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### ► To cite this version:

Coralie Arc-Chagnaud, Olivier Dupuy, Manuela Garcia, Laurent Bosquet, Romain Bouzigon, et al.. Effects of repeated cryostimulation exposures on sleep and wellness in healthy young adults. *Cryobiology*, 2024, 117, pp.104948. 10.1016/j.cryobiol.2024.104948 . hal-04746493

**HAL Id: hal-04746493**

**<https://hal.science/hal-04746493v1>**

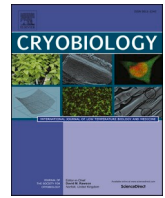
Submitted on 21 Oct 2024

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# Effects of repeated cryostimulation exposures on sleep and wellness in healthy young adults

Coralie Arc-Chagnaud<sup>a</sup>, Olivier Dupuy<sup>a,b</sup>, Manuela Garcia<sup>a</sup>, Laurent Bosquet<sup>a</sup>, Romain Bouzigon<sup>c,d</sup>, Robin Pla<sup>e</sup>, D-Day Consortium<sup>1</sup>, Benoit Dugué<sup>a,\*</sup>

<sup>a</sup> Université de Poitiers, Laboratoire MOVE (UR 20296), faculté des Sciences du Sport, Poitiers, France

<sup>b</sup> École de Kinésiologie et des Sciences de l'Activité Physique (EKSAP), Faculté de Médecine, Université de Montréal, Canada

<sup>c</sup> Société Inside the Athletes 3.0, Besançon, France

<sup>d</sup> Université de Franche-Comté, UFR STAPS Besançon, Laboratoire C3S (EA4660), Axe Sport Performance, Besançon, France

<sup>e</sup> Fédération Française de Natation, France

## ARTICLE INFO

### Keywords:

Anxiety  
Autonomic nervous system  
Cryotherapy  
Cryostimulation  
Heart rate variability  
Mood  
Sleep

## ABSTRACT

The aim of this study was to evaluate the effects of daily whole-body cryostimulation (WBC) sessions during 5 consecutive days on wellness and sleep parameters in healthy young men and women. Twenty healthy subjects (9 women; 11 men) aged  $23.1 \pm 2.6$  years old participated in this randomized protocol, with 5 consecutive days with (CRYO) or without WBC (CONT) exposure. Sleep was analyzed over the 5 nights in each condition. Sleep quality and quantity were assessed via actimetry, cerebral activity and questionnaires. Nocturnal heart rate variability (HRV) was also recorded and questionnaires were given to assess wellness and mood. Repeated WBC exposures had a beneficial impact on mood and anxiety. It also improved subjective sleep quality (scored from  $3.6 \pm 0.5$  pre to  $3.9 \pm 0.3$ ), especially in women. Also, repeated WBC sessions modulated sleep architecture by increasing slow wave sleep duration ( $+7.3 \pm 16.8$  min) during the nights without impacting other sleep parameters, nor nocturnal HRV. In conclusion, repeated WBC seems to be an effective strategy to improve slow wave sleep duration in healthy young subjects. The reported psychological improvements may better benefit women than men.

## 1. Introduction

The importance of sleep both for mental and physical health has widely been demonstrated. Health-related sleep characteristics involve appropriate schedules, sufficient duration, and especially good quality [14]. However, these elements necessary for restorative sleep are often inadequately met in the general population, impairing a wide range of body functions (neuro-endocrine, immune, cognitive and metabolic). Over the long term, sleep disruptions increase the risks of cardiovascular diseases, cancer, diabetes, and are associated with a higher risk of all-cause mortality [26]. Even among a healthy young population without severe sleep disorders (hypersomnia, insomnia, obstructive sleep apnea), poor sleep quality is frequently observed and can negatively affect mood, anxiety, and psychological well-being [1,35]. Moreover, overuse of social media by young people, as well as the

COVID-19 pandemic that they experienced twice, are two phenomena contributing to decrease sleep quality in this population.

To address these problems, strategies are required to optimize sleep quality. According to the scientific literature four types of strategies to improve sleep are available: "sleep hygiene", "nutritional", "medicinal", and "interventional" strategies. This latter category includes cold therapies, new mattress technologies and light regulation to improve sleep quality. Hypnotics can also be added as a tool to facilitate sleep. Among cold therapies, whole body cryostimulation (WBC) is known to lead to numerous beneficial effects particularly on wellness feelings, through the activation of analgesic processes and autonomous nervous system [6]. Our group previously demonstrated benefits after a cryostimulation session on perceived and measured sleep quality in healthy young athletes [12,13]. However, studies investigating how WBC may impact sleep parameters are scarce, and little is known about the effects of

\* Corresponding author. University of Poitiers, Laboratory MOVE (UR 20296), Faculty of Sport Sciences, 8 allée Jean Monnet, 86000, Poitiers, France.

E-mail address: [benoit.dugue@univ-poitiers.fr](mailto:benoit.dugue@univ-poitiers.fr) (B. Dugué).

<sup>1</sup> D-DAY consortium members. (See Appendix A)

repeated exposures. Schaal et al. [31] showed that daily WBC was able to preserve sleep quantity and prevent the increase of perceived fatigue in a context of intensified training period. Thus, WBC exposure may be considered as a potential strategy to improve sleep.

Regarding sleep analysis, numerous studies investigating sleep quality only rely on subjective indices and/or actimetry. These methods provide major parameters such as time spent in bed, total sleep time and estimated awake stages. However, these data are not based on electroencephalographic measurements. Currently, new tools exist to precisely analyze sleep structure via cerebral activity sensors, allowing the detection of the different sleep stages, such as the gold-standard polysomnography (PSG) but in a less burdensome way. Our study is one of the first to use such new validated wireless headband [3].

To briefly summarize sleep structure, it has to be remembered that total sleep over the course of the night is constituted of successive cycles lasting approximately 90 min. Each cycle is composed of 4 individual stages, characterized by distinct patterns of brain activity [25]. There are one rapid eye movement (REM) sleep stage and three non-REM sleep stages: *Stage 1* or *N1*, is the changeover from wakefulness to sleep (representing 5 % of the whole night), *Stage 2* or *N2* is light sleep (50 % of the whole night) and *Stage 3* or *N3* is the deeper sleep (20 % of the whole night) also called delta sleep or slow-wave-sleep (SWS). Deep sleep is critical to restorative sleep enabling tissue recovery and immune system strengthening, while REM-sleep (25 % of the whole night) is believed to be further involved in cognitive functions such as learning and creativity [9,27]. A good night sleep quality lies in the accurate duration of each of these stages, as well as a reduced occurrence of awakenings which may interrupt the proper progress of the night.

Therefore, the aim of this study was to evaluate the effects of daily WBC sessions during 5 consecutive days on sleep quality in healthy young men and women through the use of sleep questionnaires, actimetry and a new wireless headband device with cerebral activity sensors. We hypothesized that repeated cryostimulations will improve sleep quality and potentially enhance the mood state of the participants.

## 2. Materials and methods

### 2.1. Participants

Twenty healthy volunteers (9 women; 11 men) participated in this study (see Table 1 for their anthropometric characteristics). All participants were young ( $23 \pm 2.6$  years old) and physically active (4h and 48min  $\pm$  1h and 12min of weekly self-reported physical activity). They were unaccustomed to WBC and did not present any contraindication to cold exposure as recommended by the International Institute Refrigeration [6]. Participants were asked to refrain from stimuli that could affect autonomic nervous regulation of the cardiovascular system or the quality and quantity of sleep, such as caffeine or alcohol, during the course of the study. In practice, the volunteers were instructed to keep their regular sleeping habits (schedules for bedtime and wake-up time, number of hours spent in bed, napping habits, using their usual bed, level of light in the room). Sport practice was not allowed between the cryostimulation exposure and bedtime. All volunteers provided written consent before beginning the protocol, which was approved by the French committee for protection of persons (IDRCB: 2020-A03563-36).

**Table 1**  
Participant characteristics.

	Age (years)	Height (m)	Weight (kg)	BMI (kg/m <sup>2</sup> )	%FM
Overall (n = 20)	23.1 $\pm$ 2.6	1.72 <sup>a</sup> $\pm$ 8.9	64.4 <sup>a</sup> $\pm$ 10.2	21.5 $\pm$ 1.9	17.3 <sup>a</sup> $\pm$ 7.0
Women (n = 9)	22.7 $\pm$ 3.5	1.65 $\pm$ 3.5	56.0 $\pm$ 5.2	20.8 $\pm$ 2.1	22.4 $\pm$ 5.5
Men (n = 11)	23.4 $\pm$ 1.8	1.78 $\pm$ 7.6	71.2 $\pm$ 7.8	22 $\pm$ 1.8	13.2 $\pm$ 5.2

Data are presented as mean  $\pm$  SD.

BMI: body mass index; FM: body fat mass.

<sup>a</sup> Significant difference between men and women p < 0.05.

### 2.2. Experimental design

The experimental design is presented in Fig. 1.

This study was a randomized controlled cross-over protocol. It consisted of two experimental sessions, each one composed of 5 consecutive nights of sleep analysis, with (CRYO) or without (CONT) WBC exposure. Women participants took part to the two experimental sessions at the same phase of their menstrual cycle to minimize the effects of hormone fluctuations. During the 5 consecutive nights of the study, sleep was monitored using a cerebral activity measurement device as well as actimetry. Heart rate variability was also recorded during the sleep periods, and various perceptual measures were recorded during the entire protocol.

### 2.3. Whole body cryostimulation (WBC)

WBC sessions took place during 5 consecutive days, from monday to friday, at approximately 6:00 p.m. Each session consisted of a 5-min exposition in a cryochamber cooled at  $-90$  °C (Cryotera Center, Mecotec cryoair chamber, France). Participants entered in the cryo chamber wearing underwear, gloves, cap, a surgical mask, socks and slippers.

### 2.4. Measurements

#### 2.4.1. Perceptual measures

Before and after each experimental session, participants completed the Epworth Sleepiness Scale (ESS) and the Profile Of Mood States (POMS) questionnaire [24].

The perceived sleep quality was evaluated using the 6-item Spiegel Questionnaire [32] the morning following each night of the protocol.

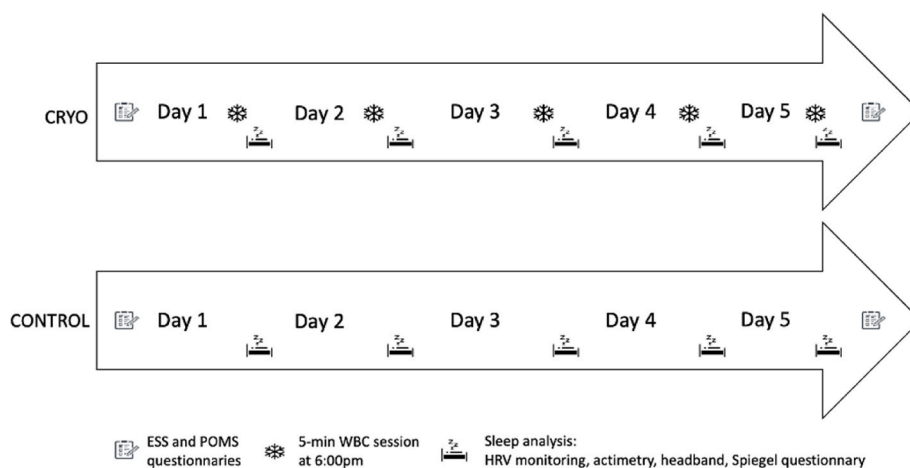
#### 2.4.2. Sleep analysis

Sleep was monitored during the 5 consecutive nights of the two experimental sessions (with and without daily WBC exposure). Sleep quality and quantity of the participants were measured using a wireless headband (Dreem 2, Paris, France), validated for sleep-related physiological signal recording and sleep stages detection [3]. This electronic device is equipped with cerebral activity sensors allowing the analysis of various sleep parameters: sleep onset latency, sleep stages (N1, N2, N3, REM) durations, wake episodes and sleep efficiency.

Actimetry was also used to quantify movements during the night. Participants were instructed to wear an actigraph (WGT3X-BT monitor, Pensacola, USA) on their non-dominant wrist during each night of the protocol. In addition to self-reported sleep schedules, this equipment allowed an estimation of the following sleep parameters: total sleep time, time in bed, sleep latency, wake episodes and sleep efficiency.

#### 2.4.3. Heart rate variability

Each night of the two experimental sessions, participants wore a Polar H10 heart rate sensor connected to a Polar watch (Polar V800, Kempele, Finland) to assess the autonomic regulation of the myocardium. R-R intervals were recorded during the night and then analyzed with Kubios HRV software (v. 3.3.1, 2019, Finland) using a “medium threshold” filter. Three types of analyses were performed: one for the signal recorded throughout the entire sleep period, another for the 4-h



**Fig. 1.** Experimental design with two randomized experimental conditions: one with an exposure to whole-body cryostimulation (CRYO) every evening and one without any exposure (CONTROL).

period of sleep starting 30 min after reported bedtime and a third for the first 10-min stationary segment in the first slow wave sleep (SWS) episode lasting at least 15 min [8]. The following parameters were retained: HR, heart rate; RR, interval beat-to-beat; PNS, index of parasympathetic nervous system activity and SNS, index of sympathetic nervous system activity; RMSSD, root-mean-square of standard deviation; SDNN, standard deviation of NN intervals; LF, low frequency; HF, high frequency.

### 2.5. Statistical analysis

Data were presented as mean  $\pm$  standard deviation (SD), and the significance level was set at  $p < 0.05$ . The Gaussian distribution of the data was assessed by the Shapiro-Wilk test. We used a two-way repeated measures ANOVA to compare the different nights in the two experimental conditions (Condition (CONT vs CRYO)  $\times$  Time (5 nights)), and a three-way ANOVA to add sex as a factor (Condition  $\times$  Time  $\times$  Sex). In the case of a non-Gaussian distribution, we used a Friedman ANOVA. Magnitude of the effects were determined using the Hedges'  $g$  effect size (ES) calculation. Statistical analysis was performed using Statistica software, and all graphs were created with GraphPad Prism5 software. The required sample size was calculated from a series of control data using G\*Power version 3.1, according to Beck [5]. Using an a priori repeated-measures design with a desired power (1-beta) set at 0.80, and an alpha risk of 0.05, twenty subjects represent a sufficient number to detect a significant difference.

**Table 2**

Sleep quality parameters.

Spiegel (score/30)		Night 1	Night 2	Night 3	Night 4	Night 5	Week
Overall	CONT	20.9 $\pm$ 3.6	21.4 $\pm$ 3.0	21.4 $\pm$ 2.9	21.9 $\pm$ 3.1	21.9 $\pm$ 3.4	21.5 $\pm$ 2.0
	CRYO	21.8 $\pm$ 2.1	22.1 $\pm$ 2.7	22.3 $\pm$ 2.8	21.9 $\pm$ 3.8	23.2 $\pm$ 3.9	22.2 $\pm$ 1.7
Women	CONT	19.6 $\pm$ 2.6	20.2 $\pm$ 3.1	19.8 $\pm$ 3.0	20.9 $\pm$ 3.8	20.2 $\pm$ 3.3	20.1 <sup>a</sup> $\pm$ 1.5
	CRYO	21.8 $\pm$ 2.2	22.7 $\pm$ 2.8	21.3 $\pm$ 3.1	19.7 $\pm$ 3.9	21.4 $\pm$ 4	21.4 <sup>a</sup> $\pm$ 1.8
Men	CONT	21.8 $\pm$ 4.0	22.3 $\pm$ 2.6	22.6 $\pm$ 2.2	22.7 $\pm$ 2.4	23.3 $\pm$ 3.0	22.5 $\pm$ 1.8
	CRYO	21.7 $\pm$ 2.1	21.6 $\pm$ 2.7	23.1 $\pm$ 2.3	23.7 $\pm$ 2.6	24.5 $\pm$ 3.3	22.9 $\pm$ 1.3
Perceived sleep quality (score/5)		Night 1	Night 2	Night 3	Night 4	Night 5	Week
Overall	CONT	3.4 $\pm$ 1.2	3.5 $\pm$ 0.9	3.5 $\pm$ 0.9	3.7 $\pm$ 0.6	3.8 $\pm$ 0.6	3.6 $\pm$ 0.5
	CRYO	3.9 $\pm$ 0.7	3.8 $\pm$ 0.7	3.9 $\pm$ 0.6	3.9 $\pm$ 0.7	4.1 $\pm$ 0.7	3.9 <sup>b</sup> $\pm$ 0.3
Women	CONT	3.5 $\pm$ 0.9	3.1 $\pm$ 1.1	3.0 $\pm$ 1.0	3.6 $\pm$ 0.7	3.7 $\pm$ 0.7	3.4 $\pm$ 0.4
	CRYO	4.0 $\pm$ 0.5	4.1 <sup>b</sup> $\pm$ 0.6	3.7 <sup>b</sup> $\pm$ 0.7	3.6 $\pm$ 0.7	3.9 $\pm$ 0.6	3.8 <sup>b</sup> $\pm$ 0.2
Men	CONT	3.3 $\pm$ 1.3	3.7 $\pm$ 0.6	3.9 $\pm$ 0.7	3.8 $\pm$ 0.4	3.9 $\pm$ 0.5	3.7 $\pm$ 0.5
	CRYO	3.8 $\pm$ 0.9	3.5 $\pm$ 0.7	4.0 $\pm$ 0.4	4.2 $\pm$ 0.6	4.2 $\pm$ 0.6	3.9 $\pm$ 0.4

Data are presented as mean  $\pm$  SD.

<sup>a</sup> Significant difference between men and women,  $p < 0.05$ .

<sup>b</sup> Significantly different than the CONT condition,  $p < 0.05$ .

## 3. Results

### 3.1. Sleep quality

Subjective sleep quality was measured through the administration of the Spiegel 6-items questionnaire (score/30), the mornings following the 5 analyzed nights. The global score includes the evaluation of various parameters: duration falling asleep, sleep quality, sleep duration, number of awakenings, dreams, and morning wellness.

The global score was higher in men than in women (interaction with sex factor) for the entire protocol, both for CONT and CRYO circumstances (ES = 0.38). Considering all participants, perceived sleep quality (score/5) was better during the CRYO compared to the CONT sessions (ES = 0.73). More specifically, women benefit more from WBC exposures in the nights 2 and 3 of the experimental sessions (interaction nights  $\times$  condition  $\times$  sex). The data are presented in Table 2.

The quantity of movements measured by actimetry was similar between the nights and was not modified by WBC exposures (Table 3).

### 3.2. Sleep structure

Sleep stages and various sleep quality indices were detected using cerebral activity recording during the nights. The data are presented in Table 4.

Various parameters did not change between CONT and CRYO conditions: sleep onset, sleep efficiency, sleep duration, REM, NREM,

**Table 3**  
Actimetry parameters.

Actimetry counts (u.a.)		Night 1	Night 2	Night 3	Night 4	Night 5	Week
Overall	CONT	108 ± 37	96 ± 35	111 ± 37	108 ± 36	124 ± 51	109 ± 32
	CRYO	92 ± 36	103 ± 43	101 ± 39	95 ± 40	119 ± 50	102 ± 35
Women	CONT	109 ± 23	106 ± 41	119 ± 36	113 ± 46	141 ± 53	118 ± 36
	CRYO	92 ± 35	113 ± 42	114 ± 31	104 ± 41.3	132 ± 47	111 ± 34
Men	CONT	107 ± 47	89 ± 27	104 ± 37	103 ± 26	108 ± 45	102 ± 29
	CRYO	91 ± 39	94 ± 45	90 ± 43	88 ± 40	107 ± 53	94 ± 35

Data are presented as mean ± SD.

**Table 4**  
Headband sleep parameters.

Sleep indices		Night 1	Night 2	Night 3	Night 4	Night 5	Week
<b>Sleep duration (min)</b>	CONT	444 ± 81	443 ± 57	426 ± 101	452 ± 56	410 ± 92	435 ± 58
	CRYO	441 ± 64	456 ± 66	452 ± 63	449 ± 51	470 ± 61	453 ± 41
<b>SOL (min)</b>	CONT	14.3 ± 6.7	13.8 ± 8.1	13.8 ± 9.6	12.7 ± 7.4	10 ± 7.1	12.9 ± 4.8
	CRYO	13.2 ± 5.9	13.3 ± 11.5	17.8 ± 15.5	12.3 ± 8.2	12.1 ± 7.4	13.8 ± 6.2
<b>Sleep efficiency (%)</b>	CONT	94.7 ± 3.4	94.7 ± 4.1	94.5 ± 4.4	94.2 ± 5	94.9 ± 3.7	94.6 ± 2.1
	CRYO	94.8 ± 2.4	95.7 ± 2.2	94.4 ± 4	95 ± 4.1	94.8 ± 3.6	95.0 ± 1.8
<b>Awakenings (n)</b>	CONT	4.2 ± 3.6	3.4 ± 2.7	3.3 ± 2.3	2.7 ± 2.8	3.3 ± 3	3.4 ± 2.3
	CRYO	3.8 ± 3.1	3.2 ± 2.6	2.8 ± 2.1	2.7 ± 2.5	3.1 ± 2.5	3.1 ± 2.2
<b>WASO (%)</b>	CONT	2.9 ± 3.5	1.8 ± 1.6	1.8 ± 1.9	1.4 ± 1.5	2.2 ± 2.5	2.0 ± 1.7
	CRYO	2.6 ± 2.3	1.8 ± 1.4	1.8 ± 1.3	1.0 ± 1.2	1.8 ± 1.9	1.8 ± 1.3
<b>REM (%)</b>	CONT	30.5 ± 7.0	29.3 ± 6.6	30.3 ± 5.2	31.8 ± 6.1	30.1 ± 4.4	30.4 ± 3.5
	CRYO	28.0 ± 7.2	29.0 ± 6.3	30.9 ± 5.5	31.0 ± 6.4	31.7 ± 4.7	30.1 ± 4.1
<b>SWS (cycle 1) duration (min)</b>	CONT	38.7 ± 11.4	41.5 ± 12	42.1 ± 17.5	42.5 ± 16	39.2 ± 12.4	40.8 ± 12
	CRYO	44.6 ± 13.3	44.9 ± 13.7	47.5 ± 21.1	42.8 ± 15.4	46.1 ± 13	45.2 <sup>a</sup> ± 11.8
<b>SWS (cycle 2) duration (min)</b>	CONT	33.6 ± 18.4	35.1 ± 16.3	32.5 ± 12.7	35.8 ± 16.1	34.4 ± 14.9	34.4 ± 11.2
	CRYO	37.9 ± 15	40 ± 15	37 ± 12.9	39.9 ± 13.8	36.9 ± 15.9	38.3 <sup>a</sup> ± 10.7
<b>SWS (1 &amp; 2) duration (min)</b>	CONT	71.8 ± 17.2	76.3 ± 23.2	74.2 ± 20.9	78 ± 21.4	73.2 ± 22.4	74.8 ± 18.6
	CRYO	82.1 ± 17.3	84.5 ± 20.7	82 ± 22.1	79.8 ± 23.6	82.5 ± 16.9	82.1 <sup>a</sup> ± 15.0

Data are presented as mean ± SD.

Abbreviations: SOL: sleep onset latency; WASO: wake after sleep onset; REM: rapid eye movement; SWS: slow wave sleep.

<sup>a</sup> Significantly different than the CONT condition,  $p < 0.05$ .

number and duration of awakenings. However, deep sleep (SWS) duration was greater in CRYO session compared to CONT (ES: 0.44). This is observable in the first and in the second cycles of the night, and the cumulated duration of deep sleep in the two first cycles (the first half of the night) is increased in the nights where WBC sessions were organized.

### 3.3. Heart rate variability (HRV)

Heart rate was recorded during the entire night and heart rate variability (HRV) was analyzed during the first 4h of sleep. This period is known to be more stable than the whole night to analyze autonomous myocardial activity [8]. This period is composed of the two first sleep cycles, where almost the totality of SWS occurs. PNS and SNS indexes relate to the activation of the parasympathetic and the sympathetic nervous system, respectively. Mean HR and RR are also correlated as RR-intervals refer to the duration between each heartbeat. We found differences between men and women for HR and RR parameters (Table 5). However, we did not observe any effect of CRYO exposures on other parameters (RMSSD, SDNN, LF/HF).

### 3.4. Mood

The POMS is a questionnaire of 65 self-report items using the 5-points Likert scale, evaluating various mood states including anxiety, angry, tiredness, and depression. A low global score indicates a better mood profile. This questionnaire was administered before (pre) and after (post) the two experimental sessions, to evaluate the effects of 5 consecutive days of WBC exposures on mood. Considering all participants, global score was higher before the CRYO session than after (7 vs 1), indicating an improved mood, but no difference was observed in the CONT condition (6 vs 4). Moreover, this positive effect of WBC seems to

benefit to a greater extent to women, (12 vs 1), who also exhibited a significantly lower anxiety level after the CRYO sessions (43 vs 38). The ANOVA revealed an interaction time x condition x sex. These results are presented in Fig. 2. The other components of the POMS did not significantly change.

## 4. Discussion

In this study, we investigated whether a series of 5-min day time WBC exposures during 5 consecutive days may affect the sleep quality and quantity during the following nights in subjectively healthy young adults. Sleep quality and quantity were assessed via actimetry, cerebral activity and questionnaires. Nocturnal heart rate variability (HRV) was also recorded and questionnaires were given to assess wellness and mood.

Globally, we observed an improvement in subjective sleep quality, especially in women, accompanied with a modulation in sleep architecture, presenting an increased duration of the slow wave sleep stage. Also, a benefit in mood and anxiety was noted in our participants.

In the following, we will discuss our results concerning the data obtained on sleep quality and structure, nocturnal HRV parameters, and changes in mood and anxiety.

### 4.1. Sleep quality and structure

Throughout the experimental sessions, including 5 consecutive nights with and without previous WBC exposure, sleep quality and quantity were subjectively and objectively recorded. Perceived sleep quality was higher in men than women throughout the protocol, without significant effect of WBC intervention. However, in women participants, whereas sleep quality score was moderate all along the CONT week, we observed an improvement in the second and third nights of the protocol

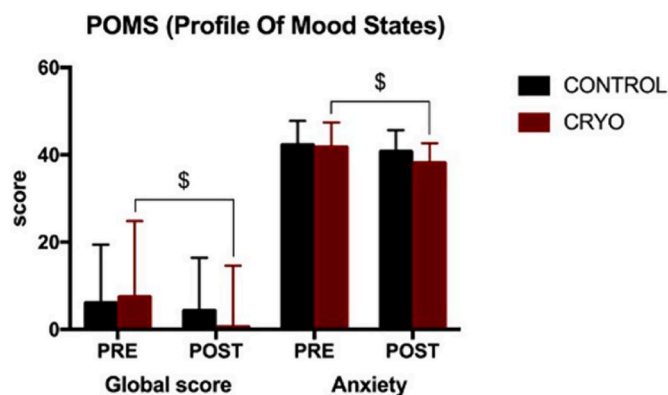
**Table 5**  
Heart rate variability parameters.

indices of HRV		Night 1	Night 2	Night 3	Night 4	Night 5	Week
HR (bpm)	CONT (W)	60 ± 9	60 ± 9	67 ± 14	64 ± 9	68 ± 12	64 <sup>a</sup> ± 9
	CONT (M)	54 ± 8	53 ± 6	55 ± 6	55 ± 7	55 ± 7	54 ± 6
	CRYO (W)	64 ± 9	62 ± 9	62 ± 8	59 ± 9	65 ± 10	62 <sup>a</sup> ± 9
	CRYO (M)	53 ± 6	54 ± 6	55 ± 6	53 ± 7	52 ± 3	53 ± 6
RR (ms)	CONT (W)	1020 ± 155	1026 ± 163	935 ± 205	964 ± 157	910 ± 160	971 <sup>a</sup> ± 160
	CONT (M)	1121 ± 127	1137 ± 113	1103 ± 111	1111 ± 125	1116 ± 120	1118 ± 109
	CRYO (W)	956 ± 146	990 ± 145	990 ± 144	1038 ± 180	952 ± 168	985 <sup>a</sup> ± 154
	CRYO (M)	1134 ± 119	1122 ± 124	1112 ± 123	1151 ± 135	1166 ± 73	1137 ± 113
PNS (u.a.)	CONT (W)	0.9 ± 1.0	0.9 ± 1.0	0.2 ± 1.6	0.8 ± 1.1	0.2 ± 1.1	0.6 <sup>a</sup> ± 1.1
	CONT (M)	1.7 ± 1.3	1.8 ± 1.3	1.3 ± 0.9	1.4 ± 1.2	1.7 ± 1.4	1.6 ± 1.3
	CRYO (W)	0.4 ± 1.0	0.7 ± 1.0	0.7 ± 1.0	1.0 ± 1.2	0.4 ± 1.2	0.6 ± 1.0
	CRYO (M)	1.8 ± 1.3	1.5 ± 1.0	1.6 ± 1.3	1.9 ± 1.6	1.7 ± 0.6	1.7 ± 1.3
SNS (u.a.)	CONT (W)	-1.1 ± 0.7	-1.0 ± 0.7	-0.4 ± 1.3	-0.8 ± 0.8	-0.3 ± 1.2	-0.7 <sup>a</sup> ± 0.9
	CONT (M)	-1.5 ± 0.8	-1.6 ± 0.6	-1.4 ± 0.6	-1.6 ± 0.7	-1.7 ± 0.9	-1.5 ± 0.7
	CRYO (W)	-0.6 ± 0.9	-0.8 ± 0.7	-0.9 ± 0.7	-1.2 ± 0.7	-0.6 ± 0.9	-0.8 <sup>a</sup> ± 0.7
	CRYO (M)	-1.5 ± 0.6	-1.5 ± 0.6	-1.6 ± 0.6	-1.6 ± 0.8	-1.7 ± 0.3	-1.5 ± 0.6
RMSSD (ms)	CONT (W)	59 ± 17	55 ± 14	55 ± 24	65 ± 19	58 ± 14	58 ± 16
	CONT (M)	75 ± 28	66 ± 29	63 ± 19	63 ± 25	72 ± 33	70 ± 29
	CRYO (W)	52 ± 14	57 ± 18	57 ± 14	62 ± 18	54 ± 23	56 ± 18
	CRYO (M)	74 ± 30	66 ± 21	69 ± 24	71 ± 37	68 ± 16	70 ± 28
SDNN(ms)	CONT (W)	57 ± 10	55 ± 7	55 ± 16	67 ± 26	56 ± 13	58 ± 13
	CONT (M)	70 ± 21	71 ± 21	64 ± 15	64 ± 19	68 ± 24	67 ± 21
	CRYO (W)	52 ± 12	57 ± 16	55 ± 12	60 ± 14	57 ± 21	56 ± 15
	CRYO (M)	68 ± 20	63 ± 16	67 ± 17	67 ± 26	66 ± 11	66 ± 19
LF (ms <sup>2</sup> )	CONT (W)	1527 ± 632	1480 ± 571	1547 ± 749	2432 ± 2266	1457 ± 642	1689 ± 930
	CONT (M)	2470 ± 1500	2615 ± 1581	2154 ± 989	2138 ± 1027	2523 ± 1687	2380 ± 1513
	CRYO (W)	1268 ± 662	1770 ± 1239	1598 ± 930	1806 ± 927	1881 ± 1518	1664 ± 1091
	CRYO (M)	2266 ± 1086	2071 ± 956	2296 ± 960	2399 ± 1805	2102 ± 790	2227 ± 1172
HF (ms <sup>2</sup> )	CONT (W)	1239 ± 710	1034 ± 331	1062 ± 578	1645 ± 960	1309 ± 498	1258 ± 479
	CONT (M)	1913 ± 1329	1882 ± 1382	1338 ± 755	1420 ± 1281	1803 ± 1652	1671 ± 1447
	CRYO (W)	1106 ± 488	1144 ± 552	1103 ± 346	1248 ± 445	1149 ± 784	1150 ± 497
	CRYO (M)	1867 ± 1468	1427 ± 889	1581 ± 1023	1773 ± 1783	1529 ± 650	1616 ± 1310
LF/HF (u.a.)	CONT (W)	1.50 ± 0.79	1.56 ± 0.67	1.55 ± 0.52	1.34 ± 0.57	1.16 ± 0.42	1.42 ± 0.56
	CONT (M)	1.34 ± 0.3	1.52 ± 0.50	1.73 ± 0.60	1.90 ± 1.10	1.64 ± 0.6	1.63 ± 0.50
	CRYO (W)	1.24 ± 0.48	1.55 ± 0.54	1.46 ± 0.59	1.44 ± 0.43	1.63 ± 0.45	1.46 ± 0.46
	CRYO (M)	1.50 ± 0.80	1.55 ± 0.40	1.69 ± 0.70	1.65 ± 0.50	1.58 ± 0.50	1.59 ± 0.50

Data are presented as mean ± SD.

Abbreviations: HR: heart rate; RR: interval beat-to-beat; PNS: index of parasympathetic nervous system activity (scored from -5 to +5); SNS: index of sympathetic nervous system activity (scored from -5 to +5); RMSSD: root-mean-square of standard deviation; SDNN: standard deviation of NN intervals; LF: low frequency; HF: high frequency.

<sup>a</sup> Significant difference between men and women for the same condition,  $p < 0.05$ .



**Fig. 2.** Profile of Mood states global score and Anxiety score, before (pre) and and after (post) experimental protocol.

due to WBC. Although there was no impact on every night of the week, WBC exposure may have punctually contributed to perceptive sleep improvement. Two studies of our group previously demonstrated sleep quality improvement the night following cryostimulation exposure [12, 13]. However, the context was different as the cryo sessions took place after an intense training, a stimulus propitious to sleep degradation. In the present study, our healthy non-athletic population did not perform intense training prior WBC sessions. Moreover, participants did not present sleep disorders, and may already have a good sleep quality.

Therefore, the use of WBC in this context did not induce any global additional benefit. Nevertheless, when studying sleep architecture using specific headbands we were able to detect changes in the duration of some sleep stages. The first half of the night (when the first two cycles of sleep occur, ~3–4h) is characterized by an elevated proportion of slow wave sleep (SWS) [2]. Our results revealed an increase of SWS duration during the nights of the CRYO session. This beneficial effect of cold exposure has recently been observed in the study of Chauvineau et al. [10] as they found that whole-body cold-water immersion enhanced SWS proportion the night following the exposure. Nevertheless, our study is the first to demonstrate the beneficial effects of one week of daily WBC on sleep stages modulation.

This is a finding of major importance for a wide range of populations: from the high-level athletes to patients suffering from various pathologies and sleep disorders, as well as the aging population. Indeed, SWS is the most restorative part of sleep and is critical for tissue growth and repair, immunity, and memory consolidation [11,19].

Therefore, athletes should better benefit from growth hormone release during a longer SWS to optimize muscle protein synthesis and tissue recovery. Patients with sleep disorders, such as primary insomnia, may also benefit from an increase quantity of SWS. Moreover, since aging is associated with a decreased proportion of SWS, older adults could also benefit from an extended SWS [4]. For such individuals, the memory decline they often experience is frequently associated with a reduced amount of SWS [4]. Deep sleep stage is strongly involved in cognitive functions, and its extension may help prevent the cognitive

decline suffered by patients with Alzheimer's disease [36]. Nevertheless, the minimal duration change in the deep sleep duration for clinical relevance is globally unknown and may vary depending on the specific condition and outcomes being measured. It is clear that the changes we observed were very modest. However, even modest changes in deep sleep duration can be clinically relevant, affecting cognitive function, physical health, emotional regulation, and overall well-being. It has also to be remembered that our volunteers did not experience sleep disturbances and it could be speculated that a WBC intervention would have clear clinical effect with subjects with affected sleep [23,34].

#### 4.2. Nocturnal HRV parameters

Repeated exposures to WBC during 5 consecutive days did not impact HRV parameters during the following nights, compared with the CONT condition. However, gender differences were noted, regardless of the experimental session. Men exhibited lower HR and therefore higher RR-interval duration than women. They also presented an apparently higher parasympathetic nocturnal activity (higher PNS and lower SNS indexes) although no significant differences were discernible in the frequency-domain parameters (LF/HF).

Regarding the impact of cryostimulation, Louis et al. [20,21] demonstrated in previous studies that resting vagal-HRV indices were increased after cold exposure (partial-body cryostimulation (PBC) and WBC), and repeated WBC exposures for 5 consecutive days stimulated the autonomous nervous system with a predominance of parasympathetic tone activation. However, in these studies HRV recording occurred immediately after the cryo session. It may explain why we did not observe any effects of WBC on HRV parameters in our study, as it was a nocturnal analysis beginning a few hours after the cryo exposure. If WBC had taken place within the hour preceding bedtime, we may have observed variations in parasympathetic activity.

Regarding inter-sex comparisons, substantial gender differences in the autonomic control of the myocardium are well-documented, with higher basal HR values in women than men [28]. Despite a greater HR, a recent meta-analysis demonstrated that women are characterized by a relative dominance of vagal activity [17]. A study also demonstrated that frequency domain ratio LF/HF was lower in women than men, indicating increased parasympathetic activation in the female population [18]. Unfortunately, only few studies have focused on gender comparison and nocturnal HRV parameters, and it seems that this study is the first to reveal such inter-sex differences in the autonomic modulation of the heart during the night. However, beyond the gender effect, hormonal variations related to the phases of the female menstrual cycle must be considered in the autonomous regulation of myocardium. In this study, we have to admit that we did not take in consideration a menstrual phase in particular, neither the use - or not - of hormonal contraceptive.

#### 4.3. Improved mood and reduced anxiety

Repeated WBC exposures had a positive effect on psychological wellness, reducing anxiety and the total mood disturbance score after the CRYO experimental sessions. These benefits were more sustained in women than in men. Such effects have already been demonstrated in patients suffering various pathologies, including depressive and anxiety disorders; spinal pain syndromes or peripheral joint disease [29,30,33]. Among athletes, winter swimmers undergoing cold exposure have shown improvements in mood and memory [15,16].

It is therefore noteworthy to observe beneficial effects of WBC even in a healthy young population without psychological or physical disturbances.

The underlying mechanisms responsible for mood improvement following repeated WBC exposure are uncertain but could be related to the increases in SWS stage and PNS activity, combined with a direct effect of the repeated WBC to modulate the level of inflammation and antioxidative status [7,22]. Therefore, the described changes might also

influence the release of specific hormones responsible for feelings of well-being. Such positive effects on psychological wellness observed in our study appeared to benefit women more than men.

## 5. Conclusion

Repeated WBC modulated sleep architecture by increasing slow wave sleep duration during the night and had a beneficial impact on mood and anxiety. It also improved subjective sleep quality, especially in young women. Therefore, the regular use of WBC could be beneficial for individuals experiencing sleep and well-being issues and could be applied in various situations. The significantly higher benefits observed in young women are noteworthy, and further studies should investigate these inter-sex differences.

## Funding

This work was partly funded by the French government through the third Future Investment Program (PIA3) of the National Agency of Research (ANR), with the reference ANR-19-STHP-0001.

## CRediT authorship contribution statement

**Coralie Arc-Chagnaud:** Writing – review & editing, Writing – original draft, Visualization, Methodology, Investigation, Formal analysis, Data curation. **Olivier Dupuy:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Project administration, Methodology, Formal analysis, Data curation, Conceptualization. **Manuela Garcia:** Writing – review & editing, Investigation, Formal analysis, Data curation. **Laurent Bosquet:** Writing – review & editing, Writing – original draft, Validation, Funding acquisition, Conceptualization. **Romain Bouzigon:** Writing – review & editing, Validation, Supervision, Resources, Methodology, Investigation. **Robin Pla:** Writing – review & editing, Resources, Project administration. **Benoit Dugué:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Project administration, Methodology, Funding acquisition, Formal analysis, Conceptualization.

## Declaration of competing interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

## Acknowledgments

The authors want to thank the volunteers for their valuable participation in the project and Alejandra et David Crol from Cryotera Poitiers, for their warm welcome in their cold room.

## Appendix A

### \*D-DAY consortium members

- Laboratoire MOVE (UR 20296), UFR STAPS Poitiers, Université de Poitiers, France: Arc-Chagnaud Coralie, Bosquet Laurent, Bretonneau Quentin, Delpech Nathalie, Dugué Benoit, Dupuy Olivier, Enéa Carina, Pichon Aurélien, Tanneau Maxence et Theurot Dimitri.

- Institut Pprime, UPR 3346, CNRS – Université de Poitiers – ISAE-ENSMA, France: Couvertier Marien, Decatoire Arnaud, Monnet Tony et Samson Mathias.

- Institut de recherche biomédicale des armées (IRBA), Brétigny-sur-Orge, France: Sauvet Fabien.

- Unité Vigilance, Fatigue, Sommeil et Santé Publique (VIFASOM, EA7330), Université de Paris, Hôtel Dieu, Paris, France: Sauvet Fabien.

- Laboratoire SEP (EA 7370), INSEP, France: Morales-Artacho Antonio, Nédelec Mathieu, Pasquier Florane, Poignard Mathilde et Ruffault Alexis.
- Fédération Française de Natation, Clichy, France: Pla Robin.
- Laboratoire Culture, Sport, Santé, Société (C3S, EA4660), UFR STAPS Besançon, Université de Franche-Comté, France: Bouzigon Romain.
- Société Aurore Concept, Noisiel, France: Bouzigon Romain.
- Société Inside the Athletes 3.0, Besançon, France: Bouzigon Romain.

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