDP and hormonal and inflammatory responses occurs under chronic ALC and SDP conditions. There are enough evidences showing significant changes on hormonal and inflammatory responses related to chronic diseases, such as obesity (55) and cancer (28).

Due to the sedative effects of ALC, a strategy that has been used to diminish the deleterious effects of SDP is ALC intake (13). However, the use of ALC before sleeping reduces rapid eye movement (REM) sleep (15, 44), increasing tiredness (2, 44, 45), reaction time (16) and the levels of cortisol and other hormones (44), as well as reducing the state of alertness (53). Moreover, combined ALC intake and SDP is very common during social events (44).

Both ALC intake and SDP are associated with the neurological response to mental stress. This stress reaction is mainly regulated by the hypothalamic–pituitary–adrenocortical (HPA) axis (28). The evaluation of the state of mood provides a rapid method of assessing psychological stress level in patients with obsessive-compulsive personality disorders (28), and in athletes (10), elders (3) and young adults (36). Previous studies demonstrated an association between fatigue and serum interleukin-6 (IL-6) levels in healthy subjects (59), and also associations in athletes between fatigue, vigor, tension, anger and cortisol level (10, 14). However, the relationship between biological markers and mood states is not entirely clear yet (19). Hence, the first aim of this study was to compare the combined and isolated effects of acute ALC intake and SDP on hormonal (cortisol, testosterone and epinephrine), inflammatory (IL-6 and -10 and C-reactive protein (CRP)) glucose and Profile of Mood States (POMS) responses in healthy male adults. Our second aim was to seek possible link between changes in mood states and hormonal and inflammatory responses through multiple stepwise regression analysis. The choice of using those hormonal and inflammatory markers is because of their common usage in similar studies as in the present work.

Materials and Methods

Subjects

Twenty-nine male subjects volunteered to participate after the project disclosure at the University campus and the social networks. The exclusion criteria included: (a) presence of metabolic diseases; (b) use of steroids; (c) ranked among the levels III and IV in Alcohol Use Disorders Identification Test (AUDIT) questionnaire (48); (d) absence of sleep disorders. In order to avoid hormonal changes cause by menstrual cycle, only male adults were recruited. After these criteria were applied, 13 volunteers were excluded due to evening chronotype and three for presenting VO_{2max} above 50 ml.kg.min^{-1}. Three others dropped out because of schedule conflict. Thus, 10 subjects (age: 23.5 ± 3.3 years; body mass: 70.2 ± 9.1 kg; height: 174.0 ± 5.1 cm; body fat: 14.9 ± 3.2%; body mass index (BMI): 23.19 ± 2.73; VO_{2max}: 44.8 ± 2.4 ml.kg^{-1}.min^{-1}) participated in the study. Clinical Trials identifier: NCT02117193.

Experimental Design

This study is characterized as a randomized controlled trial with a crossover design and blinded for the evaluators. Written informed consent was obtained from all participants before starting the experiment. This study was approved by Ethical Committee of the Federal University of Rio Grande do Sul, Porto Alegre, Brazil (acceptance number 366.465), and was conducted in accordance to the ethical standards of the Declaration of Helsinki. The subjects attended six visits to the laboratory during the study: day 1 was for familiarization and measurement of basal metabolic rate (BMR); days 2-5 were for measurements under the four experimental conditions. After a familiarization night of sleep in the laboratory in a special bedroom built for this study with noise and light controlled, volunteers performed five other visits. In the first visit (Day 1), the subjects were instructed to fast for 12 h, starting from 07:30 pm of the previous day, to determine the BMR. Thereafter, the other four visits (Days 2-5) were randomized as follows: ALC intake + normal sleep (SLE); placebo (PLA) intake + SLE; ALC intake + SDP and PLA intake + SDP. The randomization process was performed through a free access web

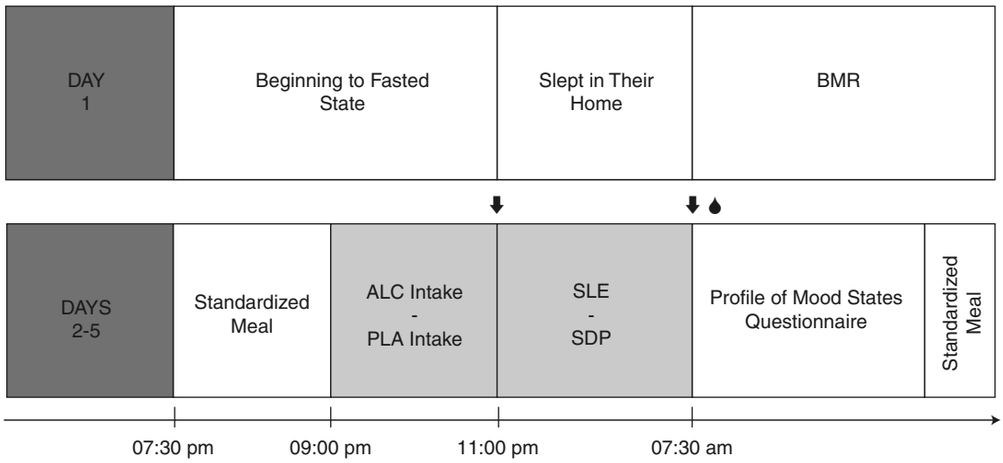

Fig. 1. Experimental design. Black arrow, BrAC measurement; black drop, blood collection.

software (www.randomizer.org). A minimum of seven days of rest was allowed between the experimental conditions.

All volunteers were instructed to not drink ALC and to sleep at least 7-8 h in the two nights before each condition, and to not perform intense physical activity within 48 h prior to data collection. On days 2-5, subjects arrived at the laboratory at 7:30 pm, and consumed a standardized meal. After 90 min, subjects started the ALC or PLA intake within 2 h from 09:00 pm to 11:00 pm). Afterwards, the SLE or SDP protocol lasted 8 h in the laboratory bedroom from 11:00 pm to 07:00 am. In the morning, breath ALC concentration (BrAC), blood collection and mood states were evaluated at 07:30 am, after which a standardized meal was consumed (Fig. 1).

Measurement of BMR

Tests for the BMR determination were performed between 08:00 am and 08:30 am with subjects submitted to a 12-h fasting, under controlled temperature (between 20 and 25°C), appropriate lighting and low noise. The protocol consisted of 10-min rest in a supine position, followed by 20 min of capturing of exhaled gases (11). For determination of VO_2 and VCO_2 values, a computerized gas analyzer using Med-Graphics Cardiorespiratory Diagnostic Systems, model CPX-D (MGC Diagnostics, Saint Paul, Minnesota, USA). The average value of VO_2 and VCO_2 (L/min) of the last 20 min of each data collection was used for data analysis. In order to obtain kcal/day values, the equation proposed by Weir was used (62).

Standardized Meals

For breakfast, bread, cream cheese and orange juice (60% carbohydrates, 25% fat and 15% protein) were provided. At dinner, pizza and orange juice (60% carbohydrates, 25% fat and 15% protein) were consumed. For this meal, calculation of the participants' meal energy content was based on 50% of the caloric expenditure of BMR. A 60-min period was respected for both meals to allow adequate ingestion and digestion processes. During meals, water was provided.

Experimental Conditions

After dinner and at rest for digestion, subjects received a drink with ALC or PLA. These conditions differed by the presence or absence of ALC in the volume of ingested fluid. A previous crossover pilot study, blinded to the subjects, was conducted to determine which beer brands had similar taste and texture between their with-ALC and without-ALC versions. In this pilot study, ten healthy male adults received four types of national commercial beers (two different brands - version with and without ALC), in a randomized and crossover trial. Subjects should indicate the presence or absence of ALC, taste and texture of the beer. For the choice of the brand, we adopted an error between the presence and absence of ALC of 30%.

For the ALC condition, volunteers ingested an amount equivalent to 1 g/kg bodyweight of ALC (7, 18, 26, 29) from the chosen commercial beer. In the PLA condition, volunteers ingested the same volume as consumed in the ALC condition, differing only by the absence of ALC in the beverage. A period of two hours was allowed for the subjects to complete the drink ingestion.

After drinking, the subjects went into SLE or SDP in the bedroom built in the laboratory for the study in which there were TV, video game, computer, internet access. Temperature, noise and luminosity were also controlled to ensure the best SLE or SDP conditions. For the SLE condition, the subjects underwent eight hours of sleep in the laboratory bedroom. In the SDP condition, the volunteers remained awake during these eight hours, engaged in activities that did not involve physical effort by playing video games, reading books, working on a computer or watching movies under supervision. Throughout both experimental conditions, water intake was, mimicking as close as possible real life conditions.

Blood Analysis

A trained professional obtained 20 mL blood samples at the antecubital region using an adapter for vacuum collection. While a vacutainer tube containing ethylenediaminetetraacetic acid (EDTA) was used to prepare glucose, epinephrine, testosterone, IL-6, IL-10 and CRP samples, a heparinized vacutainer tube was used for cortisol samples. Both blood samples were centrifuged at 1000 *g* for 10 min and the plasma aliquoted and stored at -80°C. Epinephrine, testosterone, cortisol, IL-6, IL-10 and CRP analyses were performed by enzyme-linked immunosorbent assay (ELISA) (ThermoFisher Scientific, Waltham, Massachusetts, USA) using the respective assay kits and following the manufacturers' instructions (56). Glucose analysis was performed using the colorimetric enzymatic method using the Cobas C111 equipment (Roche, Sao Paulo, Brazil) (11).

Mood States

The POMS questionnaire is an important tool to understand the psychological conditions of subjects in any situation (10, 28). This tool is made by 65-adjectives measuring six mood states, tension, depression, anger, vigor, fatigue and confusion, on a five-point Likert scale from 0 (not at all) to 4 (extreme), in relation to the context. The test takes approximately 3 min to administer in a paper-and-pencil format. The subjects were asked to state how they felt at the moment. All mood states and the Total Mood Disturbance [(Tension + Depression + Anger + Fatigue + Confusion) – Vigor] were calculated. The raw scores for each dimension were analyzed to further investigate changes in mood states (10, 28).

Statistical Analysis

Data normality was tested through the Shapiro-Wilk test. Data sphericity was tested by the Mauchly test, and Greenhouse-Geisser correction factor was used when sphericity was violated. For the BrAC, mood states and blood parameters, a repeated-measures one-way analysis of variance (ANOVA), followed by Bonferroni *post-hoc* test, was used for between-conditions comparisons. The Bonferroni adjustment was performed to multiple test correction and to control the 'family-wise error rate'. If a significance threshold of α is used, but n separate tests are performed, then the Bonferroni adjustment deems a score significant only if the corresponding *P-value* is $\leq \alpha/n$ (39). Therefore, in our study, *P-value* $\leq 0.05/6 = 0.0083$ was used to be significant in multiple comparison tests (mood states and blood parameters).

A stepwise multiple linear regression model was used to establish statistical models that were able to identify the differences in mood states (criterion variables) from hormonal and inflammatory parameters (predictor variables) in each experimental condition. This method was applied to seek explanation for the changes in mood states due to the experimental conditions. The model goodness-of-fit, which indicates how well the linear combination of the variables predict the mood states, was given by the squared multiple correlation (R^2) and the adjusted squared multiple correlation (adjusted R^2). The adjusted R^2 is reported because the R^2 can overestimate the percentage of the variance in the criterion variable that can be accounted for by the linear combination of the predictor variables, especially when the sample size is small and the number of predictors is large (21).

The relative importance of the predictors was estimated with the part correlations (part r), which provide the correlation between a predictor and the criterion after removing the effects of all other predictors in the regression equation from the predictor but not the criterion (21). A positive part correlation indicates that the predictor and the criterion are directly related, whereas a negative sign denotes an inverse relation. A *P-value* ≤ 0.05 was used to indicate statistical significance for regression and partial correlation analysis.

Results

Meals offered in the lab contained an average of 677.73 ± 124 kcal for dinner. Each volunteer consumed 1766.40 ± 218.07 ml of beer in each intervention condition. No differences were observed in the amount of ALC measured in the exhaled air test (BrAC) before SLE in the ALC + SLE condition (0.671 ± 0.350 mg/l) and SDP in the ALC + SDP condition (0.710 ± 0.391 mg/l; $P = 0.650$). In sleep conditions (ALC + SLE and PLA + SLE), subjects

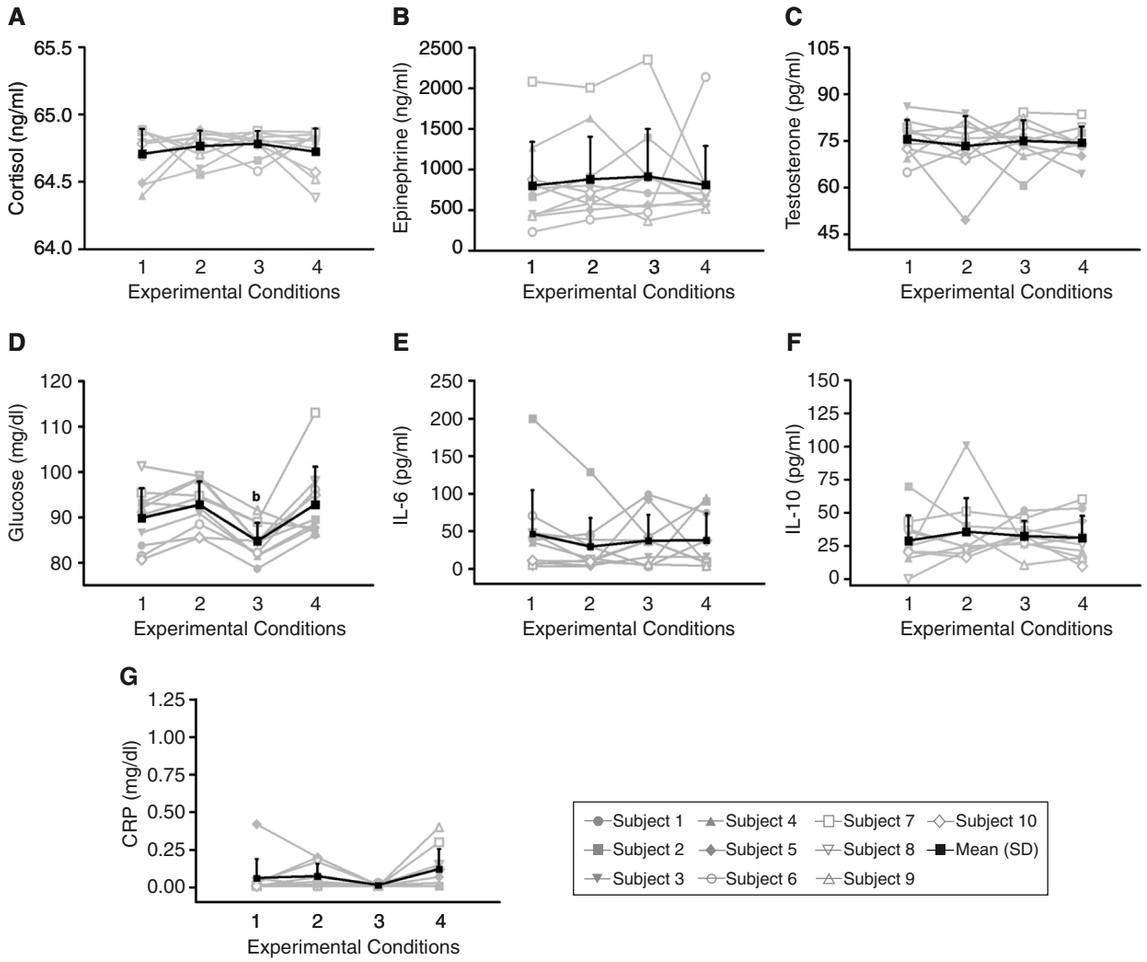

Fig. 2. Blood parameter responses after each experimental condition (mean ± standard deviation (SD)): 1. ALC + SLE; 2. PLA + SLE; 3. ALC + SDP and 4. PLA + SDP. In each subfigure, the grey lines were individual responses whereas the black line indicates the mean of the parameter, with the error bars also shown. b, indicates significant difference between PLA + SLE and ALC + SDP.

slept for 7 to 8 h. In all conditions, BrAC was normal in the morning.

Blood Parameters

Glucose levels [F (4.36) = 7.234; P = 0.001; effect size = 0.403] were lower in ALC + SDP compared to PLA + SLE (P = 0.002). However, no differences were observed in responses in cortisol [F (4.36) = 0.463; P = 0.762; effect size = 0.049], testosterone [F (4.36) = 0.113; P = 0.977; effect size = 0.012], epinephrine [F (4.36) = 0.780; P = 0.472; effect size = 0.080], IL-6 [F (4.36) = 0.978; P = 0.432; effect size = 0.098], IL-10 [F (4.36) = 0.474; P = 0.754; effect size = 0.050] and CRP [F (4.36) = 2.055; P = 0.173; effect size = 0.186) after each experimental condition (Fig. 2).

Mood States

Vigor was higher in PLA + SLE compared to ALC + SDP (P = 0.001). Fatigue was lower in PLA + SLE compared to ALC + SDP (P = 0.003) and PLA + SDP (P = 0.001). Total mood disturbance was higher in PLA + SLE compared to ALC + SDP (P = 0.005). However, no differences were observed in depression, confusion, anger and tension between the experimental conditions (Table 1).

Stepwise Multiple Regression Analysis

Multiple linear regression was performed to demonstrate which blood markers could explain the mood state changes in the experimental conditions. Only in the ALC + SDP condition, Total Mood Dis-

Table 1. Profile of mood states after each experimental condition (mean ± SD)

	ALC + SLE	PLA + SLE	ALC + SDP	PLA + SDP	F	*P-value*	ES
Tension	3.80 ± 3.73	3.60 ± 2.67	4.50 ± 2.75	2.80 ± 2.25	2.751	0.073	0.234
Depression	2.70 ± 2.86	1.50 ± 2.41	4.70 ± 4.73	3.70 ± 2.11	2.336	0.045	0.206
Confusion	5.20 ± 1.75	3.30 ± 1.33	4.70 ± 2.16	4.70 ± 2.11	3.435	0.067	0.276
Anger	4.10 ± 4.53	2.20 ± 1.98	7.70 ± 8.31	4.60 ± 3.86	2.172	0.155	0.194
Vigor	11.00 ± 4.76	12.80 ± 6.54	7.60 ± 4.24[b]	6.70 ± 5.22	12.092	<0.001	0.573
Fatigue	7.80 ± 4.68	5.30 ± 2.45	13.40 ± 4.52[b]	12.20 ± 3.11[b]	12.703	<0.001	0.585
Total mood disturbance	27.40 ± 17.98	21.30 ± 9.55	12.60 ± 16.16[b]	3.10 ± 10.04[b]	5.972	<0.001	0.399

ES, effect size; a, different to ALC + SLE; [b], different to PLA + SLE.

Table 2. Multiple stepwise regression analysis

	Situation	Blood parameter	R^2	F	R^2 adjusted	Equation	IC (95%)	*P*
Total mood	ALC + SDP	COR	0.61	12.7	0.56	-150 × COR + 9747.4 + 2718.7	-246.8;-53.2	0.007
Fatigue	ALC + SDP	COR	0.61	12.9	0.57	-37.8 × COR + 2465.1 + 681.4	-62.1;-13.5	0.007

COR, cortisol.

turbance and fatigue responses were correlated with cortisol changes. Vigor was not correlated with any blood markers in the stepwise model. Summary of results and equations of the stepwise multiple regression are presented in Table 2 and Fig. 3.

The partial correlations (part *r*) due to multiple regression results demonstrated a strong, significant and negative association between Total Mood Disturbance and cortisol (r = -0.784; P = 0.004) and fatigue and cortisol (r = -0.786; P = 0.004) in the ALC + SDP (Fig. 4).

Discussion

The novelty of our study was the assessment of the combined effects of ALC intake and SDP on hormonal, inflammatory and mood states. Our hypothesis was that the sum of these factors (ALC + SDP condition) would lead to more expressive changes in primary outcomes compared to each isolated condition (ALC + SLE and PLA + SDP). However, our results demonstrated that: (A) there was no significant effect of any condition on hormonal and inflammatory responses; (B) ALC + SDP caused a reduction in serum glucose levels; (C) Vigor, fatigue and Total Mood Disturbance were affected by the ALC + SDP condition, and (D) Total Mood Disturbance and fatigue responses were associated with changes in the cortisol levels in the ALC + SDP condition.

The endocrine system is a major contributor to the modulation of physiological and biochemical responses. However, in our study no significant changes in hormonal responses after the experimental conditions were observed, possibly due to high variability responses between the subjects in each condition (Fig. 2). These results are in agreement with previous studies regarding responses to cortisol (18, 23, 60), testosterone (23, 60) and epinephrine (18, 22, 29). However, responses of the isolated periods of SDP on these same hormones vary because epinephrine (9) and testosterone (13) reduce, while cortisol increases (13) or decreases (6, 59), probably because the SDP responses seem to be highly individualized (47).

Moreover, the types of activities during SDP periods induce different effects. Thus, physical exercise performed during SDP could cause different results from those observed at rest (36). In our study, playing video game was the main activity

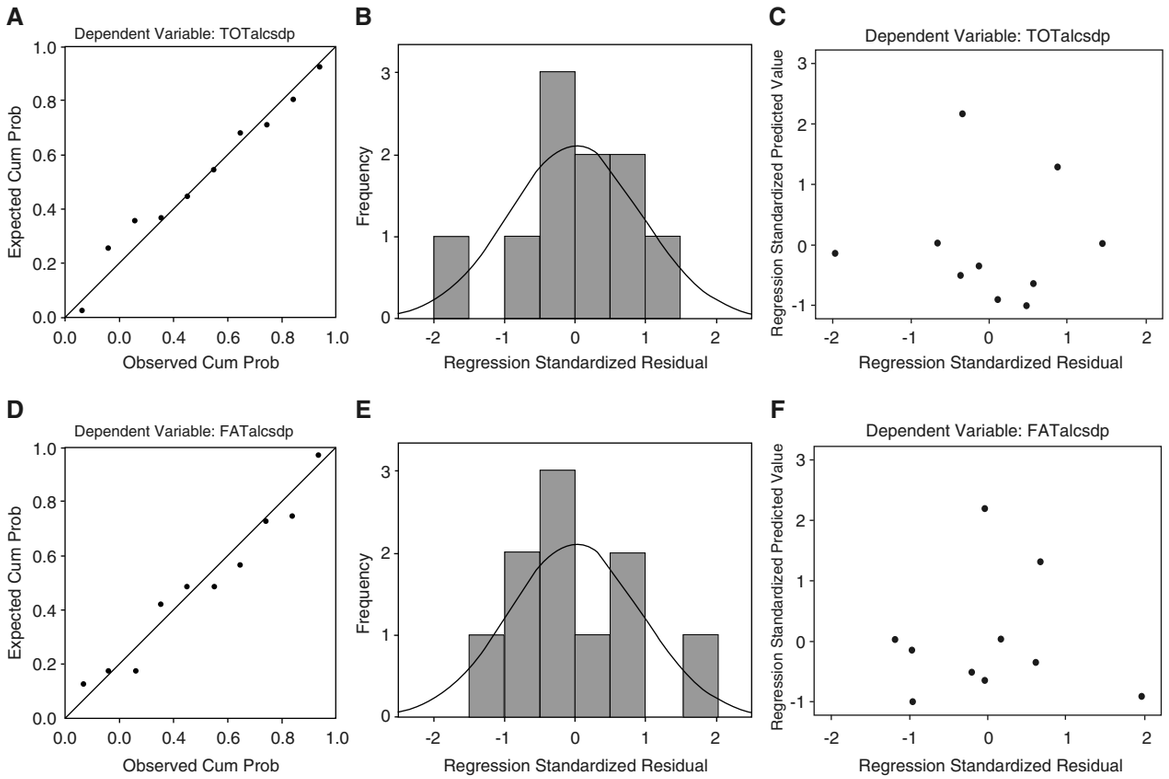

Fig. 3. Multiple stepwise regression analysis in Total Mood Disturbance in the ALC + SDP condition (A-C) and fatigue in the ALC + SDP condition (D-F).

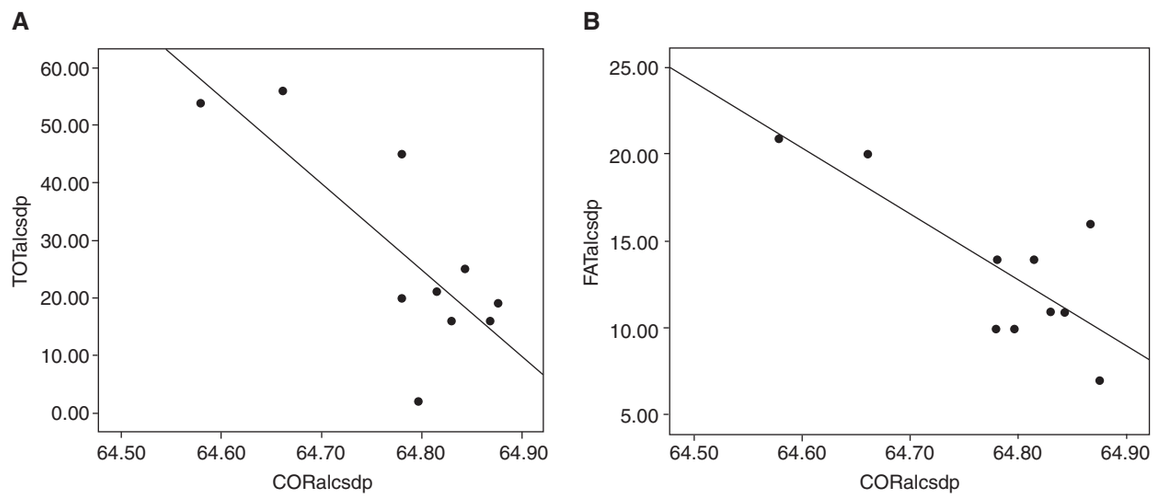

Fig. 4. Partial correlation analysis between Total Mood Disturbance (TOTalcsdp) and cortisol in ALC + SDP (CORalcsdp) (A) and fatigue (FATalcsdp) and cortisol in ALC + SDP (CORalcsdp) (B).

during the SDP period, and it was apparently insufficient to promote changes in hormonal responses. Our results, therefore, cannot be attributed to the activity performed during the SDP period.

One of the most common responses caused by ALC intake is hypoglycemia stimulated by inhibition

of hepatic gluconeogenesis (54), mainly observed in severe ALC consumption. In addition, acute lactate (7, 18, 34) and glucose responses (30, 34) vary, although they lead to considerable effects on the metabolism after moderate to severe ALC consumption (54). On the other hand, evidences indicate an increase in glucose responses after chronic SDP (13, 31, 36, 55). One likely reason could be an increase in the cortisol and epinephrine responses in chronic SDP (13, 31, 36), which leads to chronic glucose increases. Furthermore, impairment of glucose utilization by the central nervous system (31) and increases in the inflammatory profile (59) would be the prior mediators of chronic increase in glucose, which is related to metabolic diseases (50). Conversely, our study did not observe acute changes in cortisol and epinephrine after SDP.

The inverse effects of ALC and SDP on blood glucose levels are not completely understood, and their combined effects on glucose responses were still unclear. Interestingly, our results showed that the combination of ALC and SDP caused a significant reduction in the glucose level (Fig. 2D). The short period of SDP (6, 12) in our study could explain the absence of changes in glucose level in the SDP. Regardless, it is speculated that the significant glucose reduction after the ALC + SDP condition in our study, even if the values remained within normal levels, may be derived from the ALC intake and the increase in brain energy expenditure caused by the video game activity. However, this hypothesis needs to be further investigated.

Our study did not observe changes in the inflammatory response. Although chronic exposure to ALC (51) and SDP (59) cause a chronic inflammatory state, the acute effects of these conditions present divergent results. Evidence suggests that binge drinking causes a pro-inflammatory effect in the first 20 mins after ingestion, followed by an anti-inflammatory effect within 2 to 5 h after ingestion (1). In the case of acute SDP, while IL-10 and CRP did not seem to be affected (63), IL-6 increase was observed after SDP (57).

Our mood states results demonstrated higher levels of fatigue and lower level of Total Mood Disturbance in the ALC + SDP and PLA + SDP conditions. The relationship between biological markers and mood states is not completely clear (19). When seeking an explanation for changes in mood state in each experimental condition, a stepwise multiple regression analysis showed that the change in Total Mood Disturbance and fatigue in the ALC + SDP condition was associated with cortisol levels (56% and 57% respectively; Table 2). In addition, a strong, significant and negative correlation was observed between Total Mood Disturbance and cortisol levels (r = -0.784; Fig. 4A) and fatigue and cortisol (r = -0.786; Fig. 4B). Vigor changes after the ALC + SDP condition was not explained by any biological parameters measured in this study.

Previous studies reported association between fatigue and IL-6 (59), fatigue and cortisol (10), tension and cortisol (14), anger and cortisol (14) and vigor and cortisol (10). In contrast to our observations, a strong, significant and positive correlation between fatigue and cortisol was observed in swimmers during competition (10). The reasons of this opposite associations could possibly be explained by differences in subjects' characteristics and exercise session before sleeping. Although consumption of ALC before sleeping reduces REM sleep (15, 44), causing elevation of tiredness (2, 44, 45) and cortisol levels (44), no changes were observed after the ALC + SLE condition in our study.

It is important to note that we observed changes in the blood glucose level and mood state, even with a short period of SDP and a lower amount of ALC consumption than normally consumed on social occasions (42). It was still enough to cause changes in several outcomes of exercise performance (4). Yet no changes in hormonal and inflammatory responses were observed. We did not find evidences on acute effects of ALC intake and SDP on hormonal and inflammatory responses.

The relationship between chronic ALC consumption in ALC-dependent individuals and SDP in insomniacs is strong (8). Sleep disturbances are especially severe in recovering alcoholics and they are a primary contributor to relapse (8). Furthermore, ALC is often used as self-medication among insomniacs (8). Current evidence suggests that in both humans and animals, after chronic ALC exposure, sleep disruptions occur, manifested by an increase in sleep onset latency and wakefulness, and also reduction in sleeping time during the SLE period in insomnia, together with reduced wakefulness and increased sleep during the normal active period (52).

One of the main limitations of the study was a small sample size. It is important to note that blood parameters shows high variability (Fig. 2). Thus, we believe that the lack of changes in hormonal and inflammatory outcomes might be caused by a small sample size. Furthermore, there was an absence of sleep monitor *via* actigraph and sleep records one week before the experimental condition. However, all subjects were healthy and physically active and had no difficulties to sleep as reported by them. On the other hand, this study provides interesting insights regarding biochemical and mood responses after ALC intake and SDP, once this combination is very common during social events (44) and our

results demonstrated that their combination caused significant changes in important outcomes.

Conclusions

Our results showed that combined and isolated ALC intake and one night of SDP did not change the hormonal and inflammatory responses tested, and the combined effects caused a reduction in the glucose levels. Vigor, fatigue and Total Mood Disturbance were affected by each condition. Furthermore, Total Mood Disturbance and fatigue were possibly explained by changes in the cortisol levels in the combined condition. However, limitations in this study make it unwise to extrapolate the results to different groups or contexts.

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Conflict of Interest

The authors hereby declare research grants and patent licensing arrangements related to the subject matter of this article.

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