Medicine & Science IN Sports & Exercise

The Official Journal of the American College of Sports Medicine

www.acsm-msse.org

... Published ahead of Print

Effect of an Innovative Mattress and Cryotherapy on Sleep after an Elite Rugby Match

Anis Aloulou¹, Cédric Luduc², François Duforez^{3,4}, Julien Piscione⁵,
Jeremy Cheradame⁵, François Bieuzen⁶, Claire Thomas^{1,7}, Mounir Chennaoui^{4,8},
Pascal Van Beers^{4,8}, Mathieu Nedelec¹

¹French National Institute of Sport (INSEP), Laboratory of Sport, Expertise and Performance (EA 7370), Paris, France; ²Carnegie Applied Rugby Research (CARR) center, Institute for Sport, Physical Activity and Leisure, Carnegie School of Sport, Leeds Beckett University, Leeds, United Kingdom; ³Sleep and Vigilance center, Hospital Hotel-Dieu, Paris, France; ⁴University Paris Descartes, Vigilance FATigue SOMmeil unit (VIFASOM) EA 7330, France; ⁵Research Department, French Rugby Federation (FFR), Marcoussis, France; ⁶Institut National du Sport (INS) du Québec, Montréal, Canada; ⁷UMR UEVE-MD Laboratory of Exercise Biology for Performance and Health, University of Evry Val d'Essonne, STAPS Department, Evry, France; ⁸Institut de recherche biomédicale des armées (IRBA), Brétigny-sur-Orge, France

Accepted for Publication : 16 April 2020

Medicine & Science in Sports & Exercise®. Published ahead of Print contains articles in unedited manuscript form that have been peer reviewed and accepted for publication. This manuscript will undergo copyediting, page composition, and review of the resulting proof before it is published in its final form. Please note that during the production process errors may be discovered that could affect the content.

Effect of an Innovative Mattress and Cryotherapy on Sleep after an Elite Rugby Match

Anis Aloulou¹, Cédric Luduc², François Duforez^{3,4}, Julien Piscione⁵, Jeremy Cheradame⁵,
François Bieuzen⁶, Claire Thomas^{1,7}, Mounir Chennaoui^{4,8}, Pascal Van Beers^{4,8},
Mathieu Nedelec¹

¹French National Institute of Sport (INSEP), Laboratory of Sport, Expertise and Performance (EA 7370), Paris, France; ²Carnegie Applied Rugby Research (CARR) center, Institute for Sport, Physical Activity and Leisure, Carnegie School of Sport, Leeds Beckett University, Leeds, United Kingdom; ³Sleep and Vigilance center, Hospital Hotel-Dieu, Paris, France; ⁴University Paris Descartes, Vigilance FAtigue SOMmeil unit (VIFASOM) EA 7330, France; ⁵Research Department, French Rugby Federation (FFR), Marcoussis, France; ⁶Institut National du Sport (INS) du Québec, Montréal, Canada; ⁷UMR UEVE-MD Laboratory of Exercise Biology for Performance and Health, University of Evry Val d'Essonne, STAPS Department, Evry, France; ⁸Institut de recherche biomédicale des armées (IRBA), Brétigny-sur-Orge, France

Corresponding author

Mathieu Nedelec

Institution: French National Institute of Sport (INSEP), Laboratory of Sport, Expertise and Performance (EA 7370) - 11 avenue du Tremblay - 75012 Paris, France

Telephone: (+33) 1 41 74 41 00. E-mail address: mathieu.nedelec@insep.fr

Anis Aloulou was supported by a scholarship from Bultex, a company specialized in bedding.

Conflict of interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as potential conflicts of interest. The results of the present study are presented clearly, honestly, and without fabrication, falsification, or inappropriate data manipulation and do not constitute any endorsement by the American College of Sports Medicine.

Abstract

Introduction: This study aimed to explore the relationship between elite rugby union match and post-match sleep architecture and to investigate the effects of a high-heat capacity mattress (MAT) and a whole-body cryotherapy session (WBC) on post-match sleep architecture.

Methods: Nineteen elite male U23 rugby union players performed in three official matches, followed by three experimental conditions, in a randomized order: MAT, WBC, and no intervention (CONT). Match load was evaluated using global positioning system (GPS) trackers and video analyses. Sleep architecture was assessed by polysomnography (PSG). Core body temperature (CBT) and mattress surface temperature were monitored during sleep. Linear mixed-effects models were conducted to assess the effects of each experimental condition on sleep, with match load variables as covariates. **Results:** A lower wake after sleep onset ($\beta = -10.5$ min, $p < 0.01$) and higher rapid-eye-movement sleep proportion ($\beta = +2.8\%$, $p < 0.05$) were reported for MAT compared with CONT. Moreover, a lower mean CBT ($\beta = -0.135^{\circ}\text{C}$, $p < 0.001$) and mean mattress surface temperature ($\beta = -2.736^{\circ}\text{C}$, $p < 0.001$) during sleep were observed for MAT compared CONT. Whole-body cryotherapy did not affect nocturnal CBT nor interfere with sleep architecture. For every 100-m increase in high-speed running distance, a higher slow-wave sleep (SWS; $\beta = +1.1\%$, $p = 0.05$) and lower light sleep proportion ($\beta = -1.2\%$, $p < 0.05$) proportion were observed. Conversely, for every 10 supplementary collisions, a lower SWS ($\beta = -1.9$, $p = 0.09$) and higher light sleep ($\beta = +2.9\%$, $p < 0.001$) proportion were observed. **Conclusion:** MAT use had a positive effect on sleep architecture after an elite rugby union match, potentially through a more efficient nocturnal heat transfer.

Keywords: POLYSOMNOGRAPHY, CORE BODY TEMPERATURE, MUSCLE DAMAGE,
RECOVERY, HEART RATE VARIABILITY, SLEEP ARCHITECTURE

ACCEPTED

Introduction

Rugby union is a high-intensity, intermittent sport, played worldwide (1). During match play, high-speed running and collision-based activities, such as tackling, static holds, scrums, rucks, and mauls, have been shown to induce high levels of fatigue and muscle damage (2,3). Professional teams play weekly matches and train twice daily for two or more consecutive days (4), which can make player readiness for competitions challenging. Therefore, the optimal management of the recovery process is required to minimize training- and competition-induced fatigue (5). Sleep is recognized by the scientific community and by players to be a major component of the recovery process (3,6). A previous study examining rugby union players showed that the mean sleep time was reduced by approximately one hour and sleep efficiency was reduced by approximately 4.5% on nights following a match compared with nights following regular training days (7). Reductions in sleep quantity have been shown to negatively impact physical performance (8) and increase injury risk (9). Previous studies have also reported reduced sleep quality and quantity among rugby players compared with non-athletes controls (7,10). Consequently, post-match sleep conditions should be optimized to ensure that players experience the maximum levels of physical and psychological recovery (11).

Sleep and thermoregulation are closely related (12,13). Sleep initiation is associated with the maximal rate of decline in core body temperature (CBT), which occurs through an increase in cutaneous temperature and heat loss from the periphery (12). Within the sleep period, lowering the minimum CBT has been suggested to increase slow-wave sleep (SWS) and to positively impact sleep quality (14,15). Krauchi *et al.* (15) reported a higher proportion of SWS ($\approx 3.7\%$) when sleeping on a high-heat capacity mattress (MAT), which facilitates the continuous and

slow removal of body heat via conductive heat transfer. However, this study was conducted on untrained subjects. To the best of our knowledge, MAT use on sleep quality and quantity after competitions has not been examined among elite athletes.

Whole-body cryotherapy (WBC: 3 min at -110°C) has been shown to decrease cutaneous temperature during the exposure, and to slightly reduce CBT ($\approx 0.3^{\circ}\text{C}$) after the exposure (16,17). Whole-body cryotherapy has also been shown to reduce actigraphic activity during sleep, which may potentially indicate a deeper sleep (18,19). However, CBT and sleep architecture were not assessed in these studies. Moreover, it is not clear whether the acute pre-sleep WBC cooling effect persists throughout the night and interfere with sleep architecture. Earlier research stated that sleep and autonomic modulation are linked as higher delta activity during non-rapid eye movement (NREM) sleep has been associated with reduced sympathetic modulation (20). Heart rate variability (HRV) indices are higher directly after WBC exposure (21) and during the first SWS sequence following a night-time exercise (18). However, the relationship between WBC-induced parasympathetic hyperactivity during the early phase of NREM and the overall sleep architecture is still to be elucidated.

The aim of the present study was to investigate the effect of MAT - continuous cooling strategy during the night - and WBC - acute cooling intervention before the night - on sleep architecture and short-term recovery after an elite rugby union match. This study was designed to compare each intervention with a control condition. The secondary aim of this study was to investigate the potential effect of rugby match load variables on sleep architecture. We hypothesized that MAT

use and WBC would enhance sleep compared with a control condition, and that a higher match load would negatively impact sleep architecture.

Methodology

Participants

Twenty-three elite male under-23 rugby union players, competing at the highest national French level, were initially recruited. Due to substitutions related to tactical choices, injuries, and player unavailability, nineteen players who participated in at least two conditions (CONT+WBC+MAT: n=10, CONT+WBC: n=2, CONT+MAT: n=2 and WBC+MAT: n=3), or only in the CONT condition (n=2), were included in the final analysis. Ten backs (mean $\pm$ SD; age: 20.8 ± 1.0 yrs; body mass = 91.4 ± 7.4 kg; height = 183.5 ± 8.5 cm) and nine forwards (mean $\pm$ SD; age: 20.6 ± 1.3 yrs; body mass = 111.7 ± 8.7 kg; height = 189.4 ± 8.8 cm) were included. The study was conducted according to the Helsinki Declaration (1964: revised in 2001), and the protocol was approved by the local ethics committee (East III, France. Ref. 170605). All players provided a detailed medical history and underwent a medical examination, including an electrocardiogram at rest. The players also provided their written informed consent before the initiation of experiments. The inclusion criteria were as follows: i) an intermediate or moderately evening type, according to the Horne and Ostberg questionnaire (i.e. < 58 and > 31); ii) free from PSG-confirmed sleep disorders, such as sleep apnea (apnea-hypopnea index > 10), periodic limb movement syndrome, hypersomnia, or narcolepsy; and iii) free from diabetes, severe hypertension, heart disease, anorexia, and bulimia.

Experimental sessions

The study was conducted over three weekends within the same month (April 2018). The study was designed according to a within-subject crossover comparison that finally resulted in unbalanced experimental conditions order due to substitutions, injuries, and player unavailability. The design consisted of one familiarization night and three experimental conditions: 1) High heat capacity MATtress (MAT) condition, during which players slept on a specific mattress (described elsewhere); 2) Whole Body Cryotherapy (WBC) condition, during which players spent 3 min in a specific chamber, at -110°C , two hours before bedtime ($\approx 21:30$, i.e. ≈ 4 h post-match); and 3) control (CONT) condition, during which players received no intervention after the match. The players were blinded to the MAT condition, and they were informed that the aim of the study was solely to evaluate the effects of WBC on sleep. For each of the experimental weekends, the entire team arrived at the training centre on Friday, played an official match on Saturday afternoon (15:00), and left the centre on Sunday afternoon (Figure 1). Sleep was only assessed on Saturday nights, following match play. However, one night of familiarization was conducted on the very first Friday of the experimental period (22). On Saturday morning, baseline tests (BASELINE) were conducted at 09:00, before the first warm-up protocol; they were used to assess test-retest reliability. One hour after the match (around 18:00), the same testing battery (POST) was conducted as BASELINE. Dinner was served around 21:00. The meal was prepared by a nutritionist and included a variety of breads, cereals, milk/yogurts, meats, pasta/rice, fruit, and vegetables, to ensure the adequate intake of macro- and micronutrients. The same meal was replicated for each of the three conditions. The PSG equipment was assembled by a team of qualified practitioners (≈ 20 to 30 min for each player), and bedtime was set at 23:30. The following morning, wake-up was planned at 08:00. At 10:00,

after breakfast, the same testing battery was conducted (D+1) to assess recovery status. During the PSG nights, room temperature and humidity were assessed continuously, using an iButton (DS 1922L, iButtonLink, Whitewater, USA; resolution 0.0625 °C; sampling rate: 1 value per minute), which was placed on the bedroom wall. No significant differences were noted in ambient temperatures (CONT = $21.6 \pm 1.6^{\circ}\text{C}$; WBC = $20.8 \pm 1.3^{\circ}\text{C}$; MAT = $21.7 \pm 1.0^{\circ}\text{C}$) or humidity (CONT = $52.9 \pm 11.2\%$; WBC = $52.4 \pm 8.2\%$; MAT = $53.1 \pm 6.2\%$) between conditions.

Figure 1

Mattress properties

The mattress used for all conditions was composed of the following layers: i) an upper quilted panel, composed primarily of foam (thickness of 2.5 cm and density of 23 kg/m^3); ii) a core comprised of a specific foam type (nano, Bultex, France), with a thickness of 16 cm and a density of 33 kg/m^3 ; and iii) a lower quilted panel, composed primarily of foam (thickness of 1.8 cm and density of 23 kg/m^3). For the MAT condition, we added a 6-cm polyurethane, high-heat capacity layer (see Figure, Supplemental Digital Content 1, which illustrates the bedding conditions, <http://links.lww.com/MSS/C2>), with a density of 1.006 kg/m^3 which allows the slow removal of body heat via conductive heat transfer (15). All mattresses used in the study were identical in size ($100 \times 200 \times 23 \text{ cm}$). The same cover types (non-quilted textile, with a weight of 400 g/m^2) and pillows were used throughout the study to avoid potential bias. Participants were required to sleep in the same clothes for all three conditions. The mattress surface temperature

was continuously assessed with the same iButtons used to assess ambient temperatures, which were placed in the middle of the mattress, 60 cm from the top.

Match analysis

Global positioning systems (GPS) and video analyses were used to monitor the external workloads of each player during the three matches. Each player wore the same 16-Hz unit (Sensoreverywhere V2, Digital Simulation, Paris, France), in a Lycra vest that positioned the unit on the upper thoracic spine, between the scapulae. Data were downloaded after the game, and playing times were fixed for each player, to ensure that only game data were used for analysis. In this study, we focused on three GPS-derived locomotor variables: i) total covered distance (TD); ii) distance covered at high speeds (HS; $> 5 \text{ m.s}^{-1}$); and iii) distance covered at low-speeds (LS; $< 5 \text{ m.s}^{-1}$) (23). The GPS measures previously showed high levels of validity and between-device reliability for maximal sprinting and acceleration (24). In addition, video analyses were conducted via the Hudl system (Sportstec, NE), to assess the total number of collisions during the match, which was calculated as the sum of offensive and defensive tackles, rucks, and contact hits (25).

Sleep analysis

Polysomnography (PSG) recordings were obtained the night after each match using two types of portable devices (Nox A1, Resmed, United States [n=11] and Actiwave©, CamNtech Ltd., Cambridge, UK [n=8]). Each participant used the same device for all conditions. Both systems provided continuous monitoring of four electroencephalograms (F3-M2, F4-M1, C4-M1, O2-M1), two electrocardiograms (ECG), two electrooculograms (outer canthus of each eye), and two

electromyograms (chin). Contralateral mastoid leads served as references for all unipolar measurements (electroencephalograms and electrooculograms). Polysomnography data were scored by two trained technicians who were assigned all the nights from one PSG model, and were blinded to the experimental conditions. The American Academy of Sleep Medicine (AASM) criteria were employed (26). Analyses were conducted using Noxturnal software version 5.1 (Resmed, United States) and Somnologica (TM; Medcare©, Reykjavik, Iceland). The sleep variables were measured as follows: sleep onset latency (SOL) was defined as the time between lights off and sleep onset; wake after sleep onset (WASO) was defined as the total wake time during the sleep period; total sleep time (TST) was defined as the time spent asleep, as determined from sleep start to sleep end, minus WASO; sleep efficiency (SE) was defined as the TST divided by the time in bed (expressed as a percentage); and finally, light, SWS, rapid eye movement (REM) and non-rapid eye movement (NREM) were defined as the proportion of time spent in each of these sleep stages expressed as a percentage of TST.

Core body temperature (CBT)

During the afternoon, players ingested a radio telemetric pill (BodyCap, e-Celsius® Performance, France), to continuously record their nocturnal CBTs. This method has been shown to be reliable and valid (27), with an accuracy of 0.23°C, an intra-class correlation coefficient of 1.00, and a standard error of measurement of 0.03.

Nocturnal heart rate (HR) and heart rate variability (HRV)

The ECG signal was derived from positions at the right midclavicular and approximately 6 cm under the left armpit. The electrodes were connected to the polysomnographic systems (Nox A1,

Resmed, United States or Actiwave©, CamNtech Ltd, Cambridge, UK), and ECG data were continuously recorded at 200 Hz. Data were then converted into a European data format and imported into Somnologica Studio (version 2.0.1 Medcare-Embla®, Reykjavik, Iceland) for further analysis. This software has been used in previous sleep studies (28). Nocturnal HRV and HR indexes were determined using the first 5-min stationary segment (free from arousals) in the first SWS sequence that lasted for longer than 15 min. Slow wave sleep has been shown to be better for discriminating sympathovagal balance states than waking periods (29). All data acquisition and analyses were performed in accordance with established standards (30). Electrocardiogram waveforms were analyzed to obtain temporal and frequency domain components. Time-domain variables, including mean R-R interval (RRI) and the root mean square standard deviation (RMSSD) of RRI, were assessed. Frequency domains were assessed for low- (LF; 0.04–0.15 Hz) and high-frequency (HF; 0.15– 0.40 Hz) bands and the ratio of LF/HF during each 5-min spectrum. Mean HR was calculated using the following formula: HR (bpm) = $1 / \text{R-R (sec)} * 60$.

Markers of fatigue and subjective assessments

Markers of peripheral fatigue and exercise-induced muscle damage included countermovement jump performance (CMJ), blood creatine kinase concentration ([CK]), and perceived delayed-onset muscle soreness (DOMS). These indicators were obtained at BASELINE and at D+1 (Figure 1).

Countermovement jump (CMJ) performance was assessed to evaluate neuromuscular fatigue. Prior to testing, players performed a 10-minute dynamic warm-up, consisting of active mobility

and progressive lower-body loading using lunges, step-ups, and squats. Jump assessments required each participant to perform unloaded CMJs, with a wooden stick placed on their shoulders. The CMJ mean force (CMJ-F) was assessed using a portable linear encoder device (GymAware Power Tool, Kinetic Performance Technologies, Canberra, Australia) (31). Each participant performed four repetitions, pausing for approximately 3-5 s between each jump. The mean of the trials (excluding the best and the worst trial) was calculated and used as a marker of neuromuscular performance. A typical error of the mean (TEM) of 177.4 N, an intraclass correlation coefficient (ICCc) of 0.87, and a coefficient of variation (CV) of 8.6% were obtained.

Blood creatine kinase concentration ([CK]) was assessed by collecting 500 µl of blood from fingertip capillary punctures, which were stored in a microtube containing lithium heparinase (BD Microtainer, BD, New Jersey, US). Within one hour after the blood collection, 32 µl of each sample was taken from the tube, using a specific pipette, and placed on a measurement strip. Analyses were performed using a Reflotron Sprint (Roche Diagnostics, Grenzachstrasse, Switzerland). The Reflotron was calibrated according to the manufacturer's recommendations. A TEM of 65.7 IU·L⁻¹, an ICCc of 0.99, and a CV of 10.6% were obtained for [CK] measurements.

Subjective assessments

The well-being Hooper Index is a rating of general fatigue, DOMS, stress, and sleep perception. Players were asked to subjectively evaluate, twice for each condition (PRE and D+1), the four items, using a 1-7 scale, with 1 representing the most positive rating and 7 representing the most negative rating, for each variable. The Hooper index was calculated as the sum of all four scores. Each variable was presented separately (32).

Rating of perceived exertion of the session (sRPE) was assessed for each player at the end of each match. Players answered the question “How was your workout?” using a scale from 0 (rest) to 10 (maximal). The obtained score was then multiplied by the playing duration for each player (33) and recorded as arbitrary units (AU).

Perceived thermal comfort (on a scale from -2 “very uncomfortable” to +2 “very comfortable”) and *sensation* (on a scale from -3 “cold” to +3 “hot”) (34) was recorded for each player the morning after each post-match night.

The total quality of recovery (TQR) scale was reported by the players in the morning, after each post-match night. Scores varied from 6, “very, very poor recovery,” to 20, “very, very good recovery” (35).

Statistical analysis

Three types of linear mixed-effects models were fitted with regards to the dependent variables (DVs) tested and the research question. Table 1 provides an overview of the models’ designs. For the second type of model, the variations between each experimental condition and the CONT condition for each DV were adjusted according to the number of collisions and the HS distance recorded for each player. These two variables represented the most important components associated with rugby union match-related metabolic and neuromuscular fatigue (25). All models were fitted using the “*lme*” function of the *nlme* package in the R program (version 1.1.456). Data are presented as the estimate (β), the standard error (SE), the 95% confidence interval (CI),

and the P-value. For the interpretation of the models, the intercept represented the mean CONT value of the DV when the fixed effects were null. The effects of MAT and WBC on mattress surface temperature kinetics (8 time points, with 1-h intervals) compared with the CONT condition were assessed using a two-way (condition $\times$ time) repeated-measures analysis of variance (ANOVA). Multiple Student's paired t-tests, with Bonferroni correction, were then used for post hoc pairwise comparisons when a significant interaction between time and condition was observed. The level of significance was set at $p < 0.05$.

Table 1

Results

Match load, sleep schedules and baseline fatigue between conditions

Match load. Global match load variables are presented as the intercept of the mixed-effect model (see Table, Supplemental Digital Content 2, which illustrates the internal and external match load, <http://links.lww.com/MSS/C3>). Independently from the experimental conditions, total distance covered was 3643.0 ± 468.9 m, including 3416.8 ± 455.0 m of LS running distance and 226.4 ± 54.5 m of HS running distance. The number of collisions was 16.2 ± 2.9 and sRPE was 434.1 ± 65.4 AU.

A significantly higher total distance ($\beta = +980.2$ m, $p < 0.05$), LS running distance ($\beta = +899.0$ m, $p < 0.05$) and number of collisions ($\beta = +4.4$, $p = 0.05$) were reported for the WBC condition compared with the CONT condition. No significant differences were observed for HS distance ($\beta = +81.5$ m, $p = 0.17$) and sRPE ($\beta = +78.6$ AU, $p = 0.20$) between the WBC and CONT

conditions. No significant differences were observed for any match load variables between the MAT and CONT conditions (see Table, Supplemental Digital Content 2, <http://links.lww.com/MSS/C3>).

Sleep schedules. No significant differences were reported for bedtimes (intercept: 23:35 ± 00:33; Δ WBC - CONT = 00:11 min, p = 0.38; Δ MAT - CONT = 00:17 min, p = 0.17) or get up times (intercept: 08:02 ± 00:16; Δ WBC - CONT = -00:04 min, p = 0.52; Δ MAT - CONT = -00:08 min, p = 0.16) between the conditions.

Baseline fatigue markers. No significant differences between conditions were reported at BASELINE for any marker of fatigue.

Effects of MAT on sleep, temperature and fatigue markers

Polysomnography. Tables 2 and 3 provide an overview of the effects of the MAT condition on sleep architecture compared with the CONT condition. A significantly lower WASO ($\beta = -10.5$ min, $p < 0.01$) and a trend towards higher sleep efficiency ($\beta = +2.9\%$, $p = 0.11$) for the MAT condition compared with the CONT condition were observed (Table 2). A higher REM sleep proportion ($\beta = +2.8\%$, $p < 0.05$) was noted for MAT condition compared with the CONT condition (Table 3).

Table 2 and 3

Temperature, HR and HRV. A significant reduction in mean nocturnal CBT ($\beta = -0.135^{\circ}\text{C}$, $p < 0.001$) and mattress surface temperature ($\beta = -2.736^{\circ}\text{C}$, $p < 0.001$) were observed for the MAT condition compared with the CONT condition (Table 4). The two-way ANOVA revealed main effects for time and condition and for the interaction between time and condition ($p < 0.001$). Significant differences between the two conditions were observed at all-time points ($p < 0.05$), except at bedtime and 4 to 5 hours after bedtime (Figure 2). A higher nocturnal thermal comfort was perceived for the MAT ($\beta = +0.6$, 95% CI: 0.1 to 1.2, $p < 0.05$) condition compared with the CONT condition. The MAT use did not affect HR and HRV, compared with the CONT condition.

Table 4 and Figure 2

Fatigue markers. No significant differences between MAT condition and the CONT condition for TQR scores, and the variations between BASELINE and D+1 of [CK] levels, CMJ-F, and DOMS were reported.

Effects of WBC on sleep, temperature and fatigue markers

Polysomnography. Whole-body cryotherapy did not affect any of the sleep variables compared with the CONT condition (Table 2 and 3).

Temperature, HR and HRV. No significant differences between the WBC and CONT conditions were observed for mean CBT (Table 4). However, WBC showed a higher nocturnal thermal comfort ($\beta = +0.7$, 95% CI: 0.2 to 1.3, $p < 0.05$) and a colder sensation during the night ($\beta = -0.8$,

95% CI: -1.6 to -0.0, $p = 0.05$) than the CONT condition. Frequency domain analyses of HRV during the SWS sequences showed a higher normalized HF power for the WBC condition ($\beta = +9.3\%$, 95% CI: -0.1 to 18.7%, $p = 0.05$) suggesting an increased parasympathetic activity compared with the CONT condition.

Fatigue markers. No significant differences between WBC and the CONT condition for TQR scores, and the variations between BASELINE and D+1 of [CK] levels, CMJ-F, and DOMS were reported.

Effects of match load covariates on sleep architecture and fatigue markers

Table 3 provides an overview of the potential relationships between match load covariates (HS and the number of collisions) and sleep architecture DVs. We observed a higher SWS proportion ($\beta = +1.1\%$, $p = 0.05$) and a lower light sleep proportion ($\beta = -1.2\%$, $p < 0.05$) for every 100-m increase in HS during the match. For every 10 supplementary collisions during the match, the SWS proportion decreased ($\beta = -1.9\%$, $p = 0.09$) and the light sleep proportion increased ($\beta = +2.9\%$, $p < 0.001$) (Table 3).

Relationships between the match load covariates (HS and number of collisions) and Δ [CK] and Δ DOMS were observed: for every 100-m increase in HS running distance, significantly higher Δ [CK] levels ($\beta = +78.0 \text{ IU}\cdot\text{L}^{-1}$, 95% CI: 35.6 to 120.3 $\text{IU}\cdot\text{L}^{-1}$, $p < 0.001$) and Δ DOMS ($\beta = +0.3 \text{ AU}$, 95% CI: 0.1 to 0.4 AU, $p < 0.001$) were observed. Additionally, for every 10 supplementary collisions during the match, a significantly higher Δ [CK] ($\beta = +112.6 \text{ IU}\cdot\text{L}^{-1}$, 95% CI: 33.7 to 191.5 $\text{IU}\cdot\text{L}^{-1}$, $p < 0.05$) was noted.

Discussion

The aim of the present study was to investigate the effects of a high-heat capacity mattress (MAT) and whole-body cryotherapy (WBC) intervention on sleep architecture and short-term recovery after an elite rugby union match. Main results showed: i) MAT use reduced mean nocturnal CBT, and had a positive impact on sleep architecture compared with the CONT condition; ii) WBC did not affect nocturnal CBT nor interfere with sleep architecture. High-speed running and the number of collisions experienced during the match had positive and negative influences on sleep architecture, respectively.

Effects of MAT on sleep, temperature and fatigue markers

The efficacy of MAT use on sleep after participation in a rugby union match was investigated. The upper layer of the MAT tested in the present study contained a high-heat capacity material, which remained cooler than the CONT mattress during the major part of the night (Figure 2). Decreasing CBT during sleep may enhance sleep quality (14,15). Our results showed significantly lower mean CBT (-0.135°C) and mattress surface (-2.736°C) temperatures during the MAT than the CONT condition. These findings are in agreement with studies using a similar high-heat capacity mattress (15,36), and suggest an enhanced conductive body heat loss with MAT. Moreover, a significant improvement in subjective thermal comfort was reported when sleeping on the MAT. Sleeping on MAT significantly reduced WASO (-10.5 min) and increased REM sleep proportion ($+2.8\%$) compared with the CONT condition. Krauchi *et al.* (15) used a similar cooling strategy and showed a higher SWS proportion (3.7%). These results suggest potential distinct positive effects for MAT use on sleep architecture, which could be explained by differences in the population studied and/or the experimental protocol. Moreover, the mean

SWS proportion in our study during the CONT condition was higher (27.7 % vs 23.1%) and the REM sleep proportion was lower (19.4 % vs 22.1%) compared with Krauchi *et al.* (15), which may reveal a ceiling or “maximum level of SWS in a single night” (37), beyond which even MAT use cannot induce further increases in SWS. Wake after sleep onset is an indicator of sleep quality and is usually higher for elite athletes than for non-athletic individuals (38,39). Our results showed significantly lower WASO for the MAT condition compared with the CONT condition (-10.5 min), suggesting better sleep continuity for MAT. The observed increase in REM sleep during MAT use may be related to the relatively low post-match REM proportion, coupled with increased sleep continuity. Despite positive effects on sleep architecture, MAT use did not affect the short-term neuromuscular and perceptual recovery compared with the CONT condition. Future studies are required to assess the interest of chronic use of MAT on sleep and recovery.

Effects of WBC on sleep, temperature and fatigue markers

The present study showed no difference for nocturnal CBT between WBC and CONT, which suggests that the slight decrease of CBT previously reported at 30 and 60min after the exposure (16,17) does not persist during the night. A previous study by Douzi *et al.* (18) reported lower actigraphic nocturnal activity after WBC preceded by night-time running exercise. Discrepancies between studies may be explained by the different pre-intervention exercise type and timing, and the methodology of sleep assessment. One of the rationale for using WBC after exercise is to reduce sympathetic activity and increase vagal tone after the exposure (21,40) and during subsequent sleep (18). In the present study, we examined the sympathovagal balance during the first sequence of SWS (29) for WBC and CONT conditions. Our results showed

increased parasympathetic activity during this period of SWS, as inferred by higher normalized HF power. This finding is consistent with previous studies that have assessed the effects of WBC on autonomic modulation (18,21). Nevertheless, as sleep architecture and short-term recovery were not affected after an acute WBC exposure, future studies should focus on its chronic effect on recovery, e.g. during an intensified training cycle.

Relationship between match load covariates and sleep architecture

Match loads in the present study were similar to those previously reported among elite U23 players (41). To our knowledge, this is the first study to evaluate sleep architecture for a full night with PSG following a rugby match. Our results indicated that the number of collisions experienced by players during the match impaired subsequent sleep architecture. This result may be explained by the accumulation of metabolic waste in the brain, including beta-amyloid, following recurrent head impacts; such accumulation may then disrupt the inhibition of the noradrenergic signaling in the locus coeruleus and negatively impact sleep architecture (42). Conversely, increased HS running distance during the match improved SWS proportion during the following night, which is in agreement with the hypothesis that higher diurnal activity is associated with increased SWS (43).

Practical applications

The present study highlighted the importance of sleep environment and the optimization of nocturnal heat transfer via MAT for improved post-match sleep among elite athletes. Moreover, WBC did not interfere with post-match sleep. High-speed running and the number of collisions experienced during the match had positive and negative influences on sleep architecture,

respectively. Consequently, practitioners should be aware of the importance of monitoring these activities and sleep after rugby union match to ensure adequate recovery.

Limitations

Due to the real-world sports setting examined in this study, controlling the match load for each player was not feasible, and the match load was found to be slightly higher for the WBC condition compared with the CONT condition. Linear mixed-effects models were, therefore, used to assess the difference between conditions with match load variables as covariates. Due to practical issues, fatigue states were monitored only the morning after the match. Future studies are required to assess the effects of the recovery strategies on the overall post-match recovery kinetics (e.g. up to 3 days after the game).

Acknowledgments

Anis Aloulou was supported by a scholarship from Bultex, a company specialized in bedding. The authors are very grateful to the players and the managers of the “Stade Français” rugby team for their efforts and involvement in this study. Special thanks to Alexis Peeters, Florent Carton, Jean-Robert Filliard and Alexandre Dubois for their support conducting these experiments.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as potential conflicts of interest. The results of the present study are presented clearly, honestly, and without fabrication, falsification, or inappropriate data manipulation and do not constitute any endorsement by the American College of Sports Medicine.

References

1. Duthie G, Pyne D, Hooper S. Applied physiology and game analysis of rugby union. *Sports medicine*. 2003; 33(13), 973-91.
2. McLellan CP, Lovell DI, Gass GC. Biochemical and endocrine responses to impact and collision during elite rugby league match play. *The Journal of Strength & Conditioning Research*. 2011; 25(6), 1553-62.
3. Tavares F, Smith T, Driller M. Fatigue and recovery in rugby: a review. *Sports Medicine*. 2017; 47(8), 1515-30.
4. McLean BD, Coutts AJ, Kelly V, et al. Neuromuscular, endocrine, and perceptual fatigue responses during different length between-match microcycles in professional rugby league players. *International journal of sports physiology and performance*. 2010; 5(3), 367-83.
5. Mujika I, Halson S, Burke LM, et al. An Integrated, Multifactorial Approach to Periodization for Optimal Performance in Individual and Team Sports. *Int J Sports Physiol Perform*. 2018; 13(5), 538-61.
6. Venter RE. Perceptions of team athletes on the importance of recovery modalities. *Eur J Sport Sci*. 2014; 14 Suppl 1, S69-76.
7. Shearer DA, Jones RM, Kilduff LP, et al. Effects of competition on the sleep patterns of elite rugby union players. *Eur J Sport Sci*. 2015; 15(8), 681-6.
8. Azboy O, Kaygisiz Z. Effects of sleep deprivation on cardiorespiratory functions of the runners and volleyball players during rest and exercise. *Acta physiologica Hungarica*. 2009; 96(1), 29.

9. Von Rosen P, Frohm A, Kottorp A, et al. Too little sleep and an unhealthy diet could increase the risk of sustaining a new injury in adolescent elite athletes. *Scandinavian journal of medicine & science in sports*. 2016; 27(11), 1364-71.
10. Caia J, Halson SL, Scott TJ, et al. Intra-individual variability in the sleep of senior and junior rugby league athletes during the competitive season. *Chronobiol Int*. 2017; 34(9), 1239-47.
11. Dunican IC, Eastwood PR. Sleep is an important factor when considering rugby union player load. *British journal of sports medicine*. 2017; 51(22), 1640-.
12. Kräuchi K, Deboer T. The interrelationship between sleep regulation and thermoregulation. *Front Biosci*. 2010; 15, 604-25.
13. Van Someren EJ. Mechanisms and functions of coupling between sleep and temperature rhythms. *Prog Brain Res*. 2006; 153, 309-24.
14. Togo F, Aizawa S, Arai J-i, et al. Influence on human sleep patterns of lowering and delaying the minimum core body temperature by slow changes in the thermal environment. *Sleep*. 2007; 30(6), 797-802.
15. Krauchi K, Fattori E, Giordano A, et al. Sleep on a high heat capacity mattress increases conductive body heat loss and slow wave sleep. *Physiol Behav*. 2018; 185, 23-30.
16. Costello JT, Culligan K, Selfe J, et al. Muscle, skin and core temperature after -110 degrees c cold air and 8 degrees c water treatment. *PLoS One*. 2012; 7(11), e48190.
17. Zalewski P, Bitner A, Słomko J, et al. Whole-body cryostimulation increases parasympathetic outflow and decreases core body temperature. *Journal of Thermal Biology*. 2014; 45, 75-80.

18. Douzi W, Dupuy O, Tanneau M, et al. 3-min whole body cryotherapy/cryostimulation after training in the evening improves sleep quality in physically active men. *Eur J Sport Sci.* 2018; 1-8.
19. Douzi W, Dupuy O, Theurot D, et al. Partial-body cryostimulation after training improves sleep quality in professional soccer players. *BMC Res Notes.* 2019; 12(1), 141.
20. Bonnet MH, Arand DL. Heart rate variability: sleep stage, time of night, and arousal influences. *Electroencephalography and Clinical Neurophysiology.* 1997; 102(5), 390-6.
21. Schaal K, Le Meur Y, Bieuzen F, et al. Effect of recovery mode on postexercise vagal reactivation in elite synchronized swimmers. *Appl Physiol Nutr Metab.* 2013; 38(2), 126-33.
22. Agnew JHW, Webb WB, Williams RL. The First Night Effect: An Eeg Study of Sleep. *Psychophysiology.* 1966; 2(3), 263-6.
23. Lacome M, Piscione J, Hager JP, et al. Fluctuations in running and skill-related performance in elite rugby union match-play. *Eur J Sport Sci.* 2017; 17(2), 132-43.
24. Lacome M, Peeters A, Mathieu B, et al. Can we use GPS for assessing sprinting performance in rugby sevens? A concurrent validity and between-device reliability study. *Biology of Sport.* 2019; 36(1), 25-9.
25. Jones MR, West DJ, Harrington BJ, et al. Match play performance characteristics that predict post-match creatine kinase responses in professional rugby union players. *BMC sports science, medicine and rehabilitation.* 2014; 6(1), 38.
26. Berry RB, Brooks R, Gamaldo CE, et al. AASM scoring manual updates for 2017 (Version 2.4). *J Clin Sleep Med.* 2017; 13(4), 665–6.

27. Bongers CCWG, Daanen HAM, Bogerd CP, et al. Validity, Reliability, and Inertia of Four Different Temperature Capsule Systems. *Medicine & Science in Sports & Exercise*. 2018; 50(1), 169-75.
28. Song MK, Ha JH, Ryu SH, et al. The effect of aging and severity of sleep apnea on heart rate variability indices in obstructive sleep apnea syndrome. *Psychiatry Investig*. 2012; 9(1), 65-72.
29. Brandenberger G, Buchheit M, Ehrhart J, et al. Is slow wave sleep an appropriate recording condition for heart rate variability analysis? *Auton Neurosci*. 2005; 121(1-2), 81-6.
30. Task Force. Heart rate variability: standards of measurements, physiological interpretation and clinical use. Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology. *Circulation*. 1996; 93(5), 1043-65.
31. Mathieu B, Peeters A, Piscione J, et al. Usefulness of typical tests of short-duration maximal effort used to assess players readiness to perform. *Science Performance and Science Reports*. 2017.
32. Hooper SL, Mackinnon LT. Monitoring overtraining in athletes. Recommendations. *Sports medicine (Auckland, NZ)*. 1995; 20(5), 321-7.
33. Quarrie KL, Raftery M, Blackie J, et al. Managing player load in professional rugby union: a review of current knowledge and practices. *British journal of sports medicine*. 2017; 51(5), 421-7.
34. Zhang Y, Zhao R. Overall thermal sensation, acceptability and comfort. *Building and Environment*. 2008; 43(1), 44-50.
35. Kenttä G, Hassmén P. Overtraining and recovery. A conceptual model. *Sports Medicine*. 1998; 26(1), 1-16.

36. Herberger S, Kräuchi K, Glos M, et al. Effects of sleep on a high-heat capacity mattress on sleep stages, EEG power spectra, cardiac interbeat intervals and body temperatures in healthy middle-aged men. *Sleep*. 2019, DOI : 10.1093/sleep/zsz271.
37. Taylor SR, Rogers GG, Driver HS. Effects of training volume on sleep, psychological, and selected physiological profiles of elite female swimmers. *Medicine and science in sports and exercise*. 1997; 29(5), 688-93.
38. Leeder J, Glaister M, Pizzoferro K, et al. Sleep duration and quality in elite athletes measured using wristwatch actigraphy. *J Sports Sci*. 2012; 30(6), 541-5.
39. Dunican IC, Walsh J, Higgins CC, et al. Prevalence of sleep disorders and sleep problems in an elite super rugby union team. *J Sports Sci*. 2019; 37(8), 950-7.
40. Westerlund T, Uusitalo A, Smolander J, et al. Heart rate variability in women exposed to very cold air (−110°C) during whole-body cryotherapy. *Journal of Thermal Biology*. 2006; 31(4), 342-6.
41. Lacomme M, Carling C, Hager JP, et al. Workload, Fatigue, and Muscle Damage in an Under-20 Rugby Union Team Over an Intensified International Tournament. *Int J Sports Physiol Perform*. 2018; 13(8), 1059-66.
42. Asken BM, Sullan MJ, Snyder AR, et al. Factors Influencing Clinical Correlates of Chronic Traumatic Encephalopathy (CTE): a Review. *Neuropsychol Rev*. 2016; 26(4), 340-63.
43. Davenne D. Sleep of athletes – problems and possible solutions. *Biological Rhythm Research*. 2009; 40(1), 45-52.

Figure 1. Experimental design

Figure 2. Changes in mattress surface temperature over 7 hours after bed time for CONT, MAT and WBC conditions (N=10). All data are presented as mean $\pm$ SD. Significantly different from CONT: * $p < 0.05$

Supplemental Digital Content

Supplemental Digital Content 1. Figure that illustrates the bedding conditions: (a) CONT and WBC; (b) MAT. pdf

Supplemental Digital Content 2. Table that illustrates the internal and external match load variation from CONT (reference) for WBC and MAT. pdf

Figure 1

Figure 2

Table 1: Outline of models' details (the random and fixed effects) used for each dependent variable depending on the research question.

Objective	Dependent variables	Fixed effect(s)	Random effect(s)
Assess the difference in match load and sleep time between CONT and the two experimental conditions	<ul style="list-style-type: none"> - <u>Match load</u>: sRPE, TD, HS, LS and number of collisions - <u>Sleep</u>: bed time, wake up time and time in bed 	The experimental condition (categorical predictor with CONT as reference)	The player
To evaluate the effect of MAT and WBC on sleep, HRV and fatigue markers with a comparable match load between conditions	<ul style="list-style-type: none"> - <u>Sleep</u>: total sleep time, sleep onset latency, WASO, sleep efficiency, light sleep, SWS, REM and NREM - <u>HRV</u>: RMSSD, HF, LF and LF/HF - <u>Fatigue markers</u>: Δ [CK], Δ DOMS and Δ CMJ-F 	The experimental condition (categorical predictor with CONT as reference) + the number of collisions + the HS distance	The player
To compare nocturnal CBT and mattress surface temperature between the experimental conditions and CONT	<ul style="list-style-type: none"> - CBT - Mattress surface temperature 	The experimental condition (categorical predictor with CONT as reference)	The player and the slope (15 time points with 30min intervals)

CONT: control condition, MAT: high heat capacity mattress condition, WBC: whole body cryotherapy condition, sRPE: Rating of perceived exertion of the session, TD: total distance, HS: high speed running distance, LS: low speed running distance, WASO: wake after sleep onset, SWS: slow wave sleep proportion, REM: rapid eye movement sleep proportion, RMSSD: Root Mean Square Standard deviation of R-R interval, HF: high frequency band, LF: low frequency band, LF/HF : the ratio of low by high frequency, Δ [CK]: the variation from PRE to D+1 of blood creatine kinase levels, Δ DOMS: the variation from PRE to D+1 of delayed onset muscle soreness levels, Δ CMJ-F: the variation from PRE to D+1 of the force developed during a counter movement jump test, CBT: core body temperature.

Table 2. Linear mixed-effects models evaluating the effect whole body cryotherapy (WBC) and high heat capacity mattress (MAT) on sleep quality, assessed using polysomnography, compared with CONT condition (reference).

	Total sleep time (min)			Sleep onset latency (SOL) (min)			Wake after sleep onset (WASO) (min)			Sleep Efficiency (SE) (%)		
	<i>Est. (SE)</i>	<i>95% CI</i>	<i>p</i>	<i>Est. (SE)</i>	<i>95% CI</i>	<i>p</i>	<i>Est. (SE)</i>	<i>95% CI</i>	<i>p</i>	<i>Est. (SE)</i>	<i>95% CI</i>	<i>p</i>
Fixed effects												
(Intercept)	438.9 (17.0)	403.2 – 474.5	<0.001	27.8 (10.6)	5.6 – 50.0	0.02	37.9 (6.2)	24.9 – 50.8	<0.001	86.6 (2.8)	80.8 – 92.3	<0.001
CONT	Reference			Reference			Reference			Reference		
WBC	-17.9 (13.4)	-46.0 – 10.3	0.20	-4.8 (8.8)	-23.2 – 13.6	0.59	-5.0 (3.9)	-13.1 – 3.2	0.22	-1.6 (1.9)	-5.6 – 2.5	0.43
MAT	-8.8 (12.3)	-34.5 – 16.9	0.48	-3.2 (8.1)	-20.1 – 13.8	0.70	-10.5 (3.5)	-17.8 – -3.3	0.01	2.9 (1.7)	-0.7 – 6.5	0.11
HS (km)	-4.5 (33.6)	-74.7 – 65.8	0.90	-6.4 (21.1)	-50.6 – 37.7	0.76	21.5 (11.1)	-1.8 – 44.8	0.07	-0.2 (5.2)	-11.1 – 10.8	0.98
Collisions (n)	0.3 (0.7)	-1.1 – 1.7	0.67	0.1 (0.4)	-0.8 – 0.9	0.85	-0.6 (0.2)	-1.1 – -0.1	0.03	0.0 (0.1)	-0.2 – 0.3	0.69
Random part												
N		18			18			18			18	
Observations		41			41			41			41	

Table 3. Linear mixed-effects models evaluating the effect of whole body cryotherapy (WBC) and high heat capacity mattress (MAT) on sleep architecture compared with a CONT condition (reference)

	Light sleep (%)			Slow wave sleep (%)			REM sleep (%)			NREM sleep (%)		
	<i>Est. (SE)</i>	<i>95% CI</i>	<i>p</i>	<i>Est. (SE)</i>	<i>95% CI</i>	<i>p</i>	<i>Est. (SE)</i>	<i>95% CI</i>	<i>p</i>	<i>Est. (SE)</i>	<i>95% CI</i>	<i>p</i>
<i>Fixed effects</i>												
(Intercept)	50.8 (2.6)	45.3 – 56.3	<0.001	27.7 (2.7)	22.0 – 33.3	<0.001	19.4 (1.9)	15.4 – 23.3	<0.001	80.6 (1.9)	76.6 – 84.6	<0.001
CONT	Reference			Reference			Reference			Reference		
WBC	-4.1 (2.6)	-9.6 – 1.4	0.13	2.2 (2.2)	-2.5 – 6.8	0.34	0.8 (1.3)	-1.9 – 3.4	0.56	-0.7 (1.3)	-3.4 – 1.9	0.56
MAT	-2.3 (2.5)	-7.4 – 2.9	0.37	-0.1 (2.0)	-4.4 – 4.1	0.95	2.8 (1.1)	0.4 – 5.2	0.02	-2.8 (1.1)	-5.2 – -0.4	0.02
HS (km)	-11.5 (5.3)	-22.5 – -0.5	0.04	11.1 (5.4)	-0.2 – 22.3	0.05	-1.0 (3.5)	-8.4 – 6.3	0.77	1.1 (3.5)	-6.3 – 8.5	0.76
Collisions (n)	0.3 (0.1)	0.1 – 0.5	0.01	-0.2 (0.1)	-0.4 – 0.0	0.09	0.1 (0.1)	-0.1 – 0.2	0.46	-0.1 (0.1)	-0.2 – 0.1	0.47
<i>Random part</i>												
N		18			18			18			18	
Observations		41			41			41			41	

Table 4. Linear mixed-effects models evaluating the effect of whole body cryotherapy (WBC) and high heat capacity mattress (MAT) on mean core body temperature (CBT) and mattress temperature compared with CONT condition (reference).

	Core body temperature (°C)			Mattress surface temperature (°C)		
	<i>Est. (SE)</i>	<i>95% CI</i>	<i>p</i>	<i>Est. (SE)</i>	<i>95% CI</i>	<i>p</i>
<i>Fixed effects</i>						
(Intercept)	36.378 (0.043)	36.294 – 36.462	<0.001	34.857 (0.226)	34.427 – 35.286	<0.001
CONT	Reference			Reference		
WBC	0.041 (0.031)	-0.019 – 0.101	0.18	-0.030 (0.306)	-0.627 – 0.567	0.92
MAT	-0.135 (0.038)	-0.211 – -0.060	<0.001	-2.736 (0.298)	-3.315 – -2.156	<0.001
<i>Random part</i>						
N		14			18	
Observations		330			583	

Supplemental Digital Content 1. Figure that illustrates the bedding conditions: (a) CONT and WBC; (b) MAT

Supplemental Digital Content 2. Table that illustrates the internal and external match load variation from CONT (reference) for WBC and MAT

	Total distance (m)			LS running distance (m)			HS running distance (m)			Collisions (n)			sRPE (U.A)		
	<i>Est. (SE)</i>	<i>95% CI</i>	<i>p</i>	<i>Est. (SE)</i>	<i>95% CI</i>	<i>p</i>	<i>Est. (SE)</i>	<i>95% CI</i>	<i>p</i>	<i>Est. (SE)</i>	<i>95% CI</i>	<i>p</i>	<i>Est. (SE)</i>	<i>95% CI</i>	<i>p</i>
<u>Fixed effects</u>															
(Intercept)	3643.0 (490.2)	2633.5 – 4652.5	<0.001	3416.8 (455.0)	2479.8 – 4353.9	<0.001	226.4 (54.5)	114.3 – 338.5	<0.001	16.2 (2.9)	10.2 – 22.2	<0.001	434.1 (65.4)	299.3 – 568.8	<0.001
CONT	Reference			Reference			Reference			Reference			Reference		
WBC	980.2 (468.9)	14.4 – 1946.1	0.05	899.0 (426.3)	21.1 – 1776.9	0.05	81.5 (57.8)	-37.4 – 200.5	0.17	4.4 (2.1)	-0.0 – 8.8	0.05	78.6 (59.4)	-43.9 – 201.0	0.20
MAT	304.4 (468.9)	-661.5 – 1270.2	0.52	279.2 (426.3)	-598.7 – 1157.1	0.52	25.0 (57.8)	-93.9 – 144.0	0.67	-0.6 (2.1)	-5.0 – 3.8	0.78	9.5 (59.4)	-112.9 – 131.9	0.87
<u>Random part</u>															
Ngrp		19			19			19			19			19	
Observation		46			46			46			46			46	