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Minimum Time of Sleep Deprivation to Modify Mood and Sleepiness

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Abstract

Sleep is essential for physical and cognitive performance. However, it is neglected by conditions of prolonged wakefulness that can lead to drowsiness and affect the mood. Here we aimed to investigate the effect of sleep deprivation on mood and sleepiness in college students. We hypothesized that the sleep deprivation would result in hindered performance in mood and sleepiness assessments. Fourteen young adults were subjected to 38 hours of sleep deprivation and assessments of mood and somnolence using, respectively, the Brazil Mood Scale (BRAMS) and the Karolinska Sleepiness Scale (KSS) questionnaire, every six hours (total of 07 time-points of evaluation). In agreement with our predictions, the results showed worsen mood ($\chi 2$ = 118,47; $p < 0.05$), reduced vigor (χ^2 = 87,44; $p < 0,05$) and increased sleepiness (χ^2 = 92,62; $p < 0,05$) and fatigue (χ^2 = 122,35; $p < 0,05$), after 20 hours of sleep deprivation. In conclusion, we observed that sleep deprivation was associated with a mood and sleepiness since 2 hours awake, but bad mood and increased sleepiness are clearly observed after 2020 hours of sleep deprivation. Given the important public health implications of sleep loss, future research should seek to further investigate the mechanisms of sleep loss-induced mood destabilization and sleep impairment consequences.

Keywords: *Sleep; Mood Disorders; Sleep Wake Cycle; BRAMS; Wake Time*

Abbreviations

BRUMS: Brunel Mood Scale; KSS: Karolinska Sleepiness Scale; UFMG: Federal University of Minas Gerais; ICF: Free and Informed Consent Form; CEPE: Centro de Estudos em Psicobiologia e Exercícios; EEFFTO: School of Physical Education Physiotherapy and Occupational Therapy; BRAMS: Brazil Mood Scale; POMS: Profile of Mood States; BRAMST: Brazil Mood Scale Total; MEQ: Morning and Evening Questionnaire; ANOVA: Analysis of Variance; M1: Moment 1; M2: Moment 2; M3: Moment 3; M4: Moment 4; M5: Moment 5; M6: Moment 6; M7: Moment 7

Introduction

Sleep is a key factor in maintaining physical and cognitive performance [1,2]. Sleep is essential in restoring energy, cognition and metabolism, however in today's society sleep time is getting shorter. The lack of sleep generates physical, psychological, mental and social damage and is currently considered a public health problem [3].

Increased sleepiness and fatigue, mood problems and reduced psychomotor performance are common changes caused by sleep deprivation or prolonged wakefulness. The effects of sleep deprivation have been discussed in studies with workers, students, adolescents, athletes and non-athletes [1,4,5]. Lack of attention, sleepiness and mood problems have been commonly observed in the general population and have been associated with increasing situations of sleep deprivation/restriction due to increased social, occupational demands and advances in technology [6,7].

Sleepiness is a physiological phenomenon inherent to the human. However, becomes excessive at inappropriate times when the individual should be alert, such as sitting on the bus, driving a vehicle, listening to a lecture or talking to people. In a study 17 hours of sleep restriction induced sleepiness and was associated with negative mood states in adolescents [8] and adults [9]. Likewise, lack of sleep, poor sleep quality or prolonged wakefulness, can generate sleepiness and negatively affect mood. Mood is an emotional or affective state of varying and fluctuating duration and bad mood or negative mood is understood as a low energy level combined with a high level of tension [5]. Sleep deprivation and mood status were analysed in several studies, the findings demonstrated a positive association between moodiness and sleep deprivation (> 24 hours) [10,11]. Kajtna., *et al.* [10] demonstrated changes in Vigour and Fatigue among college students after 40 hours of sleep deprivation.

Some easy-to-use and low-cost instruments are used in the literature to assess mood and alert states, such as the Karolinska Sleepiness Scale (KSS) and Brunel Mood Scale (BRUMS). The KSS and BRUMS are instruments consolidated in the literature in different conditions and different groups as students [12], workers [13] and athletes [4,14]. However, in a condition of sleep deprivation, there are still few studies, with varied protocols and different results.

Aim of the Study

In this sense, the present study aimed to verify the effect of sleep deprivation on mood states and sleepiness of university students.

Materials and Methods

The study was approved (nº 1.810.015) by the Human Research and Ethics Committee of the Federal University of Minas Gerais (UFMG). All participants declared that they knew and understood the study, as well as the right to confidentiality about their identity. Participants could withdraw at any time. Everyone who accepted the conditions of participation signed the Free and Informed Consent Form (ICF).

Sample

14 male university students participated in the study, with no sleep complaints, average age of 24,4 ± 3,7 years, body mass 76,07 ± 8,62kg and height of 1,74 ± 0,05 meters. The present study included men, between 18 and 35 years old; no sleep complaints, non-smokers, classified as morning, indifferent or evening chronotype, who did not use regular medications. All participants needed to be available to stay at the study place for 38 hours. Participants who did not perform all the evaluations and those who consumed stimulating or discouraging foods in the last 24 hours preceding participation in the study were excluded.

Procedures

The study was carried out at the Centro de Estudos em Psicobiologia e Exercícios (CEPE) on Escola de Educação Física Fisioterapia e Terapia Ocupacional (EEFFTO) - UFMG. Participants remained sleep deprived for 38 hours in a constant routine protocol in a place with an ambient temperature between 23ºC and 25ºC and relative humidity between 60% to 70%.

The sleep deprivation protocols were carried out during the weekends of August and September in 2018. Participants underwent 7 assessments that started at 08:00 and ended at 20:00 the next day (M1, M2, M3, M4, M5, M6 and M7). Participants were instructed to sleep for at least 7 hours the night before the start of the experiment and to wake up 02 hours before the first assessment (moment 1: 08:00 on day 1) to avoid inertia of sleep. During the 38 hours of sleep deprivation, researchers from CEPE/UFMG monitored the participants not to sleep, nap or perform any physical activity that could interfere with the protocol or ingest some psychoactive substance. In addition, during the study protocol, habitual nutrition (current daily life) was offered to all participants.

At moment 1(M1), participants filled out an individual identification form, containing personal, biopsychosocial and health data, as well as sleep complaints in the last 30 days. During the 38 hours of sleep deprivation, participants answered the KSS scale and the BRAMS questionnaire in a quiet, bright room. Evaluations were carried out at six-hour intervals, similar to the protocols performed in previous studies [11,15], with the aim h the aim of avoiding self-learning and/or generating feelings (e.g. stress, fatigue) that could affect the responses of the mood state questionnaire. The figure 1 represents the experimental design.

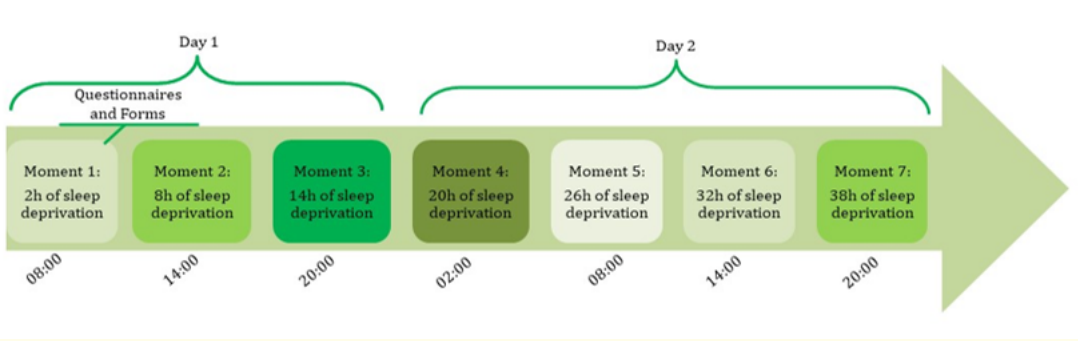

Figure 1: *Moments of data collection and hours of sleep deprivation.*

Throughout the sleep deprivation protocol, participants were allowed to use computers, cell phones, notebooks, electronic games, reading books and playing cards. However, these actions were not allowed an hour and a half (1h30min) before each assessment, so that it would not impact the assessments. In addition, there was interaction between researchers and participants, in order to keep them awake for 38 hours.

The participant was familiarized with the KSS and instructed to indicate a response according to their perception of sleepiness and/or alertness at the time of the assessment. The score ranges from 01 ("extremely alert") to 09 ("very sleepy, great effort to keep alert, fighting with sleep"), representing states of lesser or greater sleepiness. The scale presents the following question: "How are you feeling now?". The BRAMS is the Brazilian version of BRUMS, an instrument developed from a reduced version of the Profile Of Mood States (POMS). This questionnaire contains six domains with four subdomains, totaling 24 mood indicators scored according to a 5-point Likert scale: 0 = Not at all to 4 = Extremely. The final result of the mood state (BRAMS Total - BRAMST) is calculated by the sum of the Tension, Depression,

Anger, Fatigue and Confusion dimensions, and then the value of the Vigor dimension is subtracted. Values can range from -16 (negative value) to +80 (positive value) and the higher the BRAMST score, it indicates a worse mood. This instrument must be answered based on what best represents your feelings at that moment and according to the question "How are you feeling right now?". The Morning and Evening Questionnaire (MEQ) [16] was used for sample selection.

Statistical analysis

Data were presented as mean and standard deviation (mean ± SD), and confidence interval (CI95%). The normality of the data was verified using the Shapiro-Wilk test. Friedman's ANOVA was used to compare the data in the seven moments evaluated during the 38h of sleep deprivation. Bonferroni's post-hoc was used to identify possible differences between the seven moments. The correlation coefficient between mood status (BRAMST and respective domains) and sleepiness (KSS) was estimated using Spearman's correlation test. Linear regression analysis was conducted to assess the effect of sleepiness (independent variable) on mood (dependent variable). For data analysis, SPSS software version 25.0 was used and the significance value adopted was $p < 0.05$.

Results and Discussion

All participants (n = 14) had results between 31 and 69 on the MEQ, indicating that none of them had an extreme chronotype. Table 1 presents the values of mean and standard deviation of KSS, BRAMST and their respective domains, as well as the comparison of these variables on the 7 moments evaluated during sleep deprivation for 38 hours. The increase in the BRAMST and KSS scores over the seven assessment periods indicated an increase in the negative mood and sleepiness of the participants over the 38h of sleep deprivation. We also observed a negative change in mood (BRAMST) and a significant increase in sleepiness after 20 hours of sleep deprivation (M4). Likewise, the participants showed a significant worsening of the Fatigue and Vigor domains compared to previous moments, at the moment 4. Thus, we found that after 20h of sleep deprivation, the present sample showed an increase in symptoms of sleepiness, fatigue and loss of vigor.

	Day 1			**Day 2**				
	M1 (2h)	**M2 (8h)**	**M3 (14h)**	**M4 (20h)**	**M5 (26h)**	**M6 (32h)**	**M7 (38h)**	**F**
	Mean ± DP	**Mean ± DP**	**Mean ± DP**	**Mean ± DP**	**Mean ± DP**	**Mean ± DP**	**Mean ± DP**	
	($IC_{95\%}$)	**($IC_{95\%}$)**	**($IC_{95\%}$)**	**($IC_{95\%}$)**	**($IC_{95\%}$)**	**($IC_{95\%}$)**	**($IC_{95\%}$)**	
KSS	3,80 ± 1,55	4,10 ± 1,52	5,40 ± 1,27	6,50 ± 1,43^{+}	6,70 ± 1,70$^{\#}$	7,10 ± 2,13	6,60 ± 2,17	F = 9,49
	(2,69-4,90)	(3,00-5,19)	(3,59-5,41)	(5,47-7,52)	(5,48-7,92)	(5,57-8,62)	(5,04-8,15)	η^2 = 0,51
								ω = 0,96
BRAMST	-3,20 ± 6,97	-1,70 ± 8,42*	1,30 ± 8,38*	12,60 ± 13,23^{+}	15,00 ± 14,09^{+}	18,50 ± 12,96^{+}	16,00 ± 13,22*	F = 14,78
	(-9,47-1,29)	(-7,72-4,32)	(-5,59-7,19)	(3,47-22,12)	(5,30-25,50)	(9,27-27,93)	(6,52-25,28)	η^2 = 0,62
								ω = 0,99
Tension	3,10 ± 3,38	2,40 ± 3,37	2,40 ± 3,78	3,10 ± 3,96	3,40 ± 4,43	3,80 ± 4,76	3,60 ± 2,80	F = 3,50
	(0,68-5,52)	(-0,13-4,73)	(-0,42-5,02)	(0,36-6,04)	(0,33-6,67)	(0,40-7,20)	(1,50-5,50)	η^2 = 0,25
								ω = 0,88
Depression	0,50 ± 0,97	0,60 ± 1,26	0,90 ± 1,29	2,10 ± 2,38	2,00 ± 1,70	2,20 ± 2,20	1,70 ± 1,70	F = 2,57
	(-0,19-1,19)	(-0,30-1,50)	(-0,02-1,82)	(0,40-3,80)	(0,69-2,91)	(0,69-3,51)	(0,48-2,92)	η^2 = 0,22
								ω = 0,80

Anger	0,00 ± 0,00	0,30 ± 0,95	0,20 ± 0,63	0,90 ± 1,37	2,00 ± 4,29	3,10 ± 3,98	2,50 ± 3,92	F = 2,88
	-	(-0,38-0,97)	(-0,25-0,65)	(-0,08-1,88)	(-0,73-5,33)	(0,38-6,22)	(-0,31-5,31)	η^2 = 0,24
								ω = 0,49
Vigor	9,10 ± 2,18	7,50 ± 3,95	5,50 ± 3,14	3,40 ± 3,31#	3,60 ± 2,91#	4,20 ± 2,53	4,40 ± 3,81	F = 8,20
	(7,41-11,79)	(4,74-10,06)	(3,32-8,68)	(1,09-5,51)	(1,51-5,68)	(2,25-5,75)	(1,56-7,04)	η^2 = 0,48
								ω = 97
Fatigue	1,20 ± 1,48	1,60 ± 1,58	2,00 ± 2,05	8,00 ± 4,11+	8,20 ± 4,59+	10,40 ± 5,13+	10,80 ± 5,33+	F = 19,96
	(-0,01-1,61)	(0,47-2,73)	(0,53-3,47)	(5,06-10,94)	(5,92-12,48)	(6,73-14,07)	(6,99-14,61)	η^2 = 0,69
								ω = 1,00
Confu-sion	1,10 ± 1,73	0,90 ± 1,60	1,30 ± 3,47	1,90 ± 3,98	2,00 ± 4,29	3,20 ± 4,49	1,80 ± 3,46	F = 2,44
	(-0,14-2,34)	(-0,24-2,04)	(-1,06-3,86)	(-0,95-4,75)	(-0,87-5,27)	(-0,18-6,18)	(-0,76-4,16)	η^2 = 0,21
								ω = 0,36

Table 1: *Descriptive and comparative data of KSS, BRAMS and domains (BRAMST, Tension, Depression, Anger, Vigor, Fatigue and Confusion) in the seven evaluation moments. Data were expressed as mean ± SD (standard deviation) and $CI_{95\%}$ (95% confidence interval). *: Statistical difference (p < 0.05) in relation to moment 1. #: Statistical difference (p < 0.05) in relation to moments 1 and 2. +: Statistical difference (p < 0.05) in relation to moments 1, 2 and 3. $: Statistical difference (p < 0.01) in relation to the moment 4. KSS: Karolinska Sleepiness Scale. BRAMS: Brazil Mood Scale. BRAMST: Brazil Mood Scale-Total.*

Table 2 shows the values of associations between KSS and BRAMST. There was a positive correlation between sleepiness and mood at all times, except for M4 and M7 (r = 0,523; p = 0,55; r = 0,480; p = 0,08) respectively.

	KSS1	*KSS2*	*KSS3*	*KSS4*	*KSS5*	*KSS6*	*KSS7*
BRAMST1	0,842**	-	-	-	-	-	-
BRAMST2	-	0,791**	-	-	-	-	-
BRAMST3	-	-	0,651*	-	-	-	-
BRAMST4	-	-	-	0,531	-	-	-
BRAMST5	-	-	-	-	0,869**	-	-
BRAMST6	-	-	-	-	-	0,826**	-
BRAMST7	-	-	-	-	-	-	0,711*

Table 2: *Results of the correlation coefficient (r) between KSS and BRAMST in the seven evaluation moments. *: p < 0,05; **: p < 0,01. KSS: Karolinska Sleepiness Scale. BRAMST: Brazil Mood Scale-Total.*

Table 3 presents the results of associations between sleepiness and BRAMS domains in the seven evaluated moments. Fatigue showed a positive correlation with sleepiness at M2, M4, M5, M6 and M7. Depression showed a positive correlation with sleepiness only at M5. Vigor showed a negative correlation with sleepiness at M2, M3 and M7. Anger, Tension and Confusion did not correlate with sleepiness at any time.

	KSS1	*KSS2*	*KSS3*	*KSS4*	*KSS5*	*KSS6*	*KSS7*
BRAMS Tension							
Tension1	0,324	-	-	-	-	-	-
Tension2	-	0,162	-	-	-	-	-
Tension3	-	-	-0,173	-	-	-	-
Tension4	-	-	-	0,006	-	-	-
Tension5	-	-	-	-	0,181	-	-
Tension6	-	-	-	-	-	0,051	-
Tension7	-	-	-	-	-	-	-0,263
BRAMS Depression							
Depression1	0,482	-	-	-	-	-	-
Depression2	-	0,401	-	-	-	-	-
Depression3	-	-	0,433	-	-	-	-
Depression4	-	-	-	0,391	-	-	-
Depression5	-	-	-	-	0,738*	-	-
Depression6	-	-	-	-	-	0,343	-
Depression7	-	-	-	-	-	-	0,555
BRAMS Anger							
Anger1	0,00	-	-	-	-	-	-
Anger2	-	0,426	-	-	-	-	-
Anger3	-	-	0,419	-	-	-	-
Anger4	-	-	-	0,169	-	-	-
Anger5	-	-	-	-	0,386	-	-
Anger6	-	-	-	-	-	0,551	-
Anger7	-	-	-	-	-	-	0,537
BRAMS Vigor							
Vigor1	-0,723*	-	-	-	-	-	-
Vigor2	-	-0,815**	-	-	-	-	-
Vigor3	-	-	-0,586	-	-	-	-
Vigor4	-	-	-	-0,289	-	-	-
Vigor5	-	-	-	-	-0,273	-	-
Vigor6	-	-	-	-	-	-0,353	-
Vigor7	-	-	-	-	-	-	-0,721*
BRAMS Fatigue							
Fatigue1	0,585	-	-	-	-	-	-
Fatigue2	-	0,637*	-	-	-	-	-
Fatigue3	-	-	0,505	-	-	-	-
Fatigue4	-	-	-	0,702*	-	-	-
Fatigue5	-	-	-	-	0,808**	-	-
Fatigue6	-	-	-	-	-	0,928**	-
Fatigue7	-	-	-	-	-	-	0,778**
BRAMS Confusion							
Confusion1	0,558	-	-	-	-	-	-
Confusion2	-	0,183	-	-	-	-	-
Confusion3	-	-	-0,200	-	-	-	-
Confusion4	-	-	-	0,097	-	-	-
Confusion5	-	-	-	-	0,499	-	-
Confusion6	-	-	-	-	-	0,234	-
Confusion7	-	-	-	-	-	-	0,107

Table 3: *Results of the correlation coefficient (r) between KSS and BRAMS domains in the seven evaluation moments. *: p < 0,05; **: p < 0,01. KSS: Karolinska Sleepiness Scale; BRAMS: Brazil Mood Scale.*

Linear regression analysis showed that the low level of drowsiness at M1 (2 hours of sleep deprivation) was associated with mood (r^2 = 0,34; β = 2,47), at which time the participants had good alertness and mood levels. The 95% confidence limits (0,32-4,61) indicated that the slope for this population was between these limits, and the t test$_{(1,14)}$ = 2,50 showed an associated probability level of p = 0,03. At M2 and M3, we observed an association between low level of sleepiness and maintenance of mood (M2: $r^{2 = 0,50; \beta = 3,51;}$ M3: $r^{2 = 0,33; \beta = 3,53),}$ with 95% confidence intervals between 1,29-5,74 (M2); 0,44-6,61 (M3). The test $t_{(1,14)}$ for M2 it was 3,44 with a probability of p < 0,01 and for moment 4 it was 2,49 with a probability of p = 0,03.

At M5, M6 and M7 (> 26 hours) there was an increase in the level of sleepiness and association with the worsening of mood (BRAMST). At M5 (r^2 = 0,69; β = 5,96; $IC_{95\%}$ = 3,43-8,48; $t_{(1,14)}$ = 5,14; p < 0,01) and 6 (r^2 = 0,77; β = 5,47; $IC_{95\%}$ = 3,61-7,33; $t_{(1,14)}$ = 6,40; p < 0,01) we verified sleep deprivation (≥ 26 hours) impaired mood. At M7 (38 hours of sleep deprivation) there was an association between sleepiness and mood (r^2 = 0,50; β = 3,75; $IC_{95\%}$ = 1,38-6,12; $t_{(1,14)}$ = 3,45; p = 0,01), indicating that sleepiness and bad mood remained at high levels. In contrast, m4 showed no association between sleepiness and mood (r^2 = 0,27; p = 0,13).

The results of the association between sleepiness and fatigue are shown in figure 2, it is possible to observe an association in the M2 (β = 0,64; $IC_{95\%}$ = 0,15-1,12; $t_{(1,14)}$ = 2,87), M4 (β = 1,56; $IC_{95\%}$ = 0,76-2,35; $t_{(1,14)}$ = 4,28), M5 (β = 2,18; $IC_{95\%}$ = 1,51-2,86; $t_{(1,14)}$ = 7,05), M6 (β = 2,08; $IC_{95\%}$ = 1,60-2,57; $t_{(1,14)}$ = 9,31) and M7 (β = 1,62; $IC_{95\%}$ = 0,84-2,40; $t_{(1,14)}$ = 4,54). We can see that the levels of sleepiness and fatigue increased during sleep deprivation (M2, M4, M5, M6 and M7).

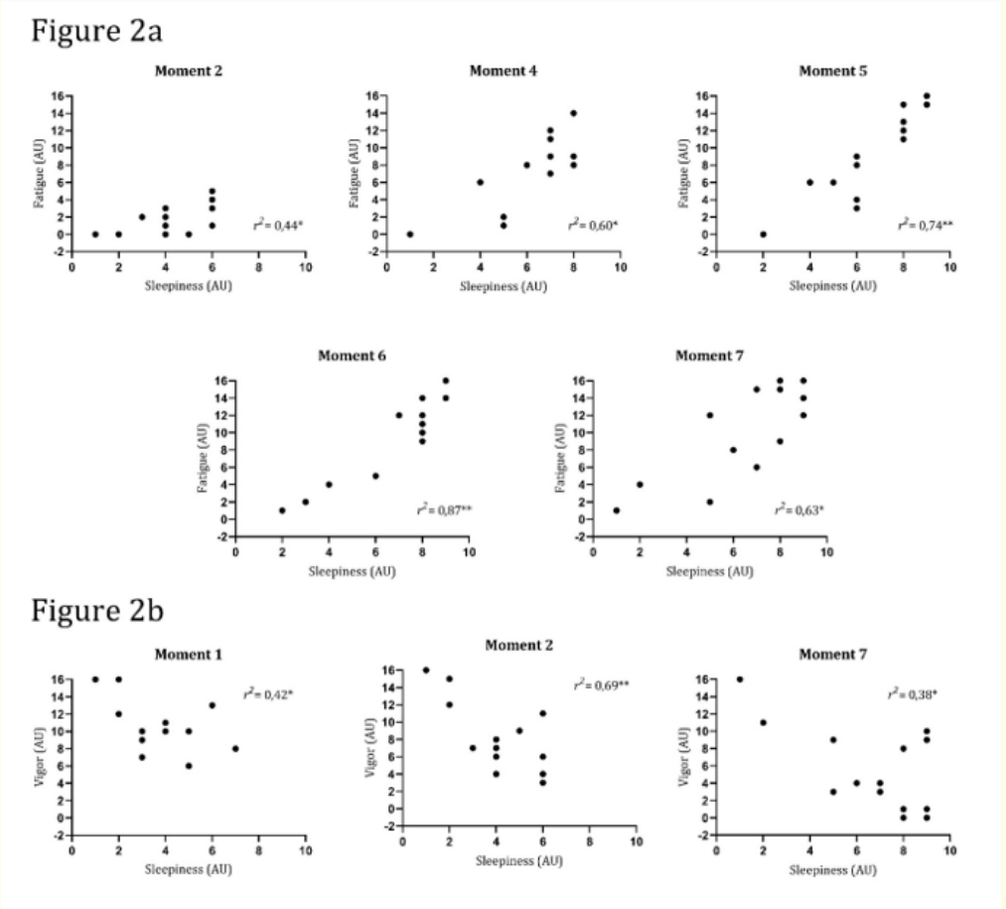

Figure 2: *Associations between BRAMS domains and sleepiness. Figure 2a: Association between sleepiness and fatigue in 14 young adults during sleep deprivation. Figure 2b: Association between Sleepiness and Vigor in 10 young adults during sleep deprivation. The data were analyzed using linear regression.*
**: p < 0.05; **: p < 0.01. UA: Arbitrary Unit.*

Figure 2b shows the results of the linear regression coefficient (r^2) of the sleepiness variable in the Vigor domain. At M2 (β = -1,73; $IC_{95\%}$ = -2,80- -0,66; $t_{(1,14)}$ = 3,52), M3 (β = -2,02; $IC_{95\%}$ = -2,95 - -0,20; $t_{(1,14)}$ = 3,13) and M7 (β = -1,17; $IC_{95\%}$ = -1,92 - -0,45 $t_{(1,14)}$ = 2,74) we observed that an increase in the level of sleepiness was associated with a reduction in Vigor during sleep deprivation. In addition, at M5 there was an association between sleepiness and the Depression domain (r^2 = 0,46; β = 0,47; $IC_{95\%}$ = 0,15-0,79; $t_{(1,14)}$ = 3,18; p < 0,01). Thus, we can infer that Depression increased after 26 hours of sleep deprivation, due to the increase in sleepiness.

The results showed an increase in sleepiness and negative mood throughout the 38h sleep deprivation. There was an association between sleepiness and total mood state, as well as between sleepiness and the Fatigue and Vigor domains. In the present study, we evaluated seven moments, allowing us to obtain robust data during the entire period of sleep deprivation, unlike previous studies in the literature that evaluated only pre and post sleep deprivation [17].

The difference between moments 1 and 7 found in the mood state score (BRAMST) corroborates with results from previous studies [1, 18]. On the other hand, our results also indicated a significant difference in BRAMST between M1 and M4, M5, M6 and M7 (Table 1), showing an increase in negative mood, especially after 20 hours of sleep deprivation. In addition, the moments of peak negative mood and sleepiness (32 hours of sleep deprivation: at 14:00 on day 2) that occurred during sleep deprivation, reinforce the need for evaluation during several moments in this condition and not only in periods before and after sleep deprivation. This is justified because the literature reports that the reduction (postprandial) and the nadir of the central temperature (02:00 to 05:00) occur during essential hours to promote sleep, reduce alertness and alter mood.

In evaluation in M4, there was a greater increase in BRAMST and Fatigue, in addition to a decrease in Vigor compared to previous moments. We believe that after 20 hours awake, associated with the circadian time coinciding with the central temperature nadir [19], can justify this worsening of the mood state. Dawson and Reid [20] observed that 17 hours of sleep deprivation is related to reduced psychomotor performance, while 24 hours of sleep deprivation was correlated with blood alcohol concentrations (0,05% and 0,10%). The duration of the reported sleep deprivation is close to M4 (20 hours), which worsened the psychobiological variables sleepiness, fatigue and vigor in the present study.

Changes in mood are often associated with sleep problems [7,17], like sleep restriction [21], sleep disorders [12] and other psychobiological factors. Studies confirm that sleep fragmentation, circadian rhythm desynchronization, sleep deprivation [22], pain symptoms in the population and overtraining in athletes [14] similarly interfere with alertness and mood. Short and Louca [15] evaluated adolescents using the POMS questionnaire, before and after 36 hours of sleep deprivation, and observed changes in Fatigue (increase of 31%), Depression (increase of 3%) and Vigor (decrease of 19%), similar to the findings of our study (Table 1).

Short and Louca [15] pointed out changes at the end of sleep deprivation in other domains such as Confusion (12% increase), Anxiety (7% increase) and Anger (2% increase) different from the findings in our study. We believe that, in the assessment seven after 38 hours of sleep deprivation, the motivation to complete the protocol by the participants, as well as the moment of the assessment (20:00) close to the time of greatest circadian performance, may have interfered in the variables. The worsening of mood in nurses after a 17-hour shift was also observed by Saadat *et al.* [13] (POMST beginning: 42,57 ± 15,26; POMST end: 70,90 ± 6,91). In our protocol, the participants remained awake performing different activities not related to work. Even though there was no demand for work, there was a worsening of mood (BRAMST beginning: -5,78 ± 7,04; BRAMST final: 12,21 ± 13,76) similar to the previous study conducted by Saadat., *et al*. [13]. Kajtna., *et al*. [10], analyzed the BRUMS domains and found that adults after 40 hours of sleep deprivation increased Fatigue (8,00 ± 3,28) compared to the pre-deprivation moment (3,33 ± 1,58), and decreased Vigor (Post: 7,11 ± 3,52 vs. Pre: 9,67 ± 2,59).

Other findings of the present study were the association between mood and sleepiness at the M1, M 2, M3, M5, M6 and M7, as well as the effect of sleepiness on the mood state (BRAMST) at the M2, M3, M5, M6 and M7. After 14h of sleep deprivation (M3) there was an association between mood and sleepiness, as well as an effect of 33% of the variable sleepiness on mood. We believe that other factors

(67%) not assessed may have interfered with this result. In this sense, the results referring to 14 hours of sleep deprivation, which corresponds to the circadian time of greatest physical performance, good alertness and better mood [23] demonstrated that the time of sleep deprivation may have caused the symptoms of excessive sleepiness or tiredness sufficient to impair the mood.

On the other hand, after 20 hours of sleep deprivation there was no association between sleepiness and mood state, however, there was a significant increase in negative mood (approximately 7.0 times greater) that possibly supplanted the increase in sleepiness (approximately 1.4 times greater), although both variables have shown a significant increase. Moment 4 (M4) refers to the circadian time close to the peak of negative mood that may have contributed to this fact [24]. However, at M5, M6 and M7, there was an association between sleepiness and mood (BRAMST) in 69%, 77% and 50% respectively. The results presented show that the mood worsened over the 38 hours of sleep deprivation due to sleepiness. A systematic review by Waters., *et al.* [25] pointed out that 76% of studies with sleep deprivation or restriction caused worsening of mood scores. Similar to the results found in our study, Wali., *et al.* [26] found an association ($r = 0,73$; $p = 0,001$) between negative mood and sleepiness in doctors after a night of sleep deprivation. In view of these results and in accordance with our findings, we believe that, although the mood state is multifactorial and presents oscillations caused by intrinsic and extrinsic factors, there is a cumulative effect of worsening mood (bad mood) during sleep deprivation.

Additionally, there was an association between sleepiness and the domains Fatigue (M2, M4, M5, M6 and M7), Vigor (M2, M3 and M7) and Depression (M5). Fatigue increased, while Vigor decreased as a result of sleep deprivation and sleepiness (Figure 2a and 2b). According to the literature [27], Vigor is characterized by animation and activity, feelings of excitement, disposition and physical energy and has an inverse relationship with other domains of humor, including fatigue. In this way, it is possible to perceive that, in addition to the level of sleepiness found, the fatigue scores (low and high) in the respective M2 and M7 can also explain the levels of Vigor.

The present study revealed that only 26 hours of sleep deprivation (assessment 5), showed a 46% association between depression and sleepiness. This result is consistent with other studies with shorter sleep restriction/deprivation protocols [7,11]. Studies report that staying awake for more than 17 hours can cause sleepiness, decreased Vigor, increased Tension, Depression, Anger, Fatigue and Confusion, which result in a momentary negative mood [1,2,13]. On the other hand, the variation in the mood state (increased tension, depression, anger, fatigue and confusion and reduced vigor) is multifactorial and can also cause sleep deprivation or prolonged wakefulness in several populations [17,28]. Thus, this feedback loop confirms this reciprocal interaction between mood and sleepiness. Detecting this cycle and acting to minimize the condition of prolonged wakefulness or sleep deprivation/restriction can improve academic performance, mood regulation, safety at work and performance when driving vehicles [12]. Therefore, assessments of mood and sleepiness should be implemented in workplaces, sports teams and study places (schools, universities, etc.) for different populations. Workers, athletes and students could benefit from becoming aware of the importance of night sleep for biological systems and psychosocial aspects. Realizing the symptoms of bad mood and sleepiness, can help control factors that interfere with physical and cognitive performance.

In the present study, we did not evaluate the concentration of the hormones melatonin and cortisol to correlate with the mood state in the circadian nadir and acrophase. The sample was composed of 14 participants and is considered small. These two points are considered limitations of the present study.

Conclusion

Sleep deprivation (38 hours) negatively impacted mood states and sleepiness. Our results indicated that 20 hours of sleep deprivation significantly increased the level of sleepiness and worsened mood. Likewise, there was an effect of sleepiness in the Fatigue, Vigor and Depression mood domains, as a result of sleep deprivation. Finally, this study provided relevant information on the effects of sleep deprivation on health and safety of the population. As a consequence, people who stay awake more than 20 hours suffer damage in states of alertness and mood.

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Conflict of Interest

The authors declare that there is no conflict of interest.

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