Whole-body cryotherapy (−110 °C) following high-intensity intermittent exercise does not alter hormonal, inflammatory or muscle damage biomarkers in trained males

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ABSTRACT

Purpose: This study examined the acute effects of a single session of Whole-body Cryotherapy (WBC) following severe intermittent running exercise on biomarkers of inflammation, muscle damage and stress.
Methods: Endurance-trained males (n = 11) were tested twice using a within-participant, balanced cross-over design that consisted of 5 × 5 min of high-intensity running (HIR) followed by either 3 min of WBC at −110 °C or a passive control condition (CON). Before the HIR and after 60 min of recovery a ramp-test was completed. At seven time points up to 24 hrs post exercise venous blood samples were analyzed for serum levels of interleukin 6 (IL-6), interleukin 10 (IL-10), c-reactive protein (CRP), soluble intercellular adhesion molecule-1 (sICAM-1), myoglobin, cortisol, and testosterone.
Results: HIR induced significant increases in all biomarkers except sICAM-1 in both recovery conditions, respectively. Compared to the CON condition WBC did not attenuate exercise- induced changes in IL-6, IL-10, sICAM-1, myoglobin, cortisol, testosterone or their ratio. Increased levels of cortisol following exercise were negatively correlated with subsequent running performance in both conditions (WBC: r = −0.61, p = 0.04; CON: r = −0.64, p = 0.04).
Conclusion: The results of this study suggest that the postulated physiological mechanisms by which WBC is proposed to improve recovery, i.e. reductions in inflammation and muscle damage, may not be accurate.

1. Introduction

Whole-body Cryotherapy (WBC) or cryostimulation is a popular and widely used recovery modality in sport and exercise medicine following intensive training and competition. It consists of brief exposures (typically 2–4 min) to very cold air (−110 °C and below) in cryogenic chambers with individuals minimally dressed [1,2]. Originally utilized in a clinical setting for treating symptoms of various rheumatic diseases, WBC is purported to reduce pain, edema, and inflammation [3]. Therefore, WBC has become very popular with both recreational and elite athletes. To date, there is a limited body of evidence regarding its efficacy and empirical data detailing the potential mechanism(s) by which this treatment could be effective is sparse [1].

Several authors have speculated that reductions in inflammation is the primary mechanism by which WBC after strenuous exercise is believed to be effective [1,2,5]. It is well established that intense exercise, especially if the athlete is unaccustomed to such modalities and/or the exercise is eccentric [6], leads to sarcomere disruptions and cell membrane damage [7]. Following cell damage leukocytes are mobilized to the injured tissue by soluble intercellular adhesion molecule-1 (sICAM-1), producing reactive oxygen species and pro-inflammatory cytokines in the injured tissue, resulting in intramuscular degradation and an amplification of muscle damage [8]. This mechanism is defined as secondary muscle damage [8] and may also be related to the increased levels of pro- and anti-inflammatory cytokines observed following exercise [9]. It is believed that exercise- induced inflammation,

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e.g. indicated by augmented Interleukin (IL)-6 impairs athletic performance [10,11]. As WBC reduces skin-, muscle- and core-temperature [12], leading to vasoconstriction and reduced blood vessel permeability to immune cells, it is plausible that fewer leukocytes are mobilized to the injured tissue, leading to a reduced pro-inflammatory response and consequently less secondary muscle damage [13]. However, the recent debate regarding the effects of WBC on modulating the expression of sICAM-1 is still inconsistent and conflicting [5,14], possibly due to the timing of the intervention post exercise [14]. To the best of our knowledge, the effect of WBC on sICAM-1 following intense exercise has not been compared to a control intervention.

Several studies have investigated the physical, psychological, and physiological effects of WBC following exercise (for a review see [1,2,15]) Many [13,16–19], but not all [20,21] have reported that WBC might facilitate the recovery process after exercise. Actually, for most biomarkers (e.g. pro- and anti-inflammatory cytokines, creatinkinase (CK)) contradictory findings have been reported in the literature. These conflicting results may be due to large differences in methodology, such as exercise duration and intensity, numbers of exercise bouts and WBC sessions and time points of biomarker assessment. Thus, practical applications and recommendations for athletes and their coaches are often difficult to conclude. As daily high-quality performance and multiple competitions per week are required in many sports, athletes often require interventions to enhance recovery within hours or a few days. To date, only a few studies [13,16,19–23] investigated the effects of a single WBC-treatment on acute recovery after high-intensity exercise. In nine well-trained runners, performing a simulated trail run peak torques of the knee extensors along with perceived sensation of pain and tiredness was not significantly different in the WBC recovery condition compared to passive rest [13]. With regard to biomarkers, no strong conclusions can be made as alterations in some biomarkers (C-reactive protein (CRP), Interleukin (IL)-1 β, IL-1 ra) indicated reduced inflammation, while others (IL-6, IL-10, tumor necrosis factor (TNF)-α) remained unchanged compared to passive recovery [19]. WBC applied after repeated sprint exercise in professional soccer players induced a greater salivary testosterone response compared to a control condition, but the changes did not result in improvements in jump performance, blood lactate, CK concentrations or perceived recovery [20]. Other studies demonstrated no beneficial effects of WBC on muscle force recovery [21] or mixed results regarding perceived pain sensation and maximal physical performance after hamstring damaging exercise [23]. As the majority of previous studies focused on performance parameters it remains unclear whether one session in combination with high-intensity exercise alters biomarkers of hormonal status, inflammation and muscle damage.

Accordingly, the aim of the present study was to investigate the acute effects of a single WBC-session during intermittent exercise on biomarkers associated with exercise-induced inflammation, muscle damage and stress. We hypothesized that a single exposure of WBC would reduce markers of inflammation and muscle damage, and alter the cortisol-testosterone ratio following high-intensity intermittent exercise

2. Methods

2.1. Participants

A convenient sample size of 11 healthy, endurance-trained, male athletes participated in the study (mean ± SD age: 25.9 ± 2.1 yrs; height: 183.4 ± 3.4 cm; mass: 76.3 ± 6.6 kg; body mass index: 22.7 ± 1.7 kg m^{-2}; body fat: 10.7 ± 1.9%; lean body mass: 68.1 ± 5.9 kg; peak oxygen uptake: 59.3 ± 5.3 mL kg^{-1} min^{-1}; performance level 3 and 4, according to De Pauw et al. [24]).. Inclusion criteria involved a history in endurance training (running) of at least 8 years with 3 or more training sessions per week as well as being familiar with high-intensity interval training, i.e. short intervals of 2–8 min at 90–95% of maximal heart rate, separated by equally short periods of recovery [25]. Athletes were excluded if they had any contraindications to WBC, such as claustrophobia, cold hypersensitivity or abrasion injuries during medical checkup as described elsewhere [2]. Participants were instructed to refrain from consuming alcohol and caffeine 24 hrs prior to the tests and to maintain their normal diet during the testing period. Intensive exercise was not permitted for up to 48 hrs prior to the two test sessions and the individual training was identical in the preceding week, respectively. Additional recovery methods, including the use of non-steroidal anti-inflammatory drugs, were not permitted. After demonstration and briefing about the potential risks, all participants provided their written informed consent. The study was conducted in accordance with the Declaration of Helsinki and approved by the ethical committee of the German Sports University Cologne.

2.2. Experimental design

This study was part of a larger project investigating effects of WBC on performance variables, and the experimental design has been described in detail elsewhere [16]. Briefly, a within-participant, balanced cross-over design (with 7-days washout) was employed. Before participation, the athletes underwent a medical checkup and familiarization with WBC. Furthermore they carried out an incremental step test (starting velocity: 2.4 m s^{-1}; increase 0.4 m s^{-1} every 5 min; treadmill gradient 1%) on a treadmill (Woodway ELG 90/200 Sport, Lörrach, Germany) until individual exhaustion to determine VO$_2$max (MetaLyzer 3b, Cortex, Leipzig, Germany) and the individual intensities for High-intensity-running (HIR) during the two main tests. The experimental protocol of these tests is presented in Fig. 1.

All athletes were tested at the same time of day on each occasion and were randomly assigned to start with either the WBC or control intervention (CON) using research randomizer (version 4.0, retrieved from http://www.randomizer.org/). The participants arrived at the laboratory at least one hour prior to the test for acclimatization. At first, an incremental exercise protocol to individual exhaustion was performed (Ramp 1). The protocol consisted of 3 submaximal 3-min steps at 3.2, 3.6 and 4.0 m s^{-1} with a treadmill gradient of 1% and 30 s of rest after each step. Thereafter velocity was increased to 4.4 m s^{-1} and remained constant while the treadmill gradient was increased by 0.5% every 30 s until exhaustion. After 5 min of recovery, HIR was carried out, consisting of 5 × 5 min at 90% of maximum velocity (V$_{max}$)
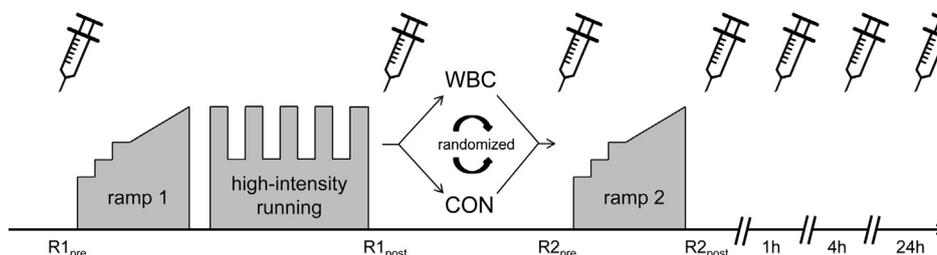

Fig. 1. Schematic presentation of the experimental randomized cross over design. denotes blood sampling at seven time points, before and after ramp test 1 (R1$_{pre}$; R1$_{post}$) and 2 (R2$_{pre}$; R2$_{post}$) and 1 (1h), 4 (4h) and 24 (24 h) hrs after the intervention for whole-body cryotherapy (WBC) and control (CON) intervention, respectively.

Table 1

Serum concentrations (mean ± SD) of cortisol, testosterone, ratio (T-C-ratio), C-reactive Protein (CRP), myoglobin, soluble intercellular adhesion molecule-1 (sICAM-1), Interleukin 6 (IL-6), Interleukin 10 (IL-10) and white blood cell count at seven time points during WBC and CON intervention.

Biomarker	Intervention	$R1_{pre}$	$R1_{post}$	$R2_{pre}$	$R2_{post}$	1 h	4 h	24 h	[time]	p-value [int.]	[time * int.]
Cortisol [ng mL^{-1}]	CON	167.0 ± 29.8	224.0 ± 58.2*	183.4 ± 50.2	173.1 ± 28.8	130.5 ± 14.6*	62.8 ± 29.1*	89.0 ± 26.2*	0.01	0.53	0.93
	WBC	158.7 ± 26.7	217.9 ± 48.6*	191.0 ± 52.2*	172.3 ± 27.3	115.6 ± 29.3*	48.7 ± 18.4*	87.1 ± 20.3*			
Testosterone [ng mL^{-1}]	CON	5.4 ± 1.9	7.0 ± 2.1*	5.0 ± 1.2	5.0 ± 1.5	4.2 ± 1.4*	3.8 ± 1.3*	5.4 ± 2.1	0.01	0.67	0.81
	WBC	5.2 ± 1.4	6.4 ± 2.2*	4.7 ± 1.6	5.3 ± 1.7	4.0 ± 1.1*	3.2 ± 1.2*	5.3 ± 2.0			
C-T-ratio [AU]	CON	0.35 ± 0.19	0.33 ± 0.12	0.30 ± 0.15	0.32 ± 0.10	0.32 ± 0.11	0.81 ± 0.54*	0.72 ± 0.47*	0.01	0.84	0.98
	WBC	0.34 ± 0.11	0.32 ± 0.16	0.28 ± 0.16	0.32 ± 0.13	0.36 ± 0.10	0.80 ± 0.52*	0.63 ± 0.21*			
Myoglobin [ng mL^{-1}]	CON	39.8 ± 10.0	84.5 ± 27.0	113.6 ± 52.8*	115.9 ± 64.3*	158.0 ± 108.3*	123.0 ± 95.8*	48.4 ± 26.6	0.01	0.36	0.73
	WBC	39.5 ± 7.7	81.7 ± 26.2	94.1 ± 35.3	93–6 ± 64.3	124.9 ± 52.2*	95.9 ± 31.4*	43.1 ± 9.7			
sICAM-1 [ng mL^{-1}]	CON	219.4 ± 25.8	217.2 ± 23.8	215.8 ± 30.5	227.0 ± 27.5	217.6 ± 20.3	213.7 ± 31.0	212.0 ± 18.9	0.01	0.96	0.27
	WBC	220.2 ± 28.2	220.8 ± 19.7	225.8 ± 26.4	225.6 ± 27.3	208.6 ± 29.0	213.9 ± 29.3	211.7 ± 19.9			
CRP [mg L^{-1}]	CON	0.37. ± 0.36	0.40 ± 0.42	0.36 ± 0.36	0.37 ± 0.35	0.35 ± 0.35	0.40 ± 0.43	0.56 ± 0.54*	0.01	0.51	0.94
	WBC	0.32 ± 0.26	0.28 ± 0.19	0.27 ± 0.18	0.27 ± 0.18	0.25 ± 0.17	0.29 ± 0.18	0.49 ± 0.32*			
IL-6 [pg mL^{-1}]	CON	0.75 ± 0.41	1.93 ± 0.51*	1.18 ± 0.28*	1.10 ± 0.31*	1.19 ± 0.42*	0.81 ± 0.32	0.60 ± 0.31	0.01	0.23	0.51
	WBC	0.85 ± 0.56	2.12 ± 0.99*	1.19 ± 0.51*	1.22 ± 0.56*	1.25 ± 0.47*	0.70 ± 0.41	0.77 ± 0.27			
IL-10 [pg mL^{-1}]	CON	1.44 ± 0.39	3.27 ± 0.97*	2.68 ± 0.61*	1.88 ± 0.31	2.25 ± 1.15	1.39 ± 0.91	1.79 ± 0.91	0.01	0.53	0.78
	WBC	1.49 ± 0.42	3.62 ± 2.03*	3.03 ± 1.39	2.16 ± 0.69*	1.98 ± 0.55*	1.46 ± 0.54	2.29 ± 1.53			
White blood cells [10^9 L^{-1}]	CON	5.4 ± 1.4	6.3 ± 1.8*	7.0 ± 2.2*	10.0 ± 2.3*	9.3 ± 2.0*	8.7 ± 1.6*	4.9 ± 1.1	0.01	0.73	0.79
	WBC	5.1 ± 1.4	6.4 ± 1.7*	7.4 ± 1.9*	10.7 ± 2.5*	9.5 ± 2.1*	9.1 ± 2.4*	5.0 ± 1.0			

* Abbreviations: indicates significant time effects (p < 0.05) compared to baseline (R1$_{pre}$) for both interventions separately.

reached during the step test, with 4 min of active recovery in between the intervals (60% of V_{max}). HIR was followed by 1 h of passive recovery, which was identically structured in both conditions except for the implementation of one 3-min session of WBC after 45 min of rest. During the recovery period the athletes remained seated in the conditioned laboratory (ambient laboratory temperature WBC: 21.7 ± 0.8 °C vs. CON: 21.7 ± 1.0 °C; humidity WBC: 36.4 ± 7.7% vs. CON: 35.8 ± 8.3%) and consumed 0.5 L of a standardized fluid intake (energy: 400 kcal consisting of 46.5 g carbohydrates, 15 g protein, 17 g fat) to avoid dehydration and to replenish depleted glycogen stores [26]. WBC was performed in a temperature-controlled cryochamber with an electrical cooling system (Zimmer MedizinSysteme GmbH, Ulm, Germany). The chamber system consists of three separate rooms with constant temperatures of −10, −60 and −110 °C and we employed a protocol similar to that previously described elsewhere [2,16]. The participants traversed the first two chambers with −10 °C and −60 °C quickly and remained slowly walking for 3 min within the room at −110 °C. During the control intervention athletes walked slowly within the laboratory for 3 min (at 21.7 ± 0.8 °C and 35.8 ± 8.3% humidity). After a total of 60 min of recovery, athletes performed a second incremental exercise (Ramp 2) with the same design as the first one. Time to exhaustion (t_{lim}) was defined as the time (sec.) from the beginning of the ramp test until the athletés volitional exhaustion [16].

2.3. Data measurement

Before and after Ramp 1 + HIR ($R1_{pre}$, $R1_{post}$) and Ramp 2 ($R2_{pre}$, $R2_{post}$) as well as 1-, 4- and 24-hrs after finishing the exercise protocol 8.5 mL venous blood samples was obtained (BD Vacutainer Blood Collection System, Beckton Dickson & Company, Plymouth, UK) from the antecubital vein following 10 min of seated rest (see Fig. 1). After collection, the samples were stored at 7 °C for ~30-min for deactivation of coagulation factors before being centrifuged (1861g for 10-min at 4 °C, Rotixa 50; Hettich Zentrifugen, Mühlheim, Germany). The serum was then aliquoted (Eppendorf type) at −80 °C until later analysis. In particular, we were interested in the inflammatory markers IL-6, IL-10, CRP and sICAM-1; the hormonal biomarkers cortisol and testosterone, and the muscle damage biomarker myoglobin. Serum levels of cortisol (ng mL^{-1}), testosterone (ng mL^{-1}), IL-6 (pg mL^{-1}), IL-10 (pg mL^{-1}), sICAM-1 (ng mL^{-1}), CRP (mg L^{-1}), and myoglobin (ng mL^{-1}) were determined using human enzyme-linked immunosorbent assay (ELISA) kits. Manufacturer instructions were followed for each of the kits and repeated freeze-thaw cycles of serum were avoided. Intra-assay coefficient of variations for cortisol, testosterone, c-reactive protein and Myoglobin (ELISA kits manufactured by DRG Instruments GmbH, Marburg, Germany) as well as sICAM-1 and IL-10 high sensitive (R&D Systems Inc, Minneapolis, USA) and IL-6 high sensitive (IBL International GmbH, Hamburg, Germany) was 3.2%, 3.3%, 4.2%, 3.9%, 5.0%, 9.3%, and 4.6%, respectively. Minimum detectable serum concentrations were 2.5 ng mL^{-1} for cortisol, 0.083 ng mL^{-1} for testosterone, 0.03 pg mL^{-1} for IL-6, 0.09 pg mL^{-1} for IL-10, 0.096 ng mL^{-1} for sICAM-1, 0.1 mg L^{-1} for CRP, and 5.0 ng mL^{-1} for myoglobin. Hematological blood analysis was performed on the day of data collection for the assessment of white blood cell count ($1 \cdot 10^{-3}$ μL^{-1}) using Sysmex KX-21 N (Sysmex Deutschland GmbH, Norderstedt, Germany).

2.4. Statistical analyses

All statistical tests were carried out using the Statistica software package for Windows® (version 13.0, StatSoft Inc., Tulsa, OK, U.S.A). The distribution of data was assessed using descriptive methods (skewness, outliers, and distribution plots) and inferential statistics (Shapiro–Wilk test). As all data were normally distributed data are presented as means ± standard deviations (SD). A two way (treatment [WBC, Control] * time [$R1_{pre}$, $R1_{post}$, $R2_{pre}$, $R2_{post}$, 1 h, 4 h, 24 h])
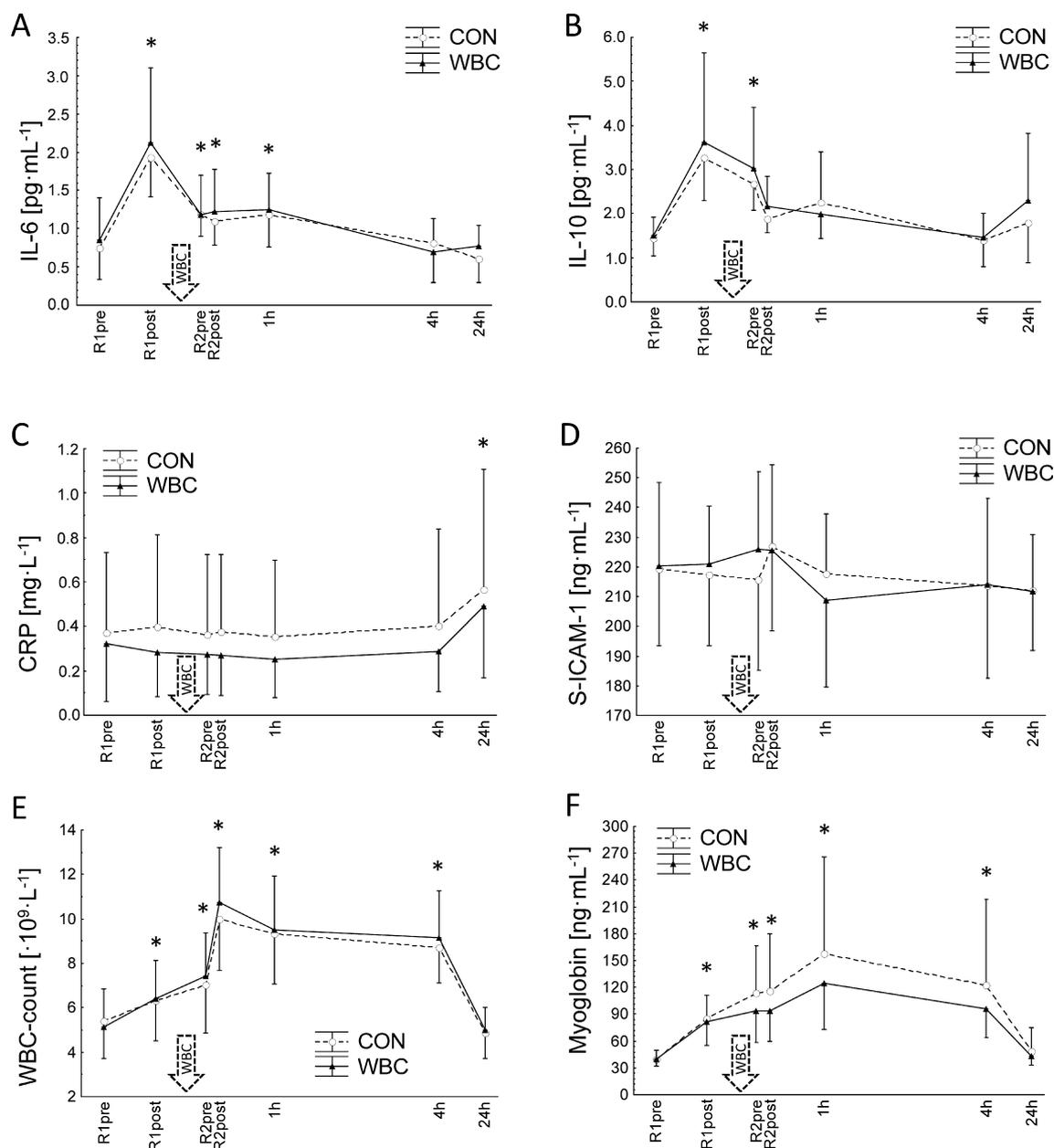
Fig. 2. Serum concentrations (mean ± SD) of interleukin 6 (IL-6; A), interleukin 10 (IL-10; B), C-reactive Protein (CRP; C), soluble intercellular adhesion molecule-1 (sICAM-1; D) and myoglobin (F) as well as white blood cell count (WBC-count; E) at seven time points ($R1_{pre}$; $R1_{post}$; $R2_{pre}$; $R2_{post}$; 1 h; 4 h; 24 h). * P < 0.05 time effect compared to baseline ($R1_{pre}$), for both interventions (whole-body cryotherapy (WBC) and control (CON)) combined.

repeated-measures analysis of variance (ANOVA) was applied to compare all biomarkers. If main effects or interactions were identified, Bonferroni post-hoc analysis was applied where appropriate. Statistical significance was accepted at P < 0.05. Person product-moment correlations were used to detect relationships between ramp tests performance decrements ($\Delta t_{lim} = t_{lim}R2 - t_{lim}R1$) and changes in cortisol, testosterone, IL-6, IL-10, sICAM-1, CRP, myoglobin, and white blood cell count from baseline ($R1_{pre}$) to $R2_{pre}$ in both recovery conditions, respectively.

3. Results

The serum concentrations of all biomarkers (mean ± SD) are detailed in Table 1. At baseline ($R1_{pre}$), similar results were recorded for all outcome measures (all p > 0.05) and all values were within normal range for healthy individuals.

3.1. Inflammatory markers

Significant time effects (p < 0.01) were observed for IL-6 (Fig. 2A) and IL-10 (Fig. 2B). No significant intervention or interaction effects were detected for IL-6 (p = 0.23 and p = 0.51) and IL-10 (p = 0.53 and p = 0.78), respectively. Compared to baseline (WBC: 0.85 ± 0.56; CON: 0.75 ± 0.41) IL-6 (pg mL^{-1}) was significantly higher at $R1_{post}$ (WBC: 2.12 ± 0.99; CON: 1.93 ± 0.51), $R2_{pre}$ (WBC: 1.19 ± 0.51; CON: 1.18 ± 0.28), $R2_{post}$ (WBC: 1.22 ± 0.56; CON: 1.10 ± 0.31) and 1 h (WBC: 1.25 ± 0.47; CON: 1.19 ± 0.42) (all p < 0.01). IL-10 (pg mL^{-1}) was elevated at $R1_{post}$ (WBC: 3.62 ± 2.03; CON: 3.27 ± 0.97, p < 0.01) and $R2_{pre}$ (WBC: 3.03 ± 1.39; CON: 2.68 ± 0.61, p < 0.01) compared to baseline (WBC: 1.49 ± 0.42; CON: 1.44 ± 0.39).

Despite an increase 24 h after exercise (p < 0.01), CRP (Fig. 2C) was not altered following WBC (intervention: p = 0.51; interaction: p = 0.94).

sICAM-1 levels following the two treatments remained similar (Fig. 2D), with no intervention ($p = 0.96$) or interaction ($p = 0.27$) effects observed. Despite observing a significant main effect for time ($p < 0.01$), no post-hoc differences (all $p > 0.05$) using the Bonferroni correction were detected.

A significant increase over time ($p < 0.01$), but no intervention ($p = 0.73$) or interaction ($p = 0.79$) effect was observed in white blood cell count (Fig. 2E). Compared to baseline (WBC: 5.1 ± 1.4; CON: 5.4 ± 1.4) white blood cell count ($10^9 \, L^{-1}$) was elevated (all $p < 0.01$) at $R1_{post}$ (WBC: 6.4 ± 1.7; CON: 6.3 ± 1.8) $R2_{pre}$ (WBC: 7.4 ± 1.9; CON: 7.0 ± 2.2), $R2_{post}$ (WBC: 10.7 ± 2.5; CON: 10.0 ± 2.3), 1 h (WBC: 9.5 ± 2.4; CON: 9.3 ± 2.0) and 4 h (WBC: 9.1 ± 2.1; CON: 8.7 ± 1.6).

3.2. Muscle damage

There was a significant increase in myoglobin [$ng \, mL^{-1}$] over time ($p < 0.01$; Fig. 2F); however no significant intervention ($p = 0.36$) or interaction ($p = 0.73$) effects were observed. Compared to baseline (WBC: 39.5 ± 7.7; CON: 39.8 ± 10.0) myoglobin was elevated at $R1_{post}$ (WBC: 81.7 ± 26.2; CON: 84.5 ± 27.0), $R2_{pre}$ (WBC: 94.1 ± 35.3; CON: 113.6 ± 52.8), $R2_{post}$ (WBC: $93–6 \pm 33.4$; CON: 115.9 ± 64.3), 1 h (WBC: 124.9 ± 52.2; CON: 158.0 ± 108.3) and 4 h (WBC: 95.9 ± 31.4; CON: 123.0 ± 95.8) (all $p < 0.01$).

3.3. Hormonal response

A significant main effect over time was also overserved for cortisol ($p < 0.01$; Fig. 3A), testosterone ($p < 0.01$; Fig. 3B), and testosterone to cortisol ratio ($p < 0.01$; Fig. 3C). Specifically, compared to baseline (WBC: 158.7 ± 26.7; CON: 167.0 ± 29.8) cortisol ($ng \, mL^{-1}$) was elevated (all $p < 0.01$) after the first ramp test (WBC: 217.9 ± 48.6; CON: 224.0 ± 58.2), at 1 h (WBC: 115.6 ± 29.3; CON: 130.5 ± 14.6), 4 h (WBC: 48.7 ± 18.4; CON: 62.8 ± 29.1) and 24 h (WBC: 87.1 ± 20.3; CON: 89.0 ± 26.2). Compared to baseline (WBC: 5.2 ± 1.4; CON: 5.4 ± 1.9) testosterone ($ng \, mL^{-1}$) was also elevated (all $p < 0.01$) after the first ramp test (WBC: 6.4 ± 2.2; CON: 7.0 ± 2.1), at 1 h (WBC: 4.0 ± 1.1; CON: 4.2 ± 1.4) and 4 h (WBC: 3.2 ± 1.2; CON: 3.8 ± 1.3). Analysis of the testosterone to cortisol ratio indicated that, compared to baseline (WBC: 0.34 ± 0.11; CON: 0.35 ± 0.19) values increased significantly at 4 h (WBC: 0.80 ± 0.52; CON: 0.81 ± 0.54, $p < 0.01$) and 24 h (WBC: 0.63 ± 0.21; CON: 0.72 ± 0.47, $p < 0.01$). Again, no significant intervention or intervention * time interaction was overserved for cortisol ($p = 0.53$ and $p = 0.93$ respectively), testosterone ($p = 0.67$ and $p = 0.81$ respectively), and the testosterone to cortisol ratio ($p = 0.84$ and $p = 0.98$ respectively).

3.4. Correlation

Performance decrements (Δt_{lim}) correlated significantly with Δcortisol after both the CON ($r = -0.64$, $p = 0.04$) and the WBC interventions ($r = -0.61$, $p = 0.04$; Fig. 4). No statistically significant correlations were detected for Δt_{lim} and the change in any of the other biomarkers (all $p > 0.05$).

4. Discussion

The current study is the most thorough investigation of the acute effects (up to 24 hrs) of a single WBC-session following intermittent high-intensity exercise on hormonal, inflammatory and muscle damage biomarkers to date. The main findings of this investigation are as follows: (1) contrary to our initial hypothesis, compared to passive recovery one session of WBC did not alter the exercise-induced inflammatory, muscle damage or hormonal response to high-intensity running in trained athletes, (2) the exercise-induced perturbations of all
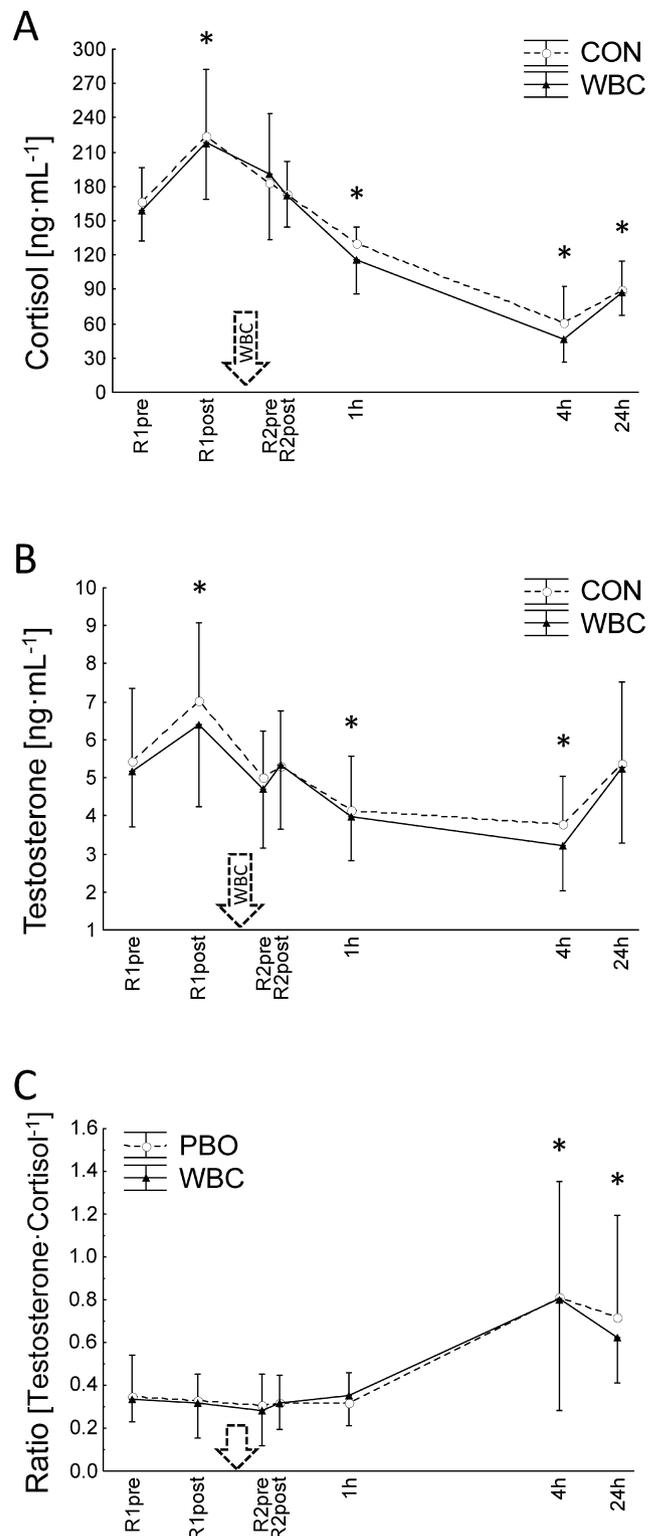
Fig. 3. Serum concentrations (mean ± SD) of cortisol (A), testosterone (B) and calculation of testosterone to cortisol ratio (C) at seven time points ($R1_{pre}$; $R1_{post}$; $R2_{pre}$; $R2_{post}$; 1 h; 4 h; 24 h). * P < 0.05 time effect compared to baseline ($R1_{pre}$), for both interventions (whole-body cryotherapy [WBC] and control [CON]) combined.

inflammatory, muscle damage, and hormonal biomarkers, except CRP and testosterone to cortisol ratio, returned to basal levels within 24 hrs, and (3) increased levels of cortisol, induced by high-intensity exercise, were negatively correlated to subsequent running performance. Collectively, these data suggest that the postulated physiological
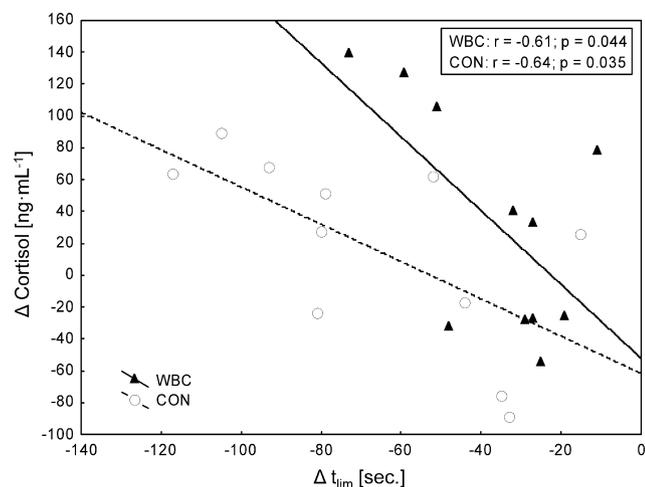
Fig. 4. Correlations between change (Δ) in time to exhaustion (t_{lim}) from ramp 1 to ramp 2 and change in serum cortisol from $R1_{pre}$ to $R2_{pre}$ in control (CON) and whole-body cryotherapy (WBC) intervention, respectively.

mechanism(s), i.e. reductions in inflammation and muscle damage, by which WBC is purported to enhance recovery from EIMD following high-intensity running in trained male athletes may not be accurate.

Reducing the inflammatory response following exercise is one of the primary reasons why WBC is applied as a recovery method [2]. In the present study, similar inflammatory responses were observed following WBC and the control intervention, with IL-6 and IL-10 peaking immediately after exercise and returning to baseline after 1–4 hrs post-exercise (see Fig. 2). Pournot and colleagues [19] have previously reported comparable IL-6 and IL-10 reactions following a simulated trail run on a treadmill in 11 well-trained distance runners. While exercise duration was longer in our investigation (56.3 ± 1.6 min vs 48 min), we did not include downhill running, that is known to induce severe structural muscle damage and inflammation due to high muscular load during eccentric contractions [13]. Selfe and colleagues [27] also reported similar IL-6 values to those of the present investigation and no effects of WBC on IL-6 concentration applied 10–16 hrs after a rugby training. Interestingly, another study reported reduced IL-6 values after 40-min of cycling in professional volleyball players following one session of WBC compared to no WBC and the authors suggested that WBC might initiate protective effects [28]. As WBC preceded exercise, it is likely that these methodological differences caused the differences in the cytokine profile compared to the present study.

Despite a main effect over time, sICAM-1 was not altered following the WBC intervention (see Fig. 2). Thus, assuming sICAM-1 plays a key-role in the inflammation response [5,14], the present data suggest that a single session of WBC is insufficient to reduce the exercise-induced inflammation. Banfi and colleagues [29] have previously reported reduced levels of sICAM-1 after five daily WBC- sessions in rugby players, yet due to the absence of a control group, it is difficult to delineate if WBC in isolation was responsible for these findings [29]. In contrast, Both Dugué and Leppänen [30] and Buemi and colleagues [31] reported increased levels of sICAM-1 after the application of cold water immersion. There is a brevity of empirical data investigating the effects of WBC on the sICAM-1, especially repeated exposures, thus this topic warrants further investigation [5,14].

In comparison to the control condition, WBC did not alter CRP (see Fig. 2). Similarly, no effect of WBC on CRP response was reported in rugby players after five days with continuous training and daily WBC-treatments [32]. Contrary, Pournot and colleagues observed reduced CRP-levels 24 hrs after one session of WBC (3 min at −110 °C) compared to passive recovery in equally trained participants [19]. Overall higher CRP-values compared to our results are most likely caused by additional eccentric contractions during downhill running.

Furthermore, Pournot reported decreased levels in pro-inflammatory cytokine IL-1β and increased levels in anti-inflammatory cytokine IL-1ra, suggesting, in contrast to our findings, that one session of WBC reduces the inflammatory process [19].

CK is probably the most frequently analyzed biomarker to identify muscle damage [33]. CK only leaks into the bloodstream when the sarcolemma is damaged and is, therefore, another commonly used biomarker of muscle damage [34]. However, the enzyme has a molecular mass of 84 kDa (kDa) and has to be transported by the lymphatic system [6]. Therefore, the onset of CK-concentration in venous blood is delayed. – Depending on the magnitude of muscle damage, CK peaks 24 to 96 hrs after exercise [35]. Therefore myoglobin, a rather small molecule (18 kDa) that is released directly to the blood flow as a result of degradation of muscle proteins [34], appears to be more feasible to detect the acute muscle damage effects of strenuous exercise. Myoglobin typically peaks within the first hours after severe exercise and is already decreasing at 24 hrs post exercise whereas CK is still rising [36,37]. To the best of our knowledge, this is the first investigation that has utilized myoglobin as marker for muscle damage after WBC. Myoglobin peaked 1 hr after exercise and returned to baseline after 24 hrs with no differences between recovery modalities (see Fig. 2). Although this is the first study to assess myoglobin, others have reported comparable results regarding CK [13,20], but also reduced levels of CK after WBC [3,18,38–40]. However, these studies have some limitations, such as small sample size [38], the lack of a control group [40] or the high probability that the results were influenced by the repeated bout effect [18,39]. Therefore, the evidence for WBC to reduce muscle damage remains very limited.

Testosterone, cortisol, and their ratio are often employed as biomarkers of anabolic status, training responses/adaptations and motivation [41]. It is well established that cortisol increases when an individual is exposed to psychophysiological stress following activation of the hypothalamic-pituitaryadrenal axis [42]. The physical exercise itself potentially increases cortisol further, though the magnitude depends on, amongst other variables, the type of exercise [43] and the environmental conditions [44] where it takes place. In the present study we observed increased levels in cortisol at baseline and exercise induced increments in cortisol and testosterone, respectively (see Fig. 3). Due to heterogeneous methodologies and conflicting results in the current literature, the effects of WBC on hormone biomarkers is still unclear [38,45–47]. However, to our knowledge there is only one study which has investigated the effects of a single WBC-session, applied immediately after exercise, on cortisol and testosterone [20]. In line with the findings of the present study, Russell and colleagues [20] detected no WBC- related changes in cortisol. Collectively, these findings suggest that a single WBC-session does not alter the stress-related hormone cortisol. However, in contrast to our findings Russell and colleagues [20] reported increased testosterone levels in the WBC-group, suggesting that higher testosterone-concentrations may facilitate recovery [20]. Moreover, the authors speculate that higher testosterone- values might indicate reduced inflammation as low serum-testosterone concentrations are related to inflammation [20]. These results and speculations cannot be confirmed by the findings of the present investigation, as testosterone responded similarly after the WBC and CON-interventions. The contrasting findings might be explained by the application of different exercise protocols. Immediately after repeated sprint exercise, Russell et al. [20] detected no time effects in cortisol or testosterone, while our data indicated significant elevations in both hormones, most likely due to higher intensity of the exercise intervention. Therefore, it is plausible to speculated that testosterone concentrations are only elevated by one session of WBC if the preceding exercise itself did not induce a significant hormonal response.

The present study also investigated the relationship between the performance decrements after HIR (i.e. Δt_{lim} R1 − R2) and the changes in biomarker-levels from baseline to the end of 60 min recovery period ($R2_{pre}$ − $R1_{pre}$). No correlations were found for all biomarkers except

cortisol, regardless of the recovery modality applied (see Fig. 4). These results suggest that reductions in cortisol after exercise and recovery lead to higher subsequent running-performance. Interestingly, despite no significant intervention or interaction effects in inflammatory, muscle damage and stress-related biomarkers, we observed improved running performance (time to exhaustion) immediately after a single WBC-session [16]. It can be speculated that these performance improvements are most likely attributed to (i) a placebo effect, (ii) perceptual reductions in pain, (iii) acute changes in muscle oxygenation, (iv) lower cardiovascular strain, or (v) a combination of these factors.

5. Limitations

Our study has limitations that warrant mention. Firstly, incorporating a range of other inflammatory cytokines (e.g. TNF-alpha, IL-8 and IL-15), and biomarkers of muscle damage (e.g. creatine kinase, lactate dehydrogenase) and stress (e.g. epinephrine, alpha amylase) would have provided further insight into the inflammatory, damage, and hormonal effects of a single WBC exposure following exercise. Secondly, despite all biomarkers except CRP and testosterone-cortisol ratio returning to basal levels a longer timeline of analysis, possibly up to 96 hrs post exercise, would have offered additional insights into the potential effects of WBC. Thirdly, although the present investigation focused on inflammatory, hormonal and muscle damage biomarkers, functional and performance measures up to 24 hrs would have added additional practical information regarding the application of WBC. Fourthly, despite employing a cross-over study design, it is possible that the small sample-size increased the potential for type II error. Furthermore, a cross-over study design might be influenced by the repeated bout effect. However, due to the participants being very familiar with the exercise protocol and the lack of any unaccustomed exercise or downhill running, i.e. eccentric muscle damage, it is very unlikely that these results were impacted by the repeated bout effect. Future research incorporating a parallel design with a larger sample size is therefore warranted. Finally, not including an active recovery group is a limitation of this study.

6. Conclusion

This study is the most thorough investigation of the effects of a single session of WBC (3 min at $-110\,°C$) on biomarkers of hormonal status, inflammation, and muscle damage after acute high-intensity exercise in trained males. Despite the expected changes in IL-6, IL-10, CRP, sICAM-1, myoglobin, cortisol, testosterone, and cortisol-testosterone ratio in the 24 hrs following exercise, and contrary to our hypotheses, our results demonstrate for the first time that WBC has no acute beneficial effect compared to passive recovery in any biomarker assessed. The results of this study suggest that the postulated physiological mechanisms by which a single exposure to WBC is speculated to improve recovery, i.e. reductions in inflammation and muscle damage, may not be accurate.

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Disclosure statement

The authors confirm there are no conflicts of interest.

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