Sleep deprivation affects post-lunch dip performances, biomarkers of muscle damage and antioxidant status

AUTHORS: Mohamed Romdhani[1,2], Omar Hammouda[3,4], Yassine Chaabouni[5,6], Kacem Mahdouani[5,6], Tarak Driss[3], Karim Chamari[7#], Nizar Souissi[1,2#]

[1] National observatory of sport, ONS, Tunis, Tunisia

[2] High institute of sport and physical education, Ksar-Said, Manouba University, Manouba, Tunisia

[3] Research Center on Sport and Movement (Centre de Recherches sur le Sport et le Mouvement, CeRSM), UPL, Univ Paris Nanterre, UFR STAPS, Nanterre, France

[4] Faculty of medicine of Sfax, Research Unit, Molecular Bases of Human Pathology, UR12ES17, Sfax, Tunisia

[5] Department of biochemistry, CHU Ibn Jazzar, Kairouan, Tunisia

[6] Laboratory of Analysis, Treatment and Valorization of Pollutants of the Environment and Products (LATVEP) Faculty of pharmacy, University of Monastir, 5000, Monastir Tunisia

[7] Athlete Health and Performance Research Centre, ASPETAR, Qatar Orthopedic and Sports Medicine Hospital, Doha, Qatar

[#] Theses authors participated equally in the paper

Corresponding author:
Omar Hammouda
Research Center on Sport and Movement (Centre de Recherches sur le Sport et le Mouvement, CeRSM), UPL, Univ Paris Nanterre, UFR STAPS, 200 avenue de la République, 92001, Nanterre, France.
TEL: + 33 782931995
E-mail: hammouda.o@ parisnanterre.fr

ABSTRACT: To compare the effects of two types of partial sleep deprivation (PSD) at the beginning (PSDBN) and the end (PSDEN) of the night on mood, cognitive performances, biomarkers of muscle damage, haematological status and antioxidant responses before and after repeated-sprint exercise in the post-lunch dip. Fourteen male athletes performed the Running-based Anaerobic Sprint Test following: (i) baseline normal sleep night, (ii) PSDBN, or (iii) PSDEN in a randomized and counter-balanced order. During each condition, participants performed simple and choice reaction time tests, the Profile of Mood States, subjective sleepiness, and the Running-based Anaerobic Sprint Test. Plasma biomarkers of muscle damage, total blood count, and antioxidant activities were measured at rest and after the repeated sprint in the three conditions. PSDEN decreased P_{max} (p=0.008; d=1.12), P_{mean} (p=0.002; d=1.33) and P_{min} (p=0.006; d=1.15), whilst PSDBN decreased P_{mean} (p=0.04; d=0.68) and P_{min} (p=0.028; d=0.58), in comparison with baseline. PSDEN exerted stronger effects on P_{max} (p=0.013; d=0.74) and P_{mean} (p=0.048; d=0.54) than PSDBN. Moreover, PSDEN increased subjective sleepiness (p<0.001; d=1.93), while PSDBN impaired choice reaction time (p<0.001, d=1.89). Both PSD types decreased resting glutathione peroxidase (p<0.001; d=5.43, d=3.86), and increased aspartate amino-transferase levels (p<0.001; d=1.36, d=1.37) respectively for PSDEN and PSDBN. PSDEN decreased repeated-sprint performances more than PSDBN in the post-lunch dip. This could be explained by the lowered mood and resting antioxidant status and the increased inflammatory profile after PSDEN. Repeated-sprint exercise resulted in greater inflammation after PSDEN, despite the decreased physical performance. The drop of resting antioxidant defence and haemoglobin concentration after PSDEN could explain the increased sleep drive at the post-lunch dip.

INTRODUCTION

Sleep is a dynamic process, and several mechanisms are not constant throughout nocturnal sleep (i.e., sleep stages, glucose metabolism, hormonal secretion, immune system) [1]. Hence, it is possible that early awakening or late bedtime will differently affect homeostasis. Several studies have tested this hypothesis [2–6]. From the above-mentioned studies, it seems that the effects of partial sleep deprivation (PSD) on various aspects of athletic performances (i.e., cognitive, physical, hormonal, haematological and inflammatory responses to the exercise) are time-of-day dependent, since evening performances decreased, but morning ones were unaffected [2–6]. In addition, subjective measurements (i.e., depression, sleepiness and mood) were more affected by PSD than objective ones (i.e., reaction-time, sprint-time and strength) [7]. However, previous research studied the effect of PSD on morning or evening performances' components and none investigated the post-lunch dip ones. Indeed, the post-lunch dip is the post-noon period characterized by a decline in attention and an increase of sleepiness [7,8]. The increase in sleep propensity still occurs in the post-lunch dip in constant routine protocols

and even without any consumed lunch [8]. Indeed, sleepiness is endogenously generated during this time of day and it is potentiated by a heavy lunch [8].

Interestingly, earlier reports showed that PSD at the end of the night (PSDEN) resulted in greater disruption of physical, cognitive and biochemical responses to the exercise than PSD at the beginning of the night (PSDBN) [2–6,9,10]. Indeed, PSDEN resulted in a greater decrease of intermittent aerobic [3] and short-term all-out performances [5,6] than PSDBN in the afternoon. In addition, PSDEN had a stronger disrupting effect on selective and constant attention than PSDBN in the afternoon [2]. Moreover, PSD shifted the immune and hormonal response to the exercise. PSDEN diminished post-exercise monocytes during the Yo-Yo intermittent recovery-test [9] and increased interleukin-6, tumour necrosis factor-alpha, growth hormone and testosterone levels during repeated-sprint in the afternoon [4]. Furthermore, PSDEN increased post-exercise creatine kinase and myoglobin levels during the Yo-Yo intermittent recovery test [10]. Also, energetic markers such as plasma glucose [1], lactate [10] and muscle glycogen [11] were affected by sleep deprivation. A more recent report indicates that PSD impaired recovery from high-intensity exercise [12]. However, despite the huge number of studies on the topic, the mechanisms by which performance decreases still need further investigations.

Judo performance is a complex process where physical, cognitive and biochemical parameters closely interact. Judo, like several other combat or team sports, is considered as a sequence of high-velocity efforts intercepted by ~10 s recovery [13]. Moreover, it is an open skills sport which requires a great level of attention and cognitive readiness. Also, anaerobic fitness is highly relevant for judo

performance [13], because higher anaerobic capacity means lesser fatigue and better recovery [14]. The excessive secretion of reactive oxygen species and biomarkers of muscle damage, and the alteration of immune state associated with high-intensity exercise, contribute to the fatigue process and cause exercise cessation [15].

As many athletes not only train but even compete in the early afternoon, the fall in alertness during the post-lunch dip could make the difference between victory and defeat [7,8]. Further understanding of the effect of PSD on markers of antioxidant status, muscle damage, and inflammation in the post-lunch dip seems of major importance for athletes, coaches and sport scientists. Thus, the current study aimed to compare the effects of PSDEN and PSDBN on mood, cognitive and repeated-sprint performances, as well as some biochemical parameters (i.e., total blood count, antioxidant defence, energetic markers and biomarkers of muscle damage), before and after repeated-sprint exercise in the post-lunch dip (i.e., 15:00 h). It was hypothesized that the two PSD types would differently affect cognitive and physical performances, as well as biomarkers of muscle damage in the post-lunch dip, due to the timing of prior nocturnal sleep.

MATERIALS AND METHODS

Participants

Fourteen men athletes volunteered to participate in this study (18.5±0.9 years, 171.9±7.4 cm, 67.5±5.7 kg, BMI: 22.8±1.4 kg.m^{-2}). They were all members of the "Regional judo team", at least black belt 1st Dan, and regularly engaged in ~2 hour/day, 5 days/week of training plus participating in competitions for at least 5 years. After receiving a complete verbal description of the
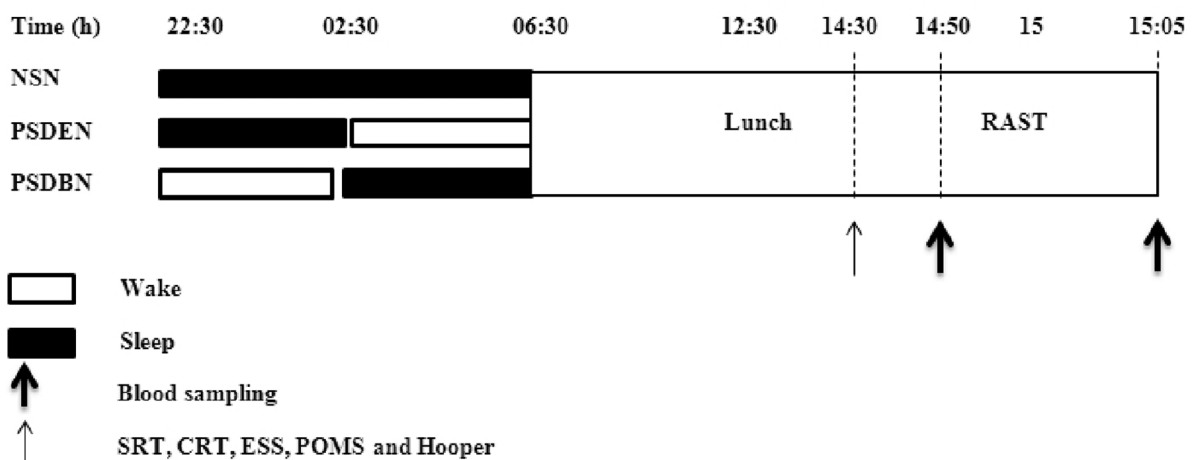

FIG. 1. Simplified experimental protocol. **NSN**; normal sleep night, **PSDEN**; partial sleep deprivation at the end of the night, **PSDBN**; partial sleep deprivation at the beginning of the night, **RAST**; Running-based Anaerobic Sprint Test, **h;** hour, **SRT**; simple reaction time, **CRT**; choice reaction time, **ESS**; Epworth Sleepiness Scale, **POMS**; Profile Of Mood States, all times are expressed in local time (GMT+1h).

protocol, the participants signed the informed consent. Participants were recruited according to their chronotype explored with the Horne and Ostberg morningness/eveningness questionnaire [16]. Only athletes with moderate and intermediate chronotypes were recruited (i.e., score between 31 and 69 in response to the morningness/ eveningness questionnaire). During the month preceding the experiment, a sleep diary was maintained (retiring and rising time, time in bed, sleep latency, waking frequency and duration, etc.). Only participants who scored ≤ 5 according to the Pittsburgh Sleep Quality Index (PSQI) [17] were recruited.

Experimental design
Participants were first familiarized with the laboratory conditions and tests, and then each participant performed the three test conditions (A, B and C one week apart) in a randomized and counter-balanced order. Session A: refers to baseline/control night where participants came to the laboratory at ~20:00 h, and took a standardized dinner at ~20:30 h. As participants were body weight and body mass index matched, a standardized meal in quality and quantity was served. Then, passive activities (i.e., watching TV, using the internet, reading books and playing cards or videos games) were allowed to keep participants awake until 22:30 h, when they were asked to go to bed (all lights and devices off). Wake-up time was set at 06:30 h, which corresponded to their daily routine schedules. Participants ate a standardized breakfast at 07:00 h, and then went to school. During school time, they were asked not to consume food but water was allowed *ad libitum*. Then, they came back to the laboratory at ~12:15 h, when they ate a standardized iso-caloric lunch, and rested until ~14:30h. Thereafter, the Profile of Mood States (POMS), Hooper Index questionnaire and the Epworth Sleepiness Scale were filled in, and then reaction times were recorded. At ~14:50 h, resting blood samples were collected. Then, the Running-based Anaerobic Sprint Test started at ~15:00 h with 5 min of passive recovery and post-exercise blood sampling. Session B: meant to verify the effect of PSDEN. Participants followed the exact same protocol as session A, but they were awakened at 02:30 h. Then, passive activities kept them awake until 07:00 h with the same schedule as in session A for the day after. Finally, session C: aimed to verify the effect of PSDBN. Participants followed the same schedule as sessions A and B, with bedtime set at 02:30 h and wake-up at 06:30 h. The experimental design is presented in Figure 1.

Naps, tobacco, alcoholic or caffeinated beverages or anything that could promote awakening were prohibited during the experimental days. Laboratory conditions were set at: temperature 24℃, humidity 35%, and luminosity (i) during tests 200 lux, and (ii) during sleep <5 lux.

Simple and choice reaction times
Participants performed simple and choice reaction times using REACT V0.9 software (Lyon 1 University, France). Simple and choice reaction time tests assess intrinsic alertness (vigilance) and motor speed.

Simple reaction time assesses the speed at which the participant responds to a simple given stimulus. However, the choice reaction time assesses how fast you can make a choice between two stimuli. The test requires pressing as fast as possible the corresponding key on the computer when the visual stimulus (yellow/blue) is shown on the screen. The reaction time performance in seconds (s) corresponded to the average of the fastest 10 of 20 responses to the stimulus. Then, the program shows the average score with the number of errors for choice reaction time.

Profile of Mood States
It is a standard validated psychological test formulated by McNair [18]. The questionnaire contains 65 words/adjectives that describe several aspects of mood. The original English version of the test was used.

Epworth Sleepiness Scale
It is a self-administered questionnaire to measure the level of daytime sleepiness. Subjective sleepiness scores were correlated with the Multiple Sleep Latency Test, during overnight polysomnography [19]. If the subjective sleepiness score exceeds 6 then the participant is considered as sleepy [19]. The original English version of the test was used.

Hooper Index questionnaire
This is a validated psychological self-reporting scale of sleep quality, fatigue, stress, and muscle pain. Each of these parameters was measured separately using a 7 points subjective rating scales ranging from 1 "very, very low" to 7 "very, very high".The total score, obtained from the sum of all sub-scales, indicates the athlete's form state or readiness to train [20]. The original English version of the test was used.

Running-based Anaerobic Sprint Test
The Running-based Anaerobic Sprint Test was developed by Draper and Whyte [21]. Its performance was correlated with the Wingate test and it can predict short-distance performances [22]. The Running-based Anaerobic Sprint Test protocol was followed according to the guidelines developed at the University Of Wolverhampton, UK. Briefly, each athlete was weighed prior to the test and warmed up for a period of 5 min followed by a 3 min recovery. Participants were instructed to perform six sprints between photocells which were placed 35 m apart on a straight race-track. Each sprint represents a maximal effort with 10 s in between for the turnaround.

From the six sprints time, the power output was calculated according to the following equation:

$$Power = Weight \times Distance^2 \div Time^3.$$

P_{max} = the highest power (i.e., the fastest sprint).
P_{min} = the lowest power (i.e., the slowest sprint).
P_{mean} = sum of all six powers ÷ 6.

Rating of perceived exertion (RPE)
This psycho-physiological scale given score represents the exertion which the athlete experiences during the exercise [23]. In the current study, the French translated version was used [24].

Blood sampling
Blood samples were collected before exercise (i.e., after 5 min of seated rest) from the right forearm vein and 5 min after exercise from the left forearm vein to avoid local inflammation caused by the first sample. Three tubes were used for blood collection. The first with heparin anticoagulant contained 5 ml of blood to measure the selected parameters, i.e. urea (URE), aspartate aminotransferase (AST), creatinine (CRE), creatine kinase (CK), uric acid (UA) and glutathione peroxidase (GPx). The second, 3 ml of blood in a tube with ethylenediaminetetraacetic acid (EDTA), was used to determine the total blood count. The total blood count values are reported as numbers, i.e., white blood cells (WBC), monocytes (MO), lymphocytes (LY), granulocytes (GR), red blood cells (RBC) and haemoglobin (HB). The third contained 3 ml of blood in a fluorinated tube to measure plasma lactate [La] and glucose (GLC). In order to eliminate inter-assay variance, all samples were analysed in the same assay run. All assays were performed in duplicate in the same laboratory with simultaneous use of a control serum from Randox on a Cobas Integra analyser (Roche Diagnostics, Switzerland). All methods used in the analysis of the current study are presented in Table 1.

Statistical analyses
All statistical tests were processed using the IBM Statistical Package for the Social Sciences (SPSS) Statistics for Windows, version 23. All values within the text and tables are reported as mean±standard deviation (SD). The Shapiro–Wilk test revealed that the data were normally distributed, and then parametric tests were performed. A one-way repeated measure analysis of variance (ANOVA) was performed for the data related to self-administered questionnaires, the Running-based Anaerobic Sprint Test, [La] and cognitive performances. For the selected biochemical parameters, a two-way repeated measure ANOVA (3 sleep conditions × 2 time-points "before and after exercise") was applied. To assess the ANOVA practical significance, partial eta-squared (ηp^2) was calculated. Once the ANOVA indicated a significant main effect of sleep condition or significant interaction, the Bonferroni post-hoc test was used. Additionally, the effect size (d) was calculated for pairwise comparison according to Cohen [25]. The magnitude of d was classified as small ($d=0.2$), moderate ($d=0.5$) or large ($d=0.8$) [26]. The level of significance was set at $p<0.05$.

Ethics
The present study was conducted according to the ethical guidelines of the Declaration of Helsinki (64th World Medical Association General Assembly, Fortaleza, Brazil, October 2013). The protocol was approved by the University of Manouba (Tunisia) Institutional Review Board.

RESULTS

Participants
According to the sleep diary (i.e., habitual sleep characteristics of the participants assessed during the month prior to the intervention), participants reported a total time in bed of 7.73±0.71 h (range 6.5-9), sleep latency of 22.73±5.56 min (range 18-29) and waking after sleep onset of 5.35±4.12 min (range 0-12). Participants scored 48.46±8.4 (range 38-66) and 3.46±1.24 (range 0-5) in the Horne and Ostberg and PSQI self-reported questionnaires respectively.

Running-based Anaerobic Sprint Test performances
The one-way repeated measures ANOVA showed a significant main effect of sleep condition on P_{max} ($F_{(2,12)}=4.6$; $p=0.032$; $\eta p^2=0.43$),

TABLE 1. Different methods used in hematological and biochemical analysis.

| Parameters	Method
Total blood count	Flow Cytometry on H18 LIGHT, Automatic Blood Cell Counter (SFRI. France)
Uric Acid	Colorimetric end point (intra and inter-assay coefficient of variation (CV) were: 1.55 % and 1.76 %, respectively)
Creatinine	Colorimetric (intra and inter-assay CV were 2.7 % and 2.9 %, respectively).
Glucose	Glucose hexokinase method (intra and inter-assay CVs were: 0.94 % and 1.3 %, respectively)
Lactate	Lactate oxidase peroxidase method (intra and inter-assay CV were: 0.9 % and 1.9 %, respectively)
Aspartate amino-transferase	Kinetic method at 340 nm
Creatine-kinase	Kinetic method at 340 nm
Urea	Kinetic enzymatic method (intra and inter-assay CV were: 0.33 % and 5.66 %, respectively).
Glutathione Peroxidase	Spectrophotometric method based on Paglia and Valentine method (1967; with kit from Randox Lab; Ransel RS. 505). The intra and inter assays CV were: 7.3% and 4.86%, respectively.
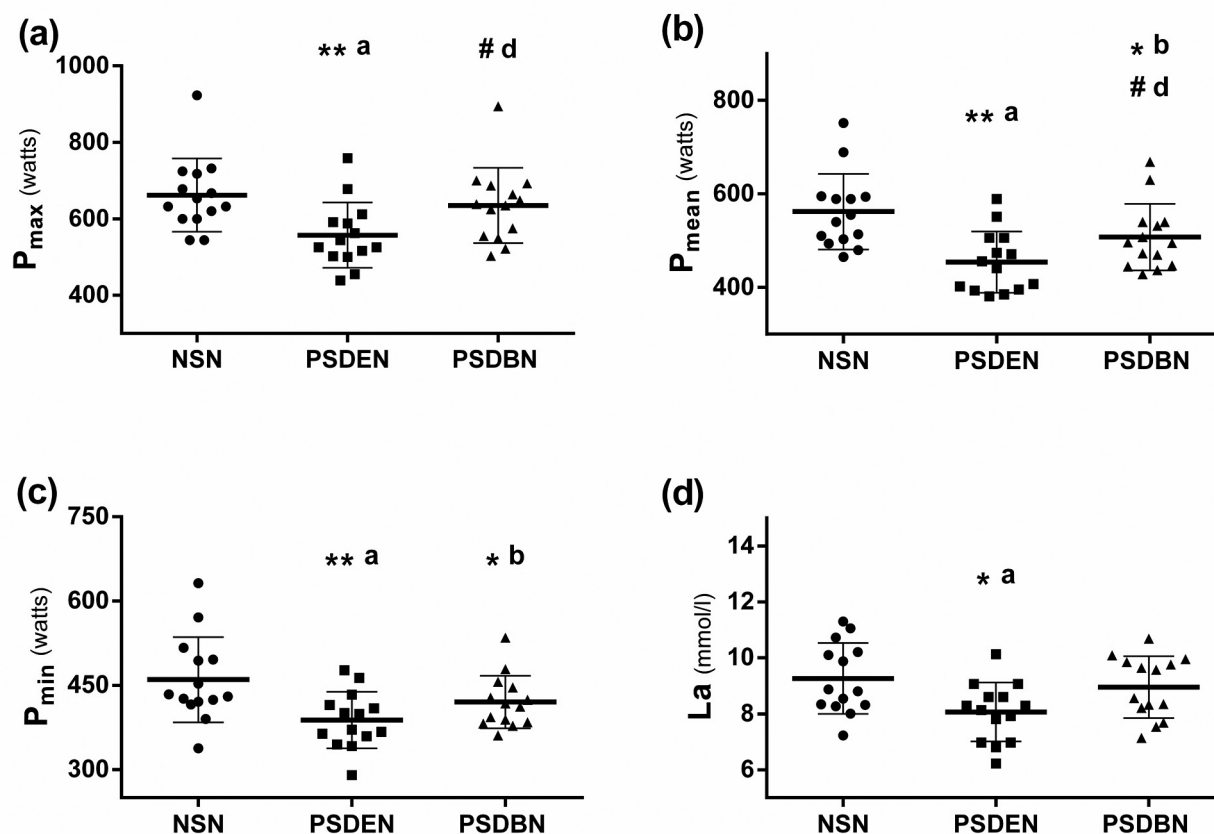
FIG. 2. Individual maximum (P_{max}; a), mean (P_{mean}; b) and minimum (P_{min}; c) powers and plasma lactate [La] concentrations (d) after normal sleep night (NSN•), partial sleep deprivation at the end of the night (PSDEN■) and Partial sleep deprivation at the beginning of the night (PSDBN▲). Significance is assessed with the one-way repeated measure ANOVA and Bonferroni post hoc test. *, ** and *** indicate a significant difference in comparison with NSN values at $p<0.05$, $p<0.01$, $p<0.001$ respectively; [a] large, [b] medium Cohen's *d* in comparison with NSN; #, ## and ### indicate a significant difference in comparison with PSDEN values at $p<0.05$, $p<0.01$ and $p<0.001$ respectively; [c] large, [d] medium Cohen's *d* in comparison with PSDEN. Bars represent the group means and standard deviations.

P_{mean} ($F_{(2,12)}=7.6$; $p=0.007$; $\eta p^2=0.56$) and P_{min} ($F_{(2,12)}=4.9$; $p=0.027$; $\eta p^2=0.45$). The Bonferroni post-hoc and Cohen tests revealed that P_{max} was lower after PSDEN compared to baseline ($p<0.01$; $d=1.12$), and to PSDBN ($p<0.05$; $d=0.54$), respectively. PSDBN was not statistically different from baseline (Figure 2a). P_{mean} was lower after PSDEN ($p<0.01$; $d=1.33$) and PSDBN ($p<0.05$; $d=0.46$) in comparison with baseline, and PSDEN resulted in a greater decrease in comparison with PSDBN ($p<0.05$; $d=0.74$) (Figure 2b). P_{min} was lower after PSDEN ($p<0.01$; $d=1.15$) and PSDBN ($p<0.05$; $d=0.58$) in comparison with baseline. The difference between PSDEN and PSDBN was not statistically different (Figure 2c). Moreover, the one-way repeated measures ANOVA showed a significant main effect of sleep condition on RPE ($F_{(2,12)}=14.3$; $p=0.001$; $\eta p^2=0.7$) and [La] ($F_{(2,12)}=4.35$; $p=0.05$; $\eta p^2=0.7$). RPE scores were higher after PSDBN in comparison with baseline ($p<0.001$; $d=1.26$) (Figure 3h). [La] decreased when ex-

ercise was performed after PSDEN compared to baseline ($p<0.05$; $d=0.82$), and PSDBN was not statistically different from baseline (Figure 2d).

Psycho-cognitive performances and self-administered questionnaires

The one-way ANOVA with repeated measures showed a significant main effect of sleep condition on simple reaction time ($F_{(2,12)}=5.5$; $p=0.023$; $\eta p^2=0.52$), choice reaction time ($F_{(2,12)}=13.9$; $p=0.001$; $\eta p^2=0.73$), subjective sleepiness ($F_{(2,12)}=11.3$; $p=0.003$; $\eta p^2=0.69$) and Hooper index ($F_{(2,12)}=3.9$; $p=0.045$; $\eta p^2=0.44$). The Bonferroni post-hoc and Cohen tests revealed that simple reaction time was higher only after PSDEN compared to PSDBN ($p<0.01$; $d=1.02$) (Figure 3a). Choice reaction time was higher only after PSDBN compared to baseline ($p<0.001$; $d=1.89$) (Figure 3b). Subjective sleepiness and Hooper index scores were higher after PSDEN
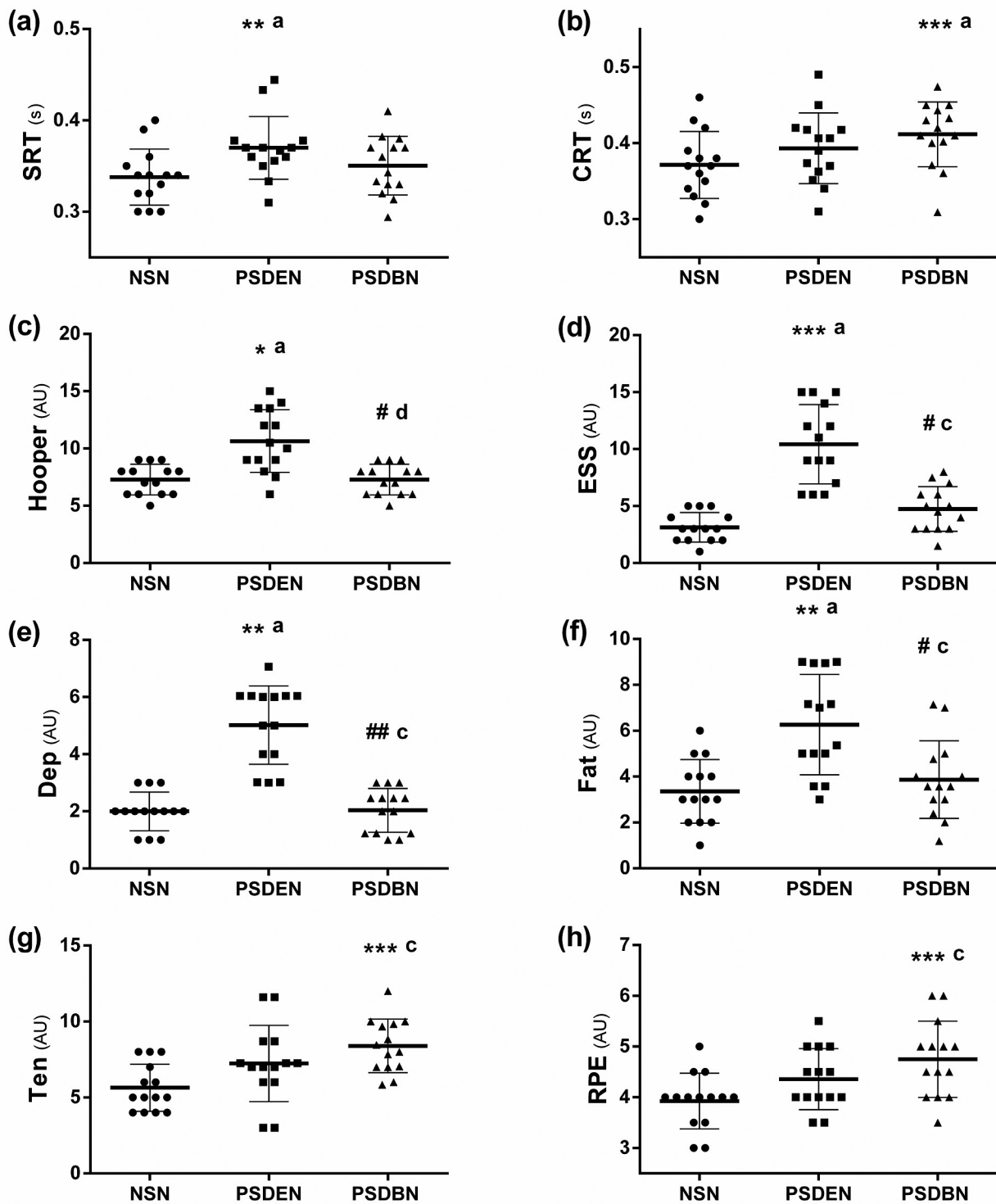
FIG. 3: Individual values of simple reaction time (**SRT; a**), choice reaction time (**CRT; b**), Hooper index (**c**), Epworth Sleepiness Scale (**ESS; d**), Depression (**Dep; e**), Fatigue (**Fat; f**), Tension (**Ten; g**) POMS sub-item and Rating of Perceived Exertion (**RPE; h**) scores after normal sleep night (NSN●), partial sleep deprivation at the end of the night (PSDEN■) and partial sleep deprivation at the beginning of the night (PSDBN▲). Significance is assessed with the one-way repeated measure ANOVA and Bonferroni post hoc test. *, **and *** indicate a significant difference in comparison with NSN values at $p < 0.05$, $p < 0.01$, $p < 0.001$ respectively; [a] large, [b] medium Cohen's *d* in comparison with NSN; #, ## and ### indicate a significant difference in comparison with PSDEN values at $p < 0.05$, $p < 0.01$ and $p < 0.001$ respectively; [c] large, [d] medium Cohen's *d* in comparison with PSDEN. Bars represent the group means and standard deviations. s; second, AU; arbitrary unit.

in comparison with baseline ($p<0.001$; $d=1.93$, $p<0.05$; $d=0.58$) and PSDBN ($p<0.05$; $d=0.86$, $d=0.43$) respectively (Figure 3c, Figure 3d). In addition, the one-way ANOVA with repeated measures showed a significant main effect of sleep condition on Fatigue ($F_{(2,12)}=6.05$; $p=0.019$; $\eta p^2=0.54$), Depression ($F_{(2,12)}=5.5$; $p=0.023$; $\eta p^2=0.52$) and Tension ($F_{(2,12)}=11.4$; $p=0.003$; $\eta p^2=0.69$) POMS sub-items scores. The Bonferroni test showed that Depression ($p=0.006$; $d=1.42$, $p=0.01$; $d=0.98$) and Fatigue ($p=0.003$; $d=2.12$, $p=0.025$; $d=1.39$) scores were higher after PSDEN (Figure 3e, Figure 3f) in comparison with baseline and PSDBN respectively. Moreover, Tension was higher after PSDBN comparatively to baseline ($p<0.001$; $d=1.61$) (Figure 3g). However, Anger, Confusion and Vigor POMS sub-items scores were unaffected.

Selected biochemical parameters
Data of the total blood count (WBC, MO, LY, GR, RBC and HB), energetic markers (CRE and GLC), biomarkers of muscle damage (AST, CK and URE), enzymatic (GPx) and non-enzymatic (UA) antioxidants, are presented in Table 2 and Figure 4. The two-way repeated measures ANOVA showed a significant main effect of sleep condition on RBC ($F_{(2,12)}=14.71$; $p<0.001$; $\eta p^2=0.53$), HB ($F_{(2,12)}=19.35$; $p<0.001$; $\eta p^2=0.61$), URE ($F_{(2,12)}=11.93$; $p=0.002$; $\eta p^2=0.64$) and GLC ($F_{(2,12)}=38.53$; $p<0.001$; $\eta p^2=0.98$). Resting GLC (Figure 4c) increased after PSDEN compared to baseline and PSDBN respectively ($p<0.001$; $d=2.5$, $d=1.61$). Resting RBC ($p<0.001$; $d=1.83$, $d=1.24$) and HB ($p<0.001$; $d=2.35$, $d=1.62$) levels decreased after PSDEN compared to baseline and PSDBN respectively (Figure 4a, Figure 4b). The two-way repeated measures ANOVA showed a significant main effect of sleep condition ($F_{(2,12)}=40.2$; $p<0.001$; $\eta p^2=0.89$) and an interaction between the effects of sleep condition and exercise ($F_{(2,12)}=4.28$; $p=0.017$; $\eta p^2=0.57$) on AST. Both types of PSD resulted in increased resting and post-exercise AST (Figure 4d). Resting and post-exercise CK increased after PSDEN due to the sleep condition ($F_{(2,12)}=14.77$; $p<0.001$; $\eta p^2=0.46$) rather than an inter-
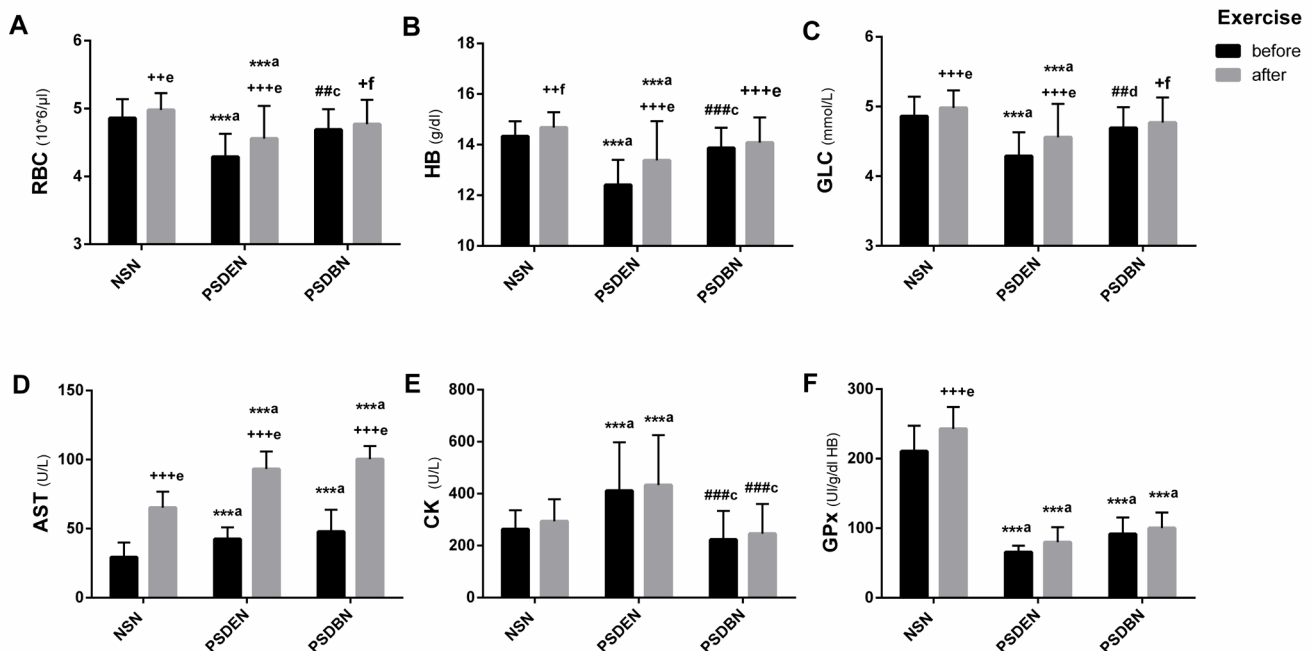

FIG. 4. A; Red blood cells (**RBC**), B; haemoglobin (**HB**), C; plasma glucose (**GLC**), D; aspartate aminotransferase (**AST**), E; creatine kinase (**CK**) and F; glutathione peroxidase (**GPx**) measured at rest and after exercise following normal sleep night (**NSN**), partial sleep deprivation at the end (**PSDEN**) and at the beginning (**PSDBN**) of the night. *** indicates a significant difference in comparison with NSN values at $p<0.001$; [a] large, [b] medium Cohen's d in comparison with NSN; # and ### indicate a significant difference in comparison with PSDEN values at $p<0.05$ and $p<0.001$ respectively; [c] large, [d] medium Cohen's d in comparison with PSDEN; +++ indicates a significant effect of exercise at $p<0.001$, [e] large, [f] medium Cohen's d in comparison with pre-exercise. Significance was determined using two-way repeated measure ANOVA. Significance for pairwise comparison is determined with Bonferroni post-hoc test. Data are presented as means ± standard deviations.

TABLE 2. Mean±SD values of leucocytes family, uric acid, creatinine and urea.

Parameters	NSN		PSDEN		PSDBN	
	Before	**After**	**Before**	**After**	**Before**	**After**
WBC (10*3/μl)	6.06±1.24	8.17±0.96[+++e]	6.57±1.74	9.25±1.47 [*b+++e]	5.47±1.18[*b##d]	8.2±2.6[+++e]
MO (10*3/ μl)	0.3±0.08	0.38±0.08	0.37±0.12[*b]	0.48±0.16[+++e]	0.27±0.05	0.4±0.11[+++e]
LY (10*3/μl)	2.06±0.55	3.63±0.5[+++e]	2.02±0.34	3.58±0.99[+++e]	1.95±0.37	3.34±0.53[+++e]
GR (10*3/μl)	3.49±1.09	4.06±0.86	4.17±1.84	5.18±1.85 [*b+++e]	3.27±0.94[##d]	4.52±2.14[+++e]
UA (μmol/l)	333.21±31.98	350.71±27.31[+f]	323.14±33.92	338.28±32.79[++e]	310.71±26.3[**b]	326.85±53.68
CRE (μmol/l)	85.64 ±11.11	96.78±14.7[++f]	79±12.41	96.14±11.32[+++e]	90.42±3.36[##d]	105.71±9.39[*b#d+++e]
URE (mmol/l)	5.16±1.42	5.19±1.39	5.7±1.48	6.16±1.49[**b]	4.25±0.95[**b ###c]	5.01±0.85[###c]

Significant difference in comparison with NSN at [*] $p<0.05$; [**] $p<0.01$; [***] $p<0.001$; [a] large, [b] medium *d* in comparison with NSN, Significant difference in comparison with PSDEN at [#] $p<0.05$; [##] $p<0.01$, [c] large, [d] medium *d* in comparison with PSDEN, Significant effect of exercise at [+] $p<0.05$; [++] $p<0.01$; [+++] $p<0.001$, [e] large, [f] medium *d* in comparison with pre-exercise. Significance was assessed with the two-way repeated measures ANOVA and the Bonferroni post-hoc and the effect size is assessed with Cohen's tests. **NSN**; normal sleep night, Partial Sleep deprivation at the end (**PSDEN**), and at the Beginning (**PSDBN**) of the night, **WBC**: white blood cells, **MO**: Monocytes, **LY**: lymphocytes, **GR**: granulocytes, **UA**: uric acid CRE: creatinine and URE: urea.

action between sleep condition and exercise (Figure 4e). Similarly, the reduction of GPx after PSDEN and PSDBN was caused by the sleep condition ($F_{(2,12)}=294.3$; $p<0.001$; $\eta p^2=0.97$) and not an interaction between sleep condition and exercise (Figure 4f).

DISCUSSION

The main findings of the current investigation are that mood state, cognitive and repeated-sprint performances were lower after partial sleep deprivation (PSD) at the end of the night (PSDEN) more than PSD at the beginning of the night (PSDBN). Both PSDEN and PSDBN reduced basal and post-exercise antioxidant capacity in the post-lunch dip. Further, the pre-post exercise changes in leukocytes and biomarkers of muscle damage were greater after PSDEN compared to PSDBN.

PSDEN decreased P_{max}, P_{min}, and P_{mean}, while PSDBN resulted in smaller decrease in P_{mean} and P_{min}, in comparison with baseline. These findings are in line with previous studies showing that PSDEN exert a stronger effects on short-term high intensity [4,5] and endurance [3] exercises than PSDBN. Moreover, as previously shown [7,11,27], PSDEN increased subjective sleepiness, the Hooper index and POMS sub-items scores. These results suggest that participants were sleepy, fatigued and not willing to perform, which probably contributed to the decrease in repeated-sprint performances especially after PSDEN. Similarly, a recent study reported higher levels of sleepiness and less motivation to train after PSD in cyclists [12].

Furthermore, the pre-post exercise changes in glucose (GLC) levels were higher after PSDEN than PSDBN, despite the fact that

participants ate the same standardized meals at the same time during the different sessions. Since overall GLC utilization is not stable during nocturnal sleep and is greatest during waking [1], it is possible that early awakening resulted in greater glycogen depletion and higher plasma GLC levels. Moreover, the increased GLC could be explained by greater insulin resistance caused by PSDEN. Indeed, a recent study reported a 20% increase in oral and intravenous insulin resistance after restriction of nocturnal sleep to 5 h (caused by earlier awakening) for 5 days [28]. In this context, a previous study showed that pre-exercise muscle glycogen was lower after 30 h of sleep deprivation, which resulted in decreased repeated-sprint performance [11]. Since repeated-sprint solicits essentially, but not exclusively, glycolytic pathways [15], the disruption of GLC metabolism caused by PSDEN could explain, at least partly, the decrease of the Running-based Anaerobic Sprint-Test performances. In this sense, post-exercise lactate plasmatic levels [La] showed a slight decrease after PSDEN. The fact that total work decreased could explain the lowered [La] levels after PSDEN. A previous study found a similar decrease in [La] levels after PSD [10].

The current data showed that resting RBC and HB counts were lower following PSDEN than PSDBN and baseline. It could be possible that the decrease of sprint power is caused by the lowered O_2 and nutrient transporter availability [29]. However, an earlier study reported that the RBC family was unaffected by PSD [9,30]. This discrepancy could be attributed to the timing of the exercise and the blood sampling, since haematological parameters and repeated-sprint

exercise [15] display a circadian rhythm. In the earlier studies, blood samples were collected at 07:00 h [30] and at 17:00 h [9]. However, in the present study, blood samples were collected in the mid-afternoon (i.e., 14:50 h). Similarly to earlier findings [9,15], repeated sprint caused leukocytosis after all sleep conditions. However, the post-exercise leukocytosis was more important after PSDEN through higher WBC and GR. Likewise, interleukin-6 and tumour necrosis factor-alpha [4] and basophil [12] levels remained elevated during the recovery period after sleep deprivation. These findings reflect the greater inflammation caused by the short-term high-intensity exercise after PSDEN.

PSDEN resulted in higher resting creatine kinase (CK) and monocyte (MO) levels, which indicates that exercise started with a higher basal level of muscle damage and inflammation. In addition, resting and post-exercise aspartate aminotransferase (AST) increased after PSDEN. The increased AST reflects the greater challenge caused by the exercise to the muscles and the liver after PSDEN. Moreover, urea (URE), which indirectly reflects the increase in ammonia – a very toxic waste for brain and muscles – increased after PSDEN. Thus, the higher levels of post-exercise URE could partly explain the higher levels of fatigue and the decrease of sprint power after PSDEN. Consequently, despite the decrease of the total work during repeated sprint, the exercise resulted in greater levels of biomarkers of muscle damage after PSDEN compared to baseline. Likewise, in the study of Mejri et al. [10], CK and myoglobin increased solely after PSDEN, suggesting greater cardiac and muscle damage when exercise was performed after PSDEN. Further, total [31] and partial [12] sleep deprivation has been shown to negatively affect recovery after exercise when compared with normal sleep. From rodent studies, it was also reported that sleep deprivation acutely down-regulated molecular markers of muscle repair and resulted in deficits of contractile function during recovery [32].

Concerning the antioxidant defence, the current results showed a decrease in resting and post-exercise glutathione peroxidase (GPx) levels after PSD. Unfortunately, technical problems occurred when measuring superoxide dismutase (SOD) levels, and data are not presented here. Similar findings were observed in an animal model [33]. Indeed, glutathione, GPx, SOD and catalase declined in response to sleep deprivation. The reduction of GPx would exacerbate the oxidative cellular damage via a positive feedback cycle of oxidant generation [34]. Indeed, Ikeda et al. [35] hypothesized that reactive oxygen species are released while awake and sleep promotes their elimination. Thus, sleep deprivation *per se* would lead to disruption of the oxidant-antioxidant balance in favour of free-radical increase. The present study findings support this theory since whenever participants were sleep deprived, PSD led to a decrease in resting and post-exercise GPx levels, and consequently decreased antioxidant defence. Additionally, uric acid, which is a non-enzymatic antioxidant, also declined in response to PSD. This suggests that when exercise is performed under sleep deficit, it would lead to lowered antioxidant defence against exercise-induced oxidative stress.

In addition to the lowered alertness, subjective sleepiness, fatigue and negative mood increased after PSD in the post-lunch dip. Accordingly, it has been reported that sleepiness increases and attention decreases in the post-lunch dip, especially after PSD [7,8]. In the present study, the decreased resting RBC and HB in the post-lunch dip could explain partly these outcomes, since it ensures oxygen and nutrient transportation to the brain [29]. Moreover, it has been proposed that glutathione is a sleep-promoting substance [35]. It could also be possible that the decrease in resting antioxidant defence around the post-lunch dip promotes sleep drive. Earlier studies reported that morning performances were unaffected by PSD, but performances of late afternoon and early evening declined [2–5]. From the current results, mid-afternoon repeated sprint performances declined after PSD. Taken together, it is safe to speculate that short-term high-intensity exercise performed later than the post-lunch dip will be affected by PSD.

PSDEN resulted in a greater disruption in the resting CK, HB and RBC than PSDBN. This discrepancy between the two types of PSD could be attributed to the longer time spent awake after PSDEN (i.e., 12 h after PSDEN vs 8 h after PSDBN) [5]. Hence, according to Borbely's sleep model [36], the longer the awakening state, the higher will be the sleep drive (i.e., process S). In addition, the post-lunch dip correspond to the secondary peak in sleepiness (i.e., process C). Taken together, both processes contributed to the enhanced sleepiness and the reduced performances. From the current study, the sharp decrease in GPx and the increased MO indicate the high level of inflammation caused by PSD, which was higher after PSDEN. Also, it could be possible that the increased cellular damage contributed to heightening subjective sleepiness [37]. Furthermore, the alteration of performances after PSDEN could be explained by the potential lack of REM sleep, which is preponderant in the late part of nocturnal sleep. In fact, REM sleep importantly intervenes in the regulation of circadian rhythms [2]. Indeed, it has been proposed that the major role of muscle atonia during REM sleep is to improve muscle efficiency and recovery [38]. In this context of sleep deprivation, the disruption of melatonin rhythm could affect the subsequent day's performances [5]; the hormone secretion peaks during the late part of nocturnal sleep, which was probably blunted (at least in part) by PSDEN in the current study. Melatonin acts as a direct free-radical scavenger in addition to its role in the stimulation of antioxidant enzyme production [39].

It is noteworthy to mention that during PSD nights, sleep was recorded only subjectively (time in bed), which could limit the current findings. In addition, the timing of light exposure during PSD nights could present another limitation. Participants were exposed to artificial light between 20h00 and 02h00 during PSDBN and between 02h30 and 07h00 during PSDEN. The light exposure during early and late night could affect melatonin secretion differently.

The present study results showed that exercising after a suboptimal nocturnal sleep will not only result in performance deficit, but also lead to greater systemic inflammation. Future studies should

focus on possible countermeasures to decrease the negative impact of PSD on performances.

moglobin could explain the increased sleep pressure related to the post-lunch dip and potentiated by previous night PSD.

CONCLUSIONS

Mood and antioxidant defence were lower, and sleepiness and inflammation were higher at rest after PSDEN than after PSDBN. Despite the lower performance after PSDEN, repeated sprint resulted in greater muscle damage. Indeed, the higher basal inflammation lowered the sprint power and enhanced the exercise-induced muscle damage. Further, the drop of resting antioxidant defence and hae-

Conflict of interest declaration

The authors have no conflict of interests.

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