BioMed Research International

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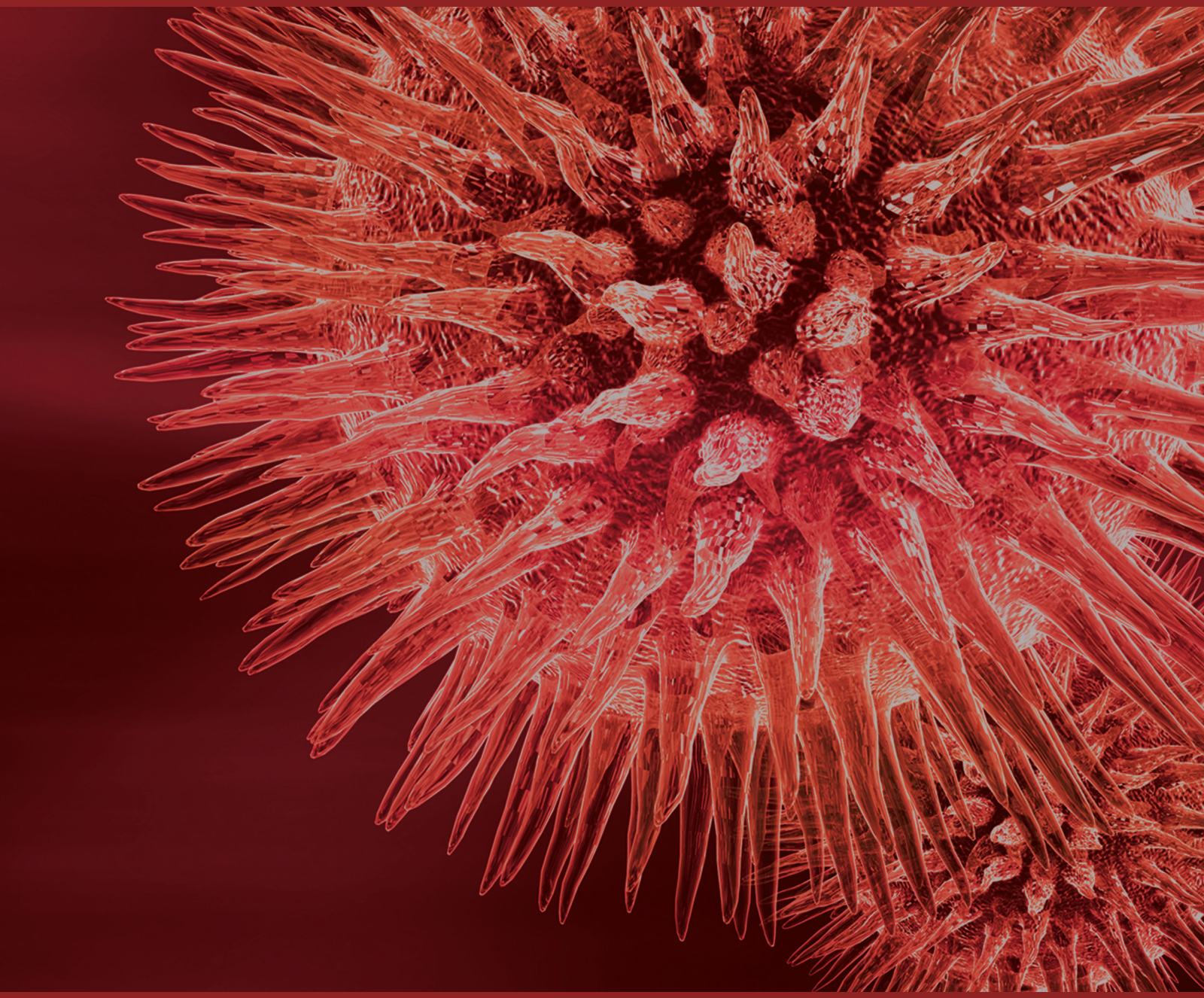
Guest Editors: Ellen Glickman, Edward J. Ryan, and David Bellar



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Contents

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Editorial

Exercise Physiology, Cognitive Function, and Physiologic Alterations in Extreme Conditions

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Exercise physiology is a heterogeneous field of study that includes a broad array of disciplines evaluating the impact of physical stressors on the physiology of man. While man is exposed to a variety of environmental conditions, it is imperative that exercise physiologists elucidate how the added stress of a terrestrial extreme impacts man's thermal, metabolic, and cognitive abilities. Research regarding environmental extremes and exercise will enhance our understanding of how to safely compete athletically, navigate in unfamiliar locations, and aid military personnel during exposure to a variety of natural settings.

The physiologic, metabolic, and cognitive responses in general are complex and differ between sexes, across the lifespan, and are impacted by heredity. When physical stress is coupled with varied ambient conditions (environmental stressors, i.e., heat, cold, altitude, hypoxia, and lower body negative pressure), there may be dramatic shifts in physiological and cognitive responses. Furthermore, this coupling of stressors may present a limitation in man's abilities to maintain homeostatic control across multiple organ systems. Such limitations may compromise safety and performance; thus further work in these areas is warranted.

Through controlled experimentation, taking into account as many confounding factors as possible (i.e., gender, menses, age, training status, circadian rhythm, and diet), we evaluate combined physiologic and cognitive responses to environmental and exercise stresses. Recently, there has been growing concern for works elucidating how heat/cold stressors influence blood flow, cognitive function, and thermoregulation at rest and during exercise. While military personnel are often deployed to areas of low barometric pressure, research has also been conducted to better understand the effects of hypoxia on cognitive function.

We invited investigators to contribute original research articles as well as review articles that will stimulate the continuing efforts to understand exercise physiology in ambient and extreme environmental conditions. Further research in these areas will allow for better precautionary and treatment guidelines in occupational and athletic settings.

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Research Article

Preservation of Cognitive Performance with Age during Exertional Heat Stress under Low and High Air Velocity

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Older adults may be at greater risk for occupational injuries given their reduced capacity to dissipate heat, leading to greater thermal strain and potentially cognitive decrements. *Purpose.* To examine the effects of age and increased air velocity, during exercise in humid heat, on information processing and attention. *Methods.* Nine young (24 ± 1 years) and 9 older (59 ± 1 years) males cycled 4 × 15 min (separated by 15 min rest) at a fixed rate of heat production (400 W) in humid heat (35°C, 60% relative humidity) under 0.5 (low) and 3.0 (high) $m \cdot s^{-1}$ air velocity wearing coveralls. At rest, immediately following exercise (end exercise), and after the final recovery, participants performed an abbreviated paced auditory serial addition task (PASAT, 2 sec pace). *Results.* PASAT numbers of correct responses at end exercise were similar for young (low = 49 ± 3; high = 51 ± 3) and older (low = 46 ± 5; high = 47 ± 4) males and across air velocity conditions, and when scored relative to age norms. Psychological sweating, or an increased sweat rate with the administration of the PASAT, was observed in both age groups in the high condition. *Conclusion.* No significant decrements in attention and speeded information processing were observed, with age or altered air velocity, following intermittent exercise in humid heat.

1. Introduction

Performing physically demanding work in hot environments and/or while wearing protective clothing increases thermal strain, which has been associated with decrements in cognitive function, such as reduced short-term memory, recall, and discrimination [1]; reaction time, reasoning, and vigilance [2]; and arithmetic ability [3]. In some cases, however, improved cognitive performance has been observed during short duration continuous aerobic exercise (i.e., 60% VO_{2peak}, 10 min duration; choice-discrimination) [4], during intense exercise (i.e., at/above anaerobic threshold; short-term memory) [5], and after bouts of continuous exercise of 20 and 40 min in duration (i.e., 70% VO_{2peak}; mathematical computations) [6]. Attempts to reconcile these conflicting reports in the literature have proposed that they are due to variances in the participant groups (e.g., hydration status, level of heat acclimatization, and degree of sleep deprivation) and/or in the methodology (e.g., environmental conditions, mode of exercise, and type of difficulty of cognitive test) between studies. Although cognitive performance has been examined during and following exercise in the heat, the analysis is typically done using either change in hydration or exercise rate (e.g., exercise at a % of VO_{2peak}, absolute work rate, or completion of predetermined work tasks) [1, 2, 7] as the independent variables. In addition, the above studies were conducted in young, healthy, active individuals (some middle-aged participants in Heckler and Croce [6]). Thus, no study has examined cognitive performance during exercise in the heat as a function of age or following intermittent

exercise at a constant rate of thermal drive, which matches the work load required in many occupational settings [8], where attention and vigilance are crucial for worker safety.

Age-related decrements in heat loss responses of sweating and skin blood flow are known to compromise the thermoregulatory efficacy of older adults [9–11]. Consequently, concerns have been raised regarding the safety of older workers in occupations which require physically demanding tasks to be performed in the heat and/or while wearing protective clothing and the need for attention/concentration in operating equipment (e.g., deep underground and surface mining, hydroelectric utilities, steel and chemical plants, and others). Age-related decrements in heat loss responses are not always reflected in measurements of local heat loss responses (e.g., sweat rate) and core temperature [11–13]. Given that health and safety heat stress guidelines are based on core temperature [14], it must be recognized that increases in thermal strain and thus potentially age-related differences in cognitive performance [15] may still be present in older adults. However, the extent to which age-related differences in thermoregulation impact cognitive performance (e.g., information processing and attention) is currently unknown, particularly under conditions that restrict evaporative heat loss (i.e., humid heat and insulative protective clothing) which can result in increases in thermal strain. Furthermore, in attempts to mitigate thermal strain in workers, ventilation systems delivering large, and in some instances thermally conditioned, air volumes have been utilized in deep underground mining [16]. Such ventilation systems are required to limit the increase in environmental temperature due to the various energy transformations and heat sources within a mine while also providing a flow to promote worker cooling. The supply of suitable ventilation has been shown effective in reducing thermal strain (i.e., core temperature) in lab studies during exercise in heat in active young adults (i.e., 25–30 years) while wearing minimal clothing [17–19]. Moreover, in a recent report, increased air velocity was shown to be effective during intermittent exercise in humid heat in young (i.e., ~25 years) and older (i.e., ~60 years) males while wearing work coveralls [13]. It remains unknown, however, whether ventilation-mediated reductions in thermal strain impact cognitive performance in young and older adults.

The primary purpose of this study was to examine the influence of aging and increased air velocity on attention and speeded information processing, following intermittent exercise in humid heat under low ($0.5\,\mathrm{m\cdot s^{-1}}$) and high ($3.0\,\mathrm{m\cdot s^{-1}}$) air velocity work conditions in young (24.1 ± 0.5 years) and older (59.4 ± 1.2 years) males. Given that cognitive stress and/or increased mental workload have been shown to increase sweating (termed psychological sweating), identifying the effects of tasks which require sustained attention and working memory on thermoregulatory and cardiovascular responses would be applicable to occupations which require concentration and attention under potentially stressful conditions [20–22]. Thus, the secondary purpose of the study was to examine the influence of increased attentional requirements and increased working memory loads on thermal and cardiovascular responses. Accordingly, thermal and cardiovascular responses, and performance on a test requiring attention and speeded information processing, were compared for young and older males following intermittent exercise in humid heat (35°C, 60% relative humidity [RH]) under low and high air velocity conditions. The low velocity may be considered similar to that typically employed in industrial settings, such as mining for general occupational hygiene related exposure control, whereas the higher air velocity (i.e., $3.0\,\mathrm{m\cdot s^{-1}}$) would be supplied to mitigate heat stress in workers. To be consistent with the required absolute work rate in many occupational settings [8], including deep underground mining, and a moderate-to-heavy work rate as defined by the American Conference of Governmental Industrial Hygienists (ACGIH) [14], a fixed rate of heat production of 400 W was utilized during exercise to maintain an equal heat load and therefore a similar level of thermal strain between groups. It was hypothesized that increased air velocity would be less effective in reducing both thermal and cardiovascular strain, as well as any cognitive decrements, in the older males as compared with young males. It was also hypothesized that psychological sweating in both young and older males would be present as a result of increased attention and working memory demands but might not be as evident during the low air velocity condition compared to high air velocity condition due to increased skin wettedness as a result of a reduced capacity for the evaporation of sweat.

2. Materials and Methods

2.1. Participants. Upon receiving approval from the University of Ottawa Health Sciences and Science Research Ethics Board, 9 young and 9 older healthy, active, nonheat acclimated, nonsmoking males, matched for height, mass, and body surface area, were recruited for the study. None of the participants had a history of respiratory, metabolic, cardiovascular, and/or hypertension disease, or skin conditions, and none were on any medication related to these conditions. All participants were moderately educated (at minimum) individuals from the general population and university communities with a wide range of occupations (e.g., students, construction, government, and general labour employees). Some participants from each group had moderate levels of scientific background due to prior research participation. All participants were informed of the experimental procedures, associated risks, and discomforts prior to providing written consent. Participant characteristics are shown in Table 1.

2.2. Experimental Design. All participants completed one preliminary screening session and two experimental sessions. During the preliminary session, participants underwent a progressive incremental test on a semirecumbent bike (one minute stages with 20 W incremental increases) to determine maximal oxygen uptake (VO_{2peak}) (AMETEK models S-3A/1 and CD 3A, resp., Applied Electrochemistry, AEI Technologies, Pittsburg, PA, USA). Participants cycled until they could no longer maintain the predetermined cadence or stopped due to volitional fatigue. During the VO_{2peak} exercise test, continuous electrocardiogram monitoring was performed in the older participants (age > 50 years) (Pulse Biomedical Inc.,

Table 1: Participant characteristics.

	Age (yr)	Height (cm)	Mass (kg)	BSA (m^2)	Body fat (%)	VO_{2peak} ($mLO_2 \cdot kg^{-1} \cdot min^{-1}$)
Young	24.1 (0.5)[a]	174.6 (3.2)	79.4 (2.6)	1.95 (0.05)	15.5 (2.7)[a]	49.2 (2.6)[a]
Older	59.4 (1.2)	174.2 (1.2)	81.5 (4.1)	1.96 (0.05)	25.6 (2.6)	40.4 (2.6)

Note: values are mean (SE). Body surface area (BSA); maximal aerobic power (VO_{2peak}).
[a]Significantly different than older males.

Norristown, PA, USA). Blood pressure was measured at every 2nd stage or every 2 min. Percent body fat was calculated using the Siri equation [23], following the measurement of body density by hydrostatic weighing.

The two experimental sessions followed a minimum of 2 weeks after the preliminary session and were separated by a minimum of 72 hours but performed at the same time of day. Prior to arriving at the laboratory, participants were instructed to eat a normal breakfast and drink water ad libitum, while refraining from caffeine and exposure to thermal stimuli for 12 and 24 hours prior to each session, respectively. Participants were also asked to refrain from alcohol and exercise for 24 hours prior to each session. Upon arrival at the laboratory, participants provided a urine sample, inserted the rectal temperature probe, sat for a 20-minute thermoneutral resting baseline, and were instrumented with a heart rate monitor and skin temperature sensors. Participants donned underwear (I_{cl} = ~0.05 clo insulation), long underwear (I_{cl} = ~0.19 clo), a t-shirt (I_{cl} = ~0.10 clo), work coveralls (I_{cl} = ~0.61 clo), socks (I_{cl} = ~0.04 clo), running shoes (I_{cl} = ~0.04 clo), work gloves, and a hard hat. Participants subsequently entered the thermal chamber (Can-Trol Environmental Systems Limited, Markham, ON, Canada) regulated at 35°C and 60% RH with an air velocity of 0.5 (low, first experimental session) or 3.0 (high, second experimental session) $m \cdot s^{-1}$ where they rested for 30 min while baseline measurements were obtained. They then performed four 15-minute bouts of cycling at a fixed rate of metabolic heat production of 400 W, a moderate-to-heavy intensity as defined by the ACGIH [14], separated by 15-minute rest periods with a final resting recovery of 30 min in duration. This rate of heat production is comparable to the absolute work rate required in occupational settings (e.g., mining) that are often performed in hot environments [8, 14].

2.3. Physiological and Cognitive Measurements

2.3.1. Metabolic Heat Production. To establish a fixed rate of metabolic heat production of 400 W, the ergometer resistance was adjusted based on the concurrent measurements of expired oxygen and carbon dioxide concentrations (AMETEK models S-3A/1 and CD 3A, resp., Applied Electrochemistry, AEI Technologies, Pittsburg, PA, USA). Metabolic heat production was taken as the difference between metabolic energy expenditure and the resistance, in Watts, on the ergometer. Metabolic energy expenditure (M) was calculated from 30-second average values for oxygen uptake (VO_2), according to the following equation.

$$M \text{ (Watts)} = \frac{\dot{V}O_2 * \left[\left(\text{RER} - 0.7/0.3 * e_c\right) + \left(1 - \text{RER}/0.3 * e_f\right)\right]}{60}, \quad (1)$$

where RER is the respiratory exchange ratio (CO_2 produced/O_2 uptake), e_c is the caloric equivalent of a liter of oxygen when carbohydrates are oxidized (21.116 kJ), and e_f is the caloric equivalent of a liter of oxygen when fat is oxidized (19.606 kJ) [24]. Stable metabolic heat production values were typically attained within the first 5 min of the first exercise bout, following which minimal adjustments were made to maintain 400 W.

Absolute oxygen uptake was not different between the young (1358.24 ± 15.39 and 1357.11 ± 15.58 $mL \cdot min^{-1}$) and older (1363.05 ± 17.67 and 1344.21 ± 17.43 $mL \cdot min^{-1}$) males nor between air velocity conditions.

2.3.2. Core Temperature. Rectal temperature (T_{re}) was measured continuously using a pediatric thermocouple probe (Mon-a-therm Nasopharyngeal Temperature Probe, Mallinckrodt Medical, St. Louis, MO, USA), inserted 12 cm past the anal sphincter. Temperature data were collected using a HP Agilent data acquisition module (Model 3497A) at a sampling rate of 15 sec and simultaneously displayed and recorded on a personal computer with LabVIEW software (Version 7.0, National Instruments Corporation).

2.3.3. Local Sweat Rate. Local sweat rate (LSR) was measured using a 3.8 cm^2 ventilated capsule affixed to the skin on the forearm (LSR_{FA}) and upper back (LSR_{UB}) with an adhesive ring and topical skin glue (Collodion HV, Mavidon Medical, Lake Work, FL, USA). Anhydrous compressed air was passed through the capsules at a rate of 1 $L \cdot min^{-1}$. Water content of the effluent air was measured using high precision dew point mirrors (Model 473, RH systems, Albuquerque, NM, USA). Local sweat rate was determined by calculating the difference in water content between the effluent and influent air for each capsule, multiplied by the flow rate, and normalized for the skin surface area under the capsule.

2.3.4. Heart Rate. Heart rate was measured continuously using a Polar coded transmitter and recorded with a Polar Advantage interface and Polar Precision Performance software (Polar Electro Oy, Finland).

2.3.5. Cognitive Function Test. Due to the short work-to-rest cycles (15 min exercise-15 min rest; according to the ACGIH guidelines [14]), a portion of the Victoria Computerized Adaptation of the paced auditory serial addition task (PASAT; 2 sec pace, 3 min test, nonstandard administration) [25], as a measure of the capacity for sustained attention and speeded information processing, was administered to participants at resting baseline (baseline, after sitting in the warm/humid environment for 20 min), at the end of the 4th/final exercise bout (end exercise), and at the end of the 30 min final recovery (end recovery). The number of correct responses for each test was recorded and scores were normalized relative to age norms for the administered condition [26] as Z scores and percentiles. During the preliminary session, the 2-second pace segment of the PASAT was administered repeatedly, with breaks, until a stable score was obtained in order to counter any practice effects during the experimental sessions. The number of repetitions required to reach a stable score (defined as 2 unchanged scores in succession) averaged 4–6 repetitions for each group. In addition, the PASAT instructions were reviewed and practice administration of the 2-second pace was completed at the beginning of each experimental session.

2.4. Statistical Analyses. A one-way ANOVA was performed to compare age, height, mass, body surface area, % body fat, and VO_{2peak} of the young versus older males. A three-way analysis of variance (ANOVA) with 1 between grouping factor (young and older) and 2 repeated factors for condition (low and high) and time (baseline, end exercise, and end recovery) was performed on T_{re}, heart rate, percent of maximum heart rate, LSR_{FA}, LSR_{UB}, and the PASAT scores (# of correct responses, Z scores, and percentiles). PASAT raw scores are reported, in addition to the normative values for the full administration of the test. To examine the effects of the PASAT administration on T_{re}, heart rate, LSR_{FA}, and LSR_{UB}, a 2-way ANOVA with 1 between grouping factor (just prior to the start of the PASAT and the average of these measures over the 3-minute duration of the PASAT) and 1 repeated factor for time (baseline, end exercise, and end recovery) was performed on the T_{re}, heart rate, LSR_{FA}, and LSR_{UB} for the young and older males during the low and high air velocity conditions. When a significant F-ratio was obtained, a Newman-Keuls post hoc procedure was used to isolate differences. Significance was assumed for $P \leq 0.05$ (trends were noted for $0.05 > P \leq 0.10$).

3. Results

3.1. Effects of Age and Air Velocity. During the low and high air velocity conditions, no overall differences were observed between the young and older males for T_{re} (Figures 1(a) and 1(c)), LSR_{FA} (Figures 2(a) and 2(c)), LSR_{UB} (Figures 2(b) and 2(d)), PASAT Z score (Figures 3(b) and 3(e)), or PASAT percentile (Figures 3(c) and 3(f)). A group x time interaction was observed for heart rate, such that heart rate was greater in the young males compared to older males at end exercise with a trend at end recovery ($P = 0.084$) (Figures 1(b) and 1(d), trend for age main effect $P = 0.067$). There were no differences between the young and older males for percent of maximum heart rate in either the low condition at baseline (young = 41.6 ± 2.5, older = 44.1 ± 2.3%), end exercise (young = 80.0 ± 3.2, older = 77.3 ± 1.2%), or end recovery (young = 62.7 ± 3.3, older = 64.9 ± 2.0%), or the high condition at baseline (young = 39.0 ± 2.1, older = 41.1 ± 1.1%), end exercise (young = 67.9 ± 2.9, older = 71.1 ± 2.0%), or end recovery (young = 46.4 ± 2.9, older = 53.3 ± 2.3%). A group x time interaction was observed for PASAT number of correct responses, such that the number of correct responses were greater at end recovery compared to baseline and end exercise for the older males (Figures 3(a) and 3(d)).

Significant differences were observed between the low and high air velocity conditions for T_{re} (Figures 1(a) and 1(c)), heart rate (Figures 1(b) and 1(d)), percent of maximum heart rate, LSR_{FA} (Figures 2(a) and 2(c)), and LSR_{UB} (Figures 2(b) and 2(d)). A condition x time effect was observed for T_{re}, heart rate, percent of maximum heart rate, LSR_{FA}, and LSR_{UB}, such that all time points were different from each other except for low versus high at baseline for T_{re}, LSR_{FA}, and LSR_{UB}, and low versus high at end exercise for LSR_{FA} and LSR_{UB}. No significant differences were observed between the low and high air velocity conditions for PASAT number of correct responses (Figures 3(a) and 3(d)), PASAT Z score (Figures 3(b) and 3(e), trend for condition main effect $P = 0.076$), or PASAT percentile (Figures 3(c) and 3(f), trend for condition main effect $P = 0.063$).

A significant effect of time was observed in the low and high air velocity conditions for T_{re} (baseline versus end exercise and end recovery), heart rate, percent of maximum heart rate, LSR_{FA}, and LSR_{UB}, (all time points different from each other), and PASAT number of correct responses (baseline and end exercise versus end recovery for older males) but not PASAT Z score (trend for time main effect $P = 0.061$) or PASAT percentile.

3.2. Effects of the PASAT Administration. The administration of the PASAT did not result in changes in T_{re}; however, it resulted in a significant reduction in heart rate for the young and older males during the high air velocity condition at end exercise (trend for older during low at end exercise, $P = 0.053$). The PASAT also resulted in a significant increase in LSR_{FA} and LSR_{UB} for the young and older males at end recovery during the high air velocity condition.

4. Discussion

Cognitive function, specifically attention and capacity of information processing, was compared using an abbreviated version of the PASAT, in young and older males prior to and following intermittent exercise, performed at a fixed rate of heat production of 400 W. This rate of heat production is equivalent to a moderate-to-high workload according to the ACGIH guidelines [14]. The young and older males performed similarly on the PASAT (i.e., absolute number of correct responses and relative to age norms [26]) prior to and following intermittent exercise while wearing work coveralls in both the low and high air velocity conditions. This was paralleled by similar thermal (i.e., T_{re} and LSR

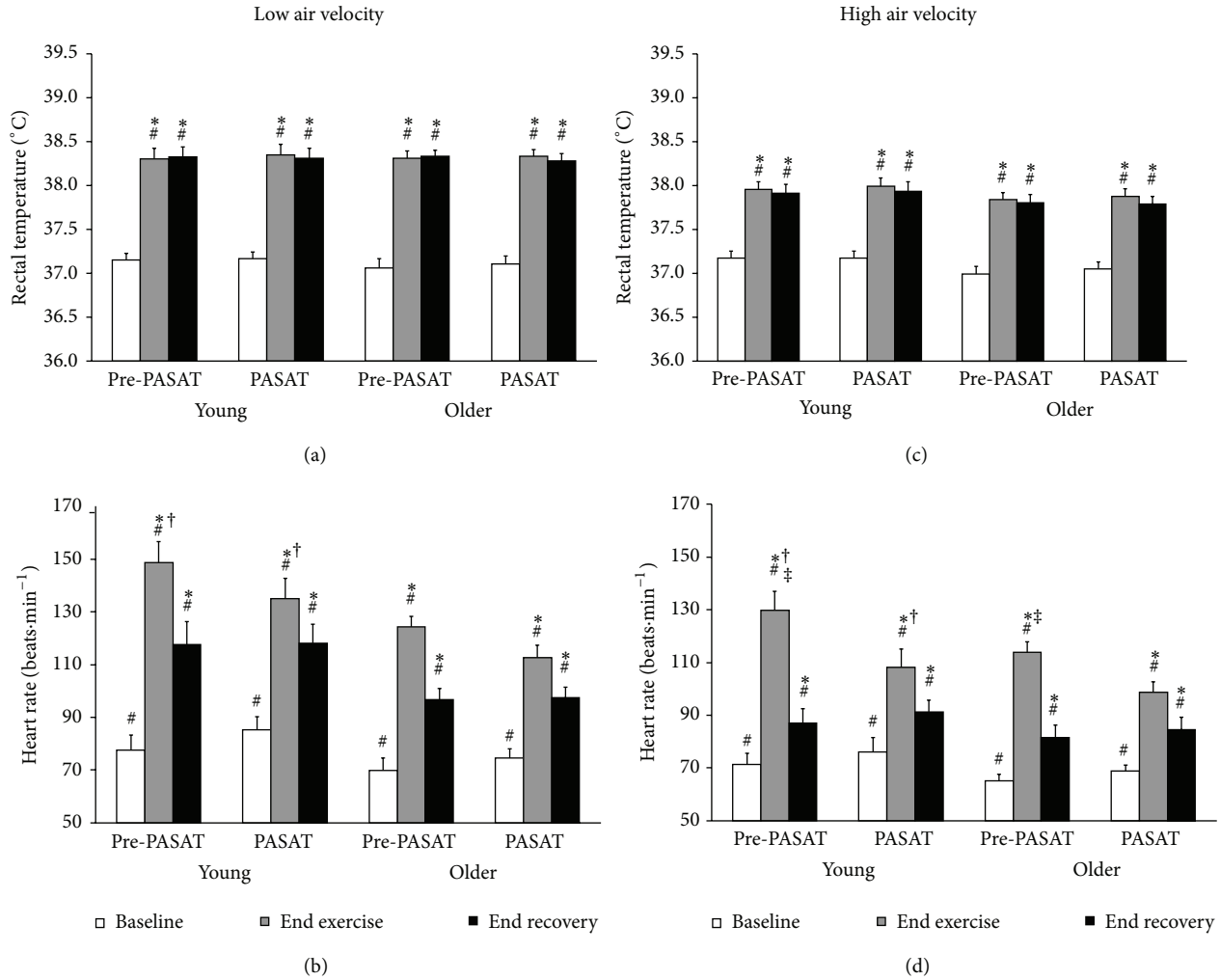

Figure 1: Rectal temperature ((a) and (c)) and heart rate ((b) and (d)) at the start of the paced auditory serial addition task (PASAT) and averaged over the 3-minute duration of the PASAT when administered at baseline (white), end of the 4th exercise bout (end exercise; grey), and end of the final recovery (end recovery; black) in young and older males under low and high air velocity. Values are mean ± SE. ∗ Main effect of time; end exercise and end recovery versus baseline for rectal temperature; end exercise and end recovery versus all time points for heart rate; # main effect of air velocity condition; † main effect of age; ‡ significant difference between pre-PASAT and PASAT within each air velocity condition and age group.

at both skin sites) and cardiovascular (i.e., percent of maximum heart rate) responses between the age groups in both air velocity conditions. In addition, neither the young nor the older males demonstrated decrements in performance on the PASAT in either air velocity condition despite the high air velocity significantly reducing thermal (i.e., T_{re} and LSR at both skin sites) and cardiovascular (i.e., heart rate and percent of maximum heart rate) strain equally in both age groups. Psychological sweating was apparent in both the young and older males at the end of recovery in the high but not the low air velocity condition.

4.1. Effects of Age and Air Velocity on Cognitive Performance. While previous studies have reported decrements in cognitive performance, such as impaired short-term memory, recall, and vigilance, during exercise in the heat in young individuals [1–3], no study has examined potential age-related differences in cognitive function during work in humid heat. Reductions in the rate of information processing have been strongly linked to age-related changes in cognitive function [27]; however, young and older adults perform similarly on tests such as the PASAT that require overlearned arithmetic skills in conjunction with rapid information processing and sustained attention [28, 29]. It was hypothesized that when subjected to thermal stress the older males might demonstrate impairments in attention and speed of information processing on a test resistant to the effects of age-related cognitive decline (PASAT). However, the young and older males demonstrated a similar cognitive performance (i.e., attention and information processing) under heat stress during both the low and high air velocity conditions. Given that the population group used in the current study likely had different work experience, heat exposure backgrounds, and/or education levels as compared to some industry workers, it would be of

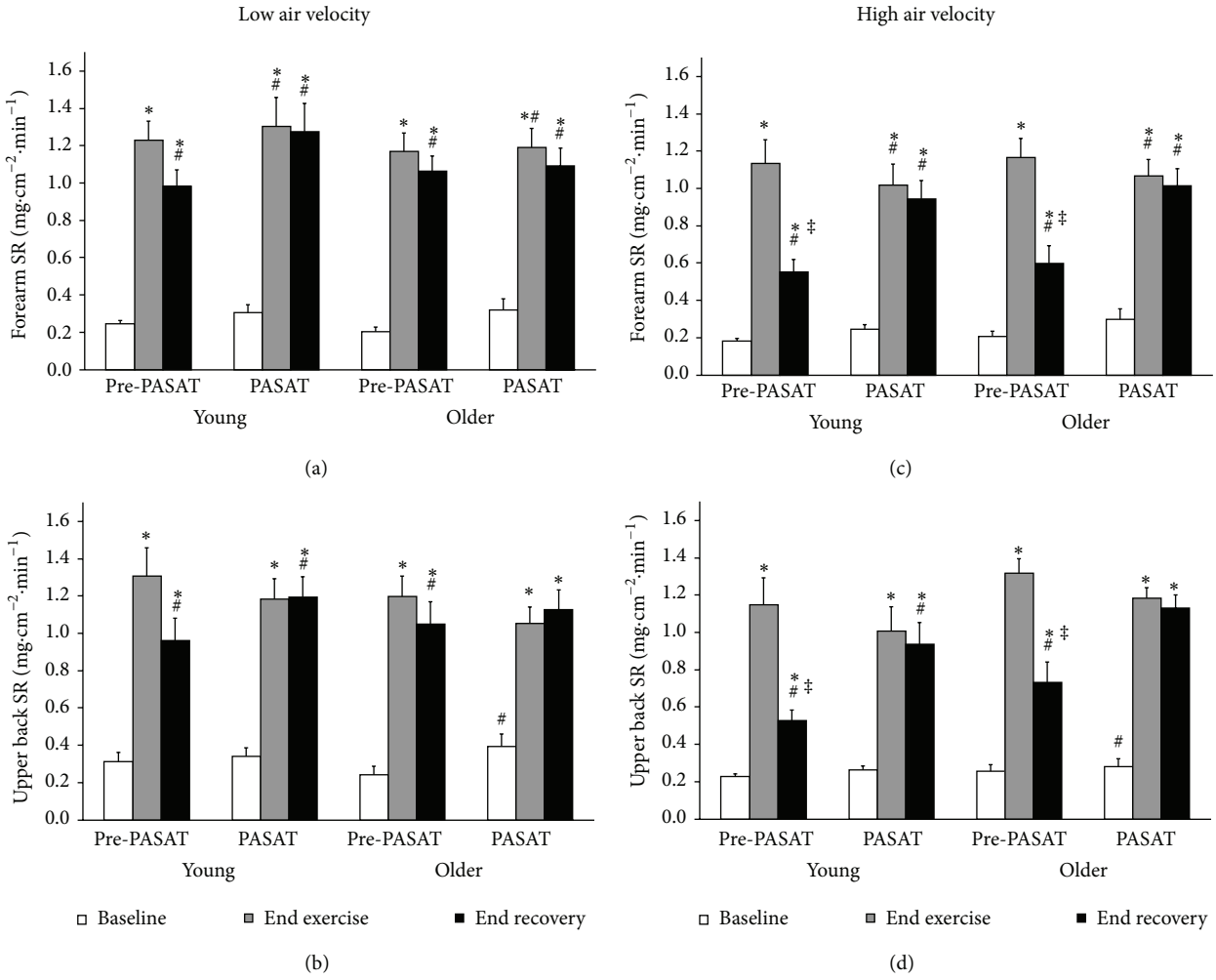

Figure 2: Local sweat rate (SR) at the forearm ((a) and (c)) and upper back ((b) and (d)) at the start of the paced auditory serial addition task (PASAT) and averaged over the 3-minute duration of the PASAT when administered at baseline (white), end of the 4th exercise bout (end exercise; grey), and end of the final recovery (end recovery; black) in young and older males under low and high air velocity. Values are mean ± SE. Notes: forearm $n = 8$ for older high condition; upper back $n = 7$ for young low condition, $n = 8$ for older low condition, and $n = 8$ for older high condition. ∗ Main effect of time; end exercise and end recovery versus all time points for pre-PASAT; end exercise and end recovery versus baseline for PASAT; # main effect of air velocity condition; ‡ significant difference between pre-PASAT and PASAT within each air velocity condition and age group.

great benefit to the occupational stakeholders if this study were repeated under field conditions and/or in the laboratory with industrial workers. Similar cognitive performance was observed between the age groups with the exception of a time effect for the older males, whereby the number of correct responses on the PASAT was significantly higher at end exercise and end recovery compared to baseline. Such an improvement in performance 30 min following exercise may be due to a practice effect over time which has previously been noted for this test [28]. The practice effects might be expected to be of a larger magnitude (despite repeated practice) due to the abbreviated quarter of the items available in the computer test being repeated at each session providing multiple exposure to a small subset of the items. It is perhaps not surprising that, given some practice, the older males were to benefit from these repeated exposures more than the young males as it has been hypothesized that generational differences in the overlearning of addition tasks may influence performance on this test [30]. Overall, the similar cognitive performance at the end of exercise between age groups was paralleled by similar thermal (i.e., T_{re} and LSR at both sites) and cardiovascular (i.e., percent of maximum heart rate) responses in both the young and older males within each of the air velocity conditions. The lack of a significant difference in cognitive performance following exertional heat stress is consistent with other studies reporting minimal or no changes in cognitive function when fluid replenishment was provided to avoid dehydration [31] or with dehydration levels of 2.6% or less body mass loss [32]. Alternatively, Szinnai et al. [32] suggested that when dehydration is developed slowly, as in the case with the current study over the duration of 2-3 hours, healthy individuals are better able to accommodate to

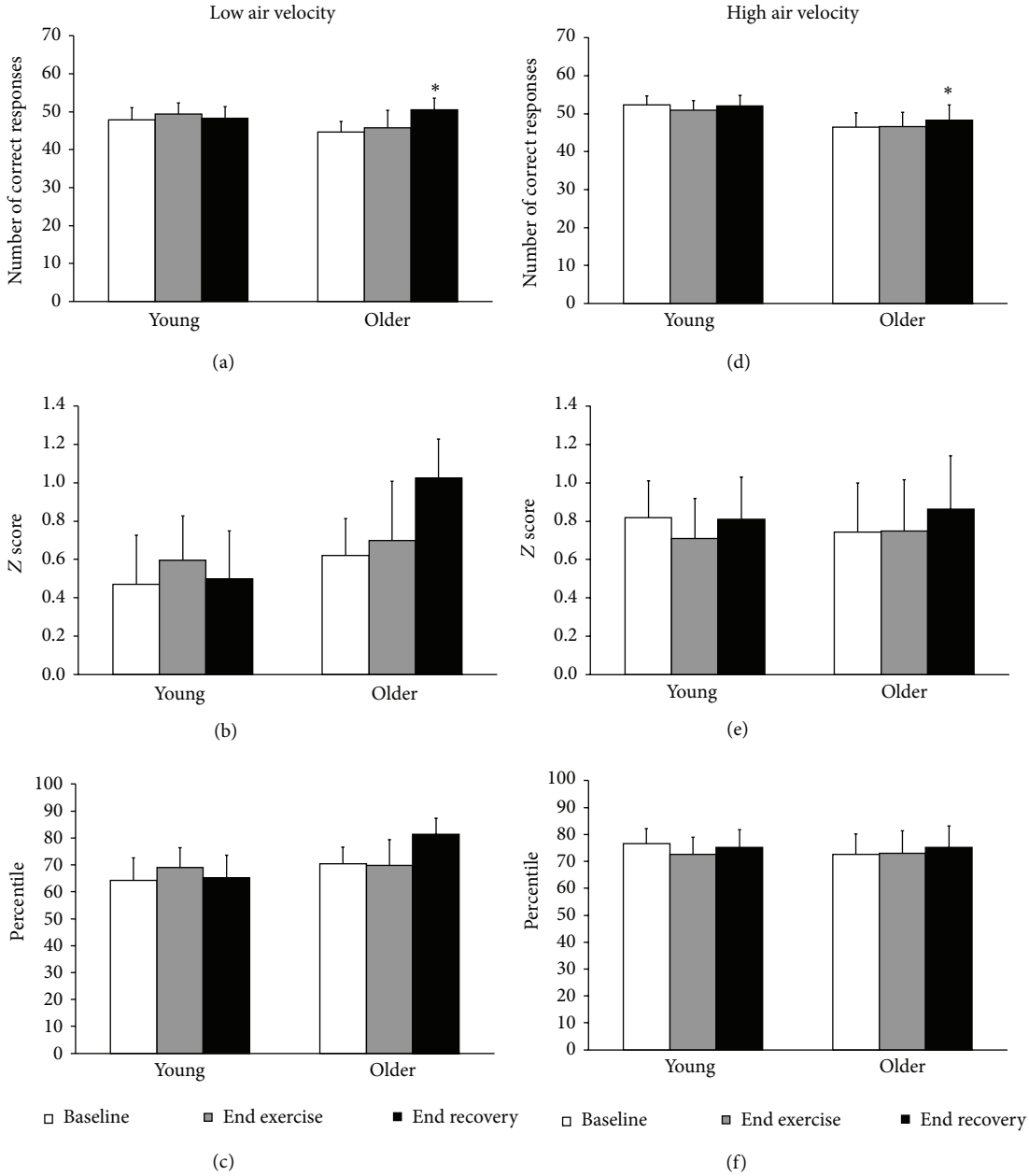

FIGURE 3: Paced auditory serial addition task # of correct responses ((a) and (d)); Z score relative to age norms ((b) and (e)); percentile relative to age norms ((c) and (f)) at baseline (white), end of the 4th exercise bout (end exercise; grey), and end of the final recovery (end recovery; black) in young and older males under low and high air velocity. Values are mean ± SE. ∗ Main effect of time; end recovery versus baseline and end exercise.

the increased tiredness and reduced alertness (e.g., exercise arousal effect) which mitigates any reductions in the capacity of information processing mechanisms. In the current study, % body mass changes were similar between the young and older males following the low (2.4 ± 0.2 and 2.7 ± 0.3%, resp.) and high (1.8 ± 0.1 and 1.9 ± 0.2%, resp.) conditions, similar to or lower than the changes reported by Szinnai et al. [32]. This was despite the high air velocity being effective in reducing the level of dehydration in both age groups (detailed hydration status changes have been previously published [13]).

The increase in air velocity during the high condition was also effective in reducing thermal (i.e., T_{re}, LSR at both skin sites) and cardiovascular (i.e., heart rate, percent of maximum heart rate) strain equally in both young and older males; however, the benefits of the high air velocity condition were not reflected in cognitive performance. Interestingly, Morley et al. [7] detected alterations in cognitive performance only at or beyond 1 hour after exercise, following 50 min of continuous treadmill exercise (i.e., walking 4.5 km·hr^{-1}) in young, active adults (~28 years) wearing thermal protective clothing. These alterations in cognitive performance were

at a high physiological stress where visceral temperatures were ~39.0°C and heart rates were ~170 bpm at the end of exercise. Thus, at the levels of thermal strain (i.e., T_{re} at end exercise of 38.0–38.3°C) and dehydration (i.e., 1.8–2.7% body mass loss) observed in the current study, young and older males were able to maintain attention during the conduct of speeded information processing without significant decrements in performance. This was despite anecdotal feedback from the participants in both groups that the PASAT was perceived as being more difficult during the low compared to high air velocity condition and the apparent presence of a practice effect for the older males. Decrements in cognitive performance, and potentially age-related differences, might have been observed with greater levels of dehydration (i.e., >3.0%), at greater levels of thermal strain (i.e., $T_{re} \geq 39.0$°C), with longer duration intermittent exercise, and/or with the completion of a full cognitive test battery that manipulates task difficulty, as compared with the partial administration of the PASAT used in the current study.

4.2. Evidence of Psychological Sweating. Eccrine sweating is most commonly associated with exercise and exposure to heat, in which thermal stressors are driving the glandular responses; however, nonthermal stresses, such as mental stress, anxiety, fear, and pain, can also result in increased sweating, also known as psychological sweating [20, 22, 33]. Administration of the PASAT has been demonstrated to be a reliable method of inducing psychological stress in the laboratory [34]. Older studies suggested that psychological sweating is only observed on the skin of the hands and feet [35, 36] and can be inhibited by thermal stimulation when both thermal and psychological stimuli are present [35]. Conversely, more recent studies have shown a larger distribution of psychological sweating in thermoneutral individuals [22] and when stimulated by passive heating [33]. Novel to the current study was the observation of psychological sweating following exertional heat stress given that the local sweating at both the forearm and upper back skin sites increased significantly as a result of the PASAT administration in both young and older males 30 min following the final exercise bout in the high air velocity condition. Therefore, the current findings dispute previous research which suggested psychological sweating to be inhibited by thermal stimuli [35], and concurred with more recent research that both thermal and psychological sweating can occur simultaneously [33]. Despite the similar sweat rates measured at both skin sites at the end of exercise prior to the PASAT, between the low and high air velocity conditions, the effectiveness of evaporative cooling was evident in the high air velocity condition as reflected in the significantly reduced core temperatures in both young and older males. Thus, for occupations that require sustained attention, vigilance, and ongoing information processing during work in the heat, it may be plausible that increased psychological sweating may subject workers to an increased sweat loss and thus risk of dehydration, when the environment permits effective evaporative heat loss from sweating.

5. Conclusions

Young and older males demonstrated similar performance on a test of attention and speeded information processing, paralleled by similar thermal and cardiovascular responses, prior to and following intermittent exercise at a fixed rate of heat production (400 W, moderate-to-heavy work as defined by the ACGIH guidelines) while wearing standard work coveralls in humid heat (35°C, 60% RH) within each of the low (0.5 m·s^{-1}) and high (3.0 m·s^{-1}) air velocity conditions. Young and older males also demonstrated similar cognitive performance between the low and high air velocity conditions despite the high air velocity significantly reducing thermal (i.e., T_{re} and LSR at both skin sites) and cardiovascular (i.e., heart rate and percent of maximum heart rate) strain equally in both young and older males. The psychological sweating in the young and older males at the end of recovery in the high but not the low air velocity condition may be indicative of a greater risk for increased dehydration under conditions which permit effective evaporative heat loss from sweating. Thus, no significant decrements in attention and speeded information processing were observed, with age or altered air velocity, following intermittent exercise in humid heat.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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Research Article

Human Monocyte Heat Shock Protein 72 Responses to Acute Hypoxic Exercise after 3 Days of Exercise Heat Acclimation

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The aim of this study was to determine whether short-term heat acclimation (STHA) could confer increased cellular tolerance to acute hypoxic exercise in humans as determined via monocyte HSP72 (mHSP72) expression. Sixteen males were separated into two matched groups. The STHA group completed 3 days of exercise heat acclimation; 60 minutes cycling at 50% $\dot{V}O_{2peak}$ in 40°C 20% relative humidity (RH). The control group (CON) completed 3 days of exercise training in 20°C, 40% RH. Each group completed a hypoxic stress test (HST) one week before and 48 hours following the final day of CON or STHA. Percentage changes in HSP72 concentrations were similar between STHA and CON following HST1 ($P = 0.97$). STHA induced an increase in basal HSP72 ($P = 0.03$) with no change observed in CON ($P = 0.218$). Basal mHSP72 remained elevated before HST2 for the STHA group ($P < 0.05$) and was unchanged from HST1 in CON ($P > 0.05$). Percent change in mHSP72 was lower after HST2 in STHA compared to CON ($P = 0.02$). The mHSP72 response to hypoxic exercise was attenuated following 3 days of heat acclimation. This is indicative of improved tolerance and ability to cope with the hypoxic insult, potentially mediated in part by increased basal reserves of HSP72.

1. Introduction

Heat acclimation induces an increase in basal stores of the evolutionarily conserved molecular chaperone heat shock protein 72 (HSP72) [1, 2]. Additionally, HSP72 is induced by exposure to hypoxia at rest in humans [3]. These data demonstrate a degree of commonality in stress adaption and thus the potential to exploit cross acclimation in humans in preparation for exposures to different physiological stressors.

An increase in the basal stores of HSP72 represents an improvement in a cell's ability to tolerate stress without the need for *de novo* protein synthesis [4] and is an accepted marker in an organism's adaptation to stress [5]. It is possible that invoking the heat shock response (HSR) via exposure to one stress may induce a degree of tolerance to a second, different stressor [6]. This cross acclimation is well documented *in vivo* and *in vitro* in animal models (for a review see Horowitz, 2007 [6]). For example, hemodynamic recovery is enhanced in heat acclimated animals exposed to a hypoxic stressor (such as ischemia reperfusion) compared to control animals [7]. However, to date very little is known about the evocation of acclimation *in vivo* in humans. Taylor et al. [8] indicated that increased basal stores of monocyte HSP72 (mHSP72) during 5 daily resting hypoxic exposures were associated with reduced oxidative stress after submaximal exercise in normoxia. However the absence of normoxic [8] or normothermic [1] controls in human *in vivo* studies often makes it difficult to determine whether the intervention alone led to the increase in mHSP72.

HSP72 can be released into the circulation in response to stress and may serve as part of the immune response [9, 10]. Changes in circulating HSP72 (eHSP72) have been proposed to have effects on the cytokine cascade and therefore affect the inflammatory response *in vivo* [11]. eHSP72 is released following exercise in an intensity and duration dependent manner [12, 13] and heat acclimation has been shown to

reduce basal eHSP72 and attenuate eHSP72 responses after subsequent exercise-heat stress [14–16]. However both the tissue of release and physiological relevance of eHSP72 release during exercise and following heat acclimation remain unclear. Furthermore, the response of eHSP72 to hypoxic exercise in humans remains undefined in the literature.

Heat acclimation is a complex process involving actions at both the whole body and the cellular level [17]. Regimens for humans are traditionally medium (8–14 days) or long term (>15 days) in their nature [18]. It is now accepted that many of the beneficial adaptations to heat stress are cardiovascular in nature, for example, reduced exercising heart rate (HR) and core temperature (T_{core}) and increased sweat rates, and occur rapidly over the initial 3–5 days of acclimation [19]. A 3-day heat acclimation protocol has also been shown to increase the basal levels of HSP72 mRNA alongside small increases in HSP72 protein [14]. Therefore shorter term protocols may be more appropriate and logistically easier to implement in preparation for exposure to hypoxic-based stressors.

To date, no research has examined whether in humans the initial phase of heat acclimation and the associated increase in basal levels of HSP72 may confer tolerance to a subsequent exposure to a different stressor. Thus the primary aim of this study was to determine whether short-term heat acclimation (STHA) in humans could induce an increase in basal mHSP72 and precondition against a subsequent bout of acute hypoxic exercise when compared to a normothermic control group. Secondly, the eHSP72 response to acute hypoxic stressors before and after STHA was examined. It was hypothesized that 3 days of heat acclimation would increase basal stores of mHSP72 and that the increased availability of mHSP72 would attenuate the response of this cytoprotective protein following a bout of acute hypoxic exercise.

2. Methods

2.1. Participant Characteristics. Sixteen healthy males provided signed informed consent prior to participation in this study, which was granted approval by the Coventry University Ethics committee, and were divided into 2 matched groups based on their aerobic capacity (Figure 1). All were physically active, nonsmokers with no prior history of cardiorespiratory illness. Laboratory attendance time was kept constant within each participant in order to minimize the effects of circadian variation on performance and the known diurnal variation in mHSP72 [20]. Caffeine [21] and alcohol consumption were barred from all meals and beverages for 72 h prior to each laboratory visit. Participants were required to maintain a food and activity diary as accurately as possible for 3 days prior to each experimental visit and then requested to replicate this prior to subsequent visits [22]. Additionally, participants refrained from all supplementation (i.e., vitamins, ergogenic aids) throughout the study period. Participants were requested to abstain from prolonged thermal exposures (baths, saunas, steam rooms, and tanning devices) and vigorous physical activity for seven days prior to the preliminary testing and throughout the remaining experimental program. Participants who had visited or resided at altitudes in excess of 1000 m [3] or climates with ambient temperatures in excess of 30°C [23, 24] or had experienced high pressure environments, for example, hyperbaria, within the three months prior to study commencement were excluded during recruitment due to the possible influence of such environments on basal HSP72 expression. Participants fasted for 2 hours prior to each trial and did not eat until the final blood withdrawal of each trial. Compliance for all the aforementioned experimental controls was monitored via questionnaires administered before, during, and after the extended experimental study period and was reported as 100% in all participants.

2.2. Experimental Design. Participants reported to the laboratory on six occasions, outlined in Figure 1. The first involved assessment of preliminary measures of anthropometry, lactate threshold, and $\dot{V}O_{2peak}$. Participants then returned 7 days later to undergo an exercise hypoxic stress test (HST; visit 2). At least 7 days after the HST participants completed 3 days of either STHA or control acclimation (CON; visits 3, 4, and 5) and returned 48 hours after the final acclimation session to complete a final HST (HST2, visit 6). A fractional inspired oxygen level of 0.14 (equivalent to ~3000 meters above sea level) was selected for all hypoxic trials and heat acclimation temperature of 40°C for the STHA group as they are close to the acute habitable limits for nonacclimatized individuals. These environmental conditions were chosen to reflect conditions regularly experienced on sojourns by athletic populations, adventure tourists, and the military.

2.3. Visit 1: Preliminary Testing. The initial visit involved preliminary tests for resting hemoglobin concentration, anthropometry to measure height, weight, and estimated body fat followed by the measurement of lactate threshold and peak oxygen uptake ($\dot{V}O_{2peak}$).

Peak oxygen uptake was determined using an incremental exercise test to volitional exhaustion on a cycle ergometer (Monark Ergomedic 874e, Monark Exercise AB, Vansbro, Sweden) whilst breathing room air. Resting blood lactate (Biosen C-Line Analyser, EKF Diagnostics, Germany) was determined from a finger capillary whole blood sample following a 10-minute seated rest period. The test began at a workload of 70 W for 4 minutes and was then increased by 35 W every 4 mins until a blood lactate value of >4 mmol·L^{-1} was reached. Thereafter, workload increased 35 W every 2 minutes until volitional exhaustion. A cadence of 70 rev·min^{-1} was maintained throughout. Expired gas was collected into 200 L Douglas bags between minutes 3 and 4 of each 4-minute stage and then minutes 1-2 of each 2-minute stage. Expired gas samples were analyzed to determine CO_2 and O_2 content, using a Servomex infrared and paramagnetic gas analyzer, respectively (model 1400, Servomex, Crowthorne, UK), and gas volume, via a Harvard Dry Gas meter (Cranlea and Company, Birmingham, UK). Peak oxygen consumption was considered to be achieved if at least two of the following criteria were met: (i) a respiratory exchange ratio of >1.1, (ii) a heart rate greater than 95% of age predicted maximum (220-age), and (iii) a final blood lactate

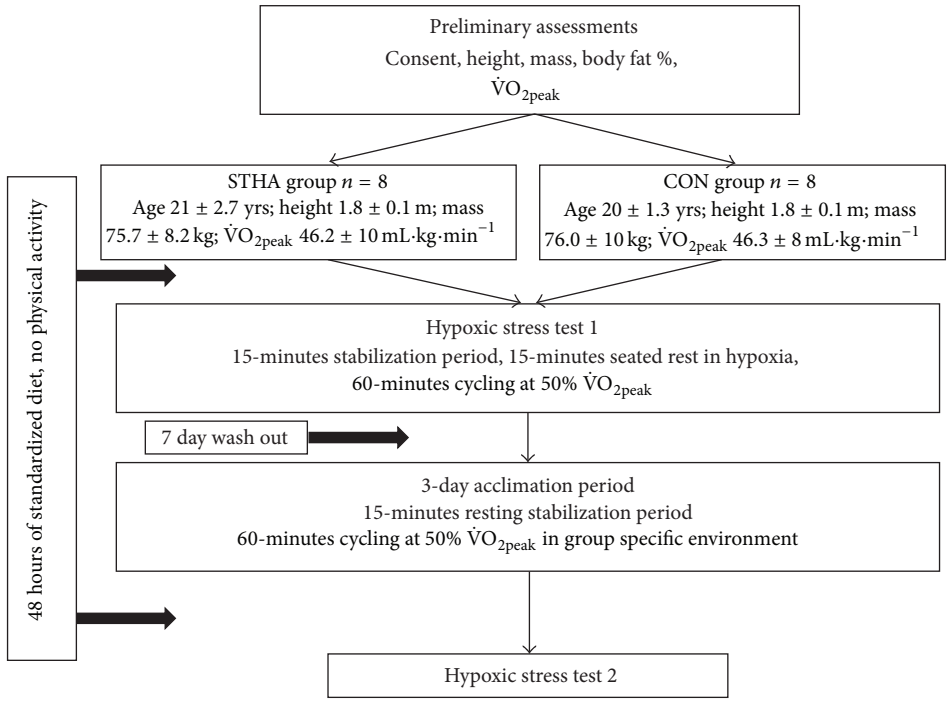


Figure 1: Experimental schematic. See text for details. STHA = short-term heat acclimation; CON = control.

value in excess of 8 mmol·L^{-1}. This protocol has shown a CV of <1.5% for oxygen consumption in our laboratory.

2.4. Participant Preparation. The remaining 5 laboratory visits consisted of identical procedures and measurements. Upon arrival to the laboratory participants voided their bladder to provide a sample for assessment of urine specific gravity (USG; Visual Refractometer, Index Instruments, Cambridge, UK) and osmolality (Osmocheck, Vitech Scientific, Partridge Green, West Sussex, UK). Participants were considered euhydrated if these values were <1.030 and 600 mOsmol·kg^{-1}, respectively [25]. They then measured their own nude body mass (Seca 899 scales, Seca, Hamberg, Germany), inserted a rectal thermometer 10 cm past the anal sphincter (Grant Instruments, Shepreth, UK), and attached a heart rate monitor to their chest (Suunto T6c, Suunto, Vaanta, Finland). Whilst seated, skin thermistors (Grant Instruments, Shepreth, UK) were fitted to the upper arm, upper thigh, chest, and calf using a micropore tape to enable continuous monitoring of skin temperature. Participants were then seated on a cycle ergometer (Monark Ergomedic 874e, Monark Exercise AB, Vansbro, Sweden) and completed a 15-minute resting period. At the end of the rest period a 7 mL venous sample was collected from an antecubital vein into potassium coated EDTA vacutainers (VACUETTE, Greiner Bio-One, Stonehouse, UK) for the immediate assessment of monocyte HSP72 (mHSP72). Heparinized capillary sample tubes were collected in triplicate and centrifuged (Hawksley Micro Hematocrit Centrifuge, Hawksley & Sons Ltd., UK) and hematocrit was determined by reading from the hematocrit reader (Hawksley Micro). Hemoglobin was determined via a calibrated B-Hemoglobin Photometer (Hemocue Ltd., UK) and corrected plasma volume was calculated [26].

2.5. Exercise Measurements. Participants completed 60 minutes of cycle exercise at an intensity corresponding to 50% normoxic $\dot{V}O_{2peak}$ in each of the 5 testing sessions. Measurement of heart rate, core and skin temperatures, arterial oxygen saturation (SpO_2) and recording of RPE [27], and thermal sensation (TS; [28]) were noted at the end of rest after the venous blood withdrawal and at 10-minute intervals throughout the exercise period. Arterial Hb oxygen saturation (SpO_2) was recorded during respiratory gas collections using a finger-clip pulse oximeter (3100 WristOx, Nonin Medical, Inc., Plymouth, MN, USA). The sensor has a reported accuracy of ±2 digits (manufacturers guide). Physiological strain was calculated using the physiological strain index (PSI; [28]). Sweat losses were determined by the change in nude body mass before and after exercise, with sweat volume and mass assumed as being the same (i.e., 1 mL = 1 g; [1]). Upon completion of each exercise bout a final venous sample (7 mL) was collected while participants remained on the cycle ergometer as previously described.

2.6. Visits 2 and 6: Hypoxic Tolerance Test. At least 5 days after the preliminary visit, participants returned to the laboratory for the baseline hypoxic stress test (HST1, visit 2 [29]). This procedure was repeated 48 hours after the final acclimation session (HST2, visit 6). After instrumentation, participants were seated on a cycle ergometer (Monark Ergomedic 874e) and completed a 15-minute normoxic resting period whilst

breathing through a mouthpiece and 30 mm diameter connector (Harvard Ltd, Eldenbridge, UK) attached to a two-way nonrebreathable valve (Harvard Ltd, Eldenbridge, UK). Ethylene clear vinyl tubing was used to connect the inspiratory side of the valve to a series of 1000 L Douglas bags. During all hypoxic trials the 1000 L Douglas bags were filled with hypoxic gas ($F_IO_{2,}$ = 0.14) generated by an oxygen filtration device (Hypoxico HYP123 hypoxicator, New York, USA) prior to the start of all testing. After the initial 15-minute normoxic rest period participants completed a further 15-minute resting wash-in period in hypoxic conditions and 60 minutes of cycle exercise at an intensity corresponding to 50% normoxic $\dot{V}O_{2peak}$. Physiological and subjective variables were collected after each 15-minute rest period and every 10 minutes throughout exercise. Venous samples were collected as previously described at the end of the normoxic rest period and immediately upon completion of exercise. Exercise was terminated if arterial oxygen saturation fell below 70% and heart rate reached 95% $\dot{V}O_{2peak}$ for 3 consecutive minutes or if the participant requested to stop the trial.

2.7. Visits 3, 4, and 5: Intervention Period. At least 1 week after the initial HST, participants reported to the laboratory to undergo 3 days of heat acclimation (40°C, 20% RH; STHA) or exercise training (18°C, 20% RH; CON). Venous samples were collected as previously described on day 1 and day 3 after a 15-minute seated rest period outside the heat chamber. A final venous sample was collected with participants remaining seated on the cycle ergometer as soon as exercise was terminated. Participants entered the environmental chamber and completed a further 15-minute rest period before commencing cycling at 70 rpm and against a resistance sufficient to elicit 50% normoxic $\dot{V}O_{2peak}$ for 60 minutes [30, 31]. Physiological and subjective variables were collected at the end of the rest period and at 10-minute intervals throughout exercise.

2.8. Monocyte HSP72 Concentrations. An IgG1 isotype and concentration-matched FITC-conjugated negative control were used in order to assess nonspecific binding. Briefly, cells obtained after red cell lysis were fixed and permeabilised (AbD Serotec, UK) and a negative control (FITC, AbD Serotec, UK) or FITC-conjugated anti-HSP72 antibody (SPA-810, clone-C92F3A-5, Assay Designs, USA) was added to a final concentration of 100 μg/mL; this was used to label 1×10^6 cells according to the manufacturer's instructions and then incubated for 30 min in the dark. Samples were then analysed on a BD FACSCalibur (BD Biosciences) by flow cytometry with monocytes gated for forward/side scatter properties and further discriminated separately by CD14 expression in order to objectively determine the correct gate position for each participant sample. Mean florescence intensity (MFI) was calculated using CellQuest Pro software (BD Biosciences) with a total of 15000 cells counted. Fluorescence gained with the anti-HSP72 antibody divided by the fluorescence gained with the isotype-matched negative control. Results are reported as percentage change in MFI.

2.9. Circulating HSP72. eHSP72 was analysed with a preprepared enzyme-linked immunosorbent assay (ELISA) kit (StressExpress HSP72 ELISA kit, Stressgen Bioreagents, Canada). The HSP72 concentration was assessed via sample absorbance at 450 nm using a microplate reader (ELx800, BioTek Instruments, Inc., USA) and the KC Junior software package (v.1.41.3, BioTek Instruments, Inc., USA). A log-to-log scale of recombinant HSP72 standard concentration and absorbance measures were plotted to determine a line of best fit. The linear equation generated was then used to obtain inducible HSP72 concentration ($ng \cdot mL^{-1}$) from the absorbance of each sample. The sensitivity of the ELISA kit was 500 $pg \cdot mL^{-1}$ and both the inter- and intra-assay coefficient of variation was less than 10% (StressExpress HSP72 ELISA kit, Stressgen Bioreagents, Canada).

2.10. Data Analysis and Statistics. All statistical procedures were carried out using SPSS (version 20). Data are presented as means (SD) in the text and tables and as means (SD) in the figures. The primary outcome variables of interest in this experiment were the mHSP72 and eHSP72 responses to the HST.

One participant in the CON group was below the detection limit of the eHSP72 assay at rest throughout all trials and was removed from the analysis. Two members of STHA were below the detection limit of the eHSP72 assay prior to HA3 and were removed from the statistical analysis for the 3-day acclimation period.

All data was checked for skewness and kurtosis prior to analysis. A mixed model two-factor repeated measures ANOVA was used to make all group x time comparisons throughout each HST and to assess between and within group differences upon completion of the first and last acclimation day. F values were adjusted for sphericity where appropriate, and main and interaction effects were investigated by Tukey's HSD test. In order to investigate the relationship between preexercise and postexercise induced expression of mHSP72, a linear regression analysis was performed. Effect sizes for changes in mHSP72 were calculated using Cohen's d and used to compare the effectiveness of the STHA intervention with CON. The significance level for statistical tests was set at $P < 0.05$.

3. Results

3.1. Physiological and Perceptual Responses to the Acclimation Period. Participants in both groups were considered hydrated prior to each acclimation session with no differences in nude body mass (CON: day 1 = 75.8 ± 10.4 kg, day 2 = 75.8 ± 10.2 kg, day 3 = 75.9 ± 10.4 kg; STHA: day 1 = 77.4 ± 6.4 kg, day 2 = 77.4 ± 7.1 kg, day 3 = 77.3 ± 6.5 kg; $P > 0.05$) or USG (CON: day 1 = 1.009 ± 0.004, day 2 = 1.011 ± 0.007, day 3 = 1.008 ± 0.010; STHA: day 1 = 1.006 ± 0.005, day 2 = 1.010 ± 0.006, day 3 = 1.009 ± 0.008; $P > 0.05$) upon arrival to the laboratory each day. All participants in CON ($n = 8$) completed the full 60 minutes of cycling on each acclimation day. In STHA, 3 of 8 participants failed to complete the 60 minutes on day 1 (mean ± SD; 55.5 ± 6.2 mins) compared to 1

Table 1: Peak and mean exercising (mean ± SD) physiological and thermoregulatory measures during the 3-day acclimation period tests for the control (CON) and short-term heat acclimation (STHA) groups.

Measure	Change in NBM (kg)	Peak HR (bts·min^{-1})	Mean HR (bts·min^{-1})	Peak T_{core} (°C)	Mean T_{core} (°C)	Peak T_{skin} (°C)	Mean T_{skin} (°C)	Peak T_{body} (°C)	Mean T_{body} (°C)	Peak PSI (A.U)	Mean PSI (A.U)
CON											
Day 1	0.6 ± 0.2	151 ± 21	144 ± 18	38.0 ± 0.2	37.7 ± 0.5	33.8 ± 1.3	33.0 ± 1.0	37.2 ± 0.2	36.8 ± 0.2	5.5 ± 1.1	4.6 ± 0.8
Day 2	0.6 ± 0.3	151 ± 23	142 ± 19	38.0 ± 0.2	37.7 ± 0.5	33.7 ± 1.0	32.6 ± 1.3	37.0 ± 0.5	36.7 ± 0.2	5.6 ± 1.3	4.5 ± 0.8
Day 3	0.6 ± 0.4	149 ± 23	143 ± 20	38.1 ± 0.4	37.7 ± 0.2	33.6 ± 0.9	32.8 ± 0.7	37.2 ± 0.3	36.7 ± 0.3	5.7 ± 1.4	4.5 ± 0.9
STHA											
Day 1	1.2 ± 0.5*	180 ± 13*	165 ± 14*	38.8 ± 0.3*	38.1 ± 0.2*	36.6 ± 1.0*	35.9 ± 1.0*	38.3 ± 0.3*	37.6 ± 0.3*	8.3 ± 1.0*	6.0 ± 0.8*
Day 2	1.4 ± 0.5*	176 ± 13*	162 ± 14*	38.7 ± 0.4*	38.0 ± 0.2*	36.6 ± 0.9*	36.1 ± 0.4*	38.3 ± 0.4*	37.6 ± 0.2*	8.1 ± 1.2*	5.9 ± 0.9*
Day 3	1.5 ± 0.5*	173 ± 13*	160 ± 13*	38.5 ± 0.3*	37.8 ± 0.1*	36.2 ± 1.0*	35.7 ± 0.7*	38.1 ± 0.4*	37.4 ± 0.3*	7.8 ± 1.1*	5.7 ± 0.8*

NBM = nude body mass; PSI = physiological strain index. *Difference between experimental groups ($P < 0.01$).

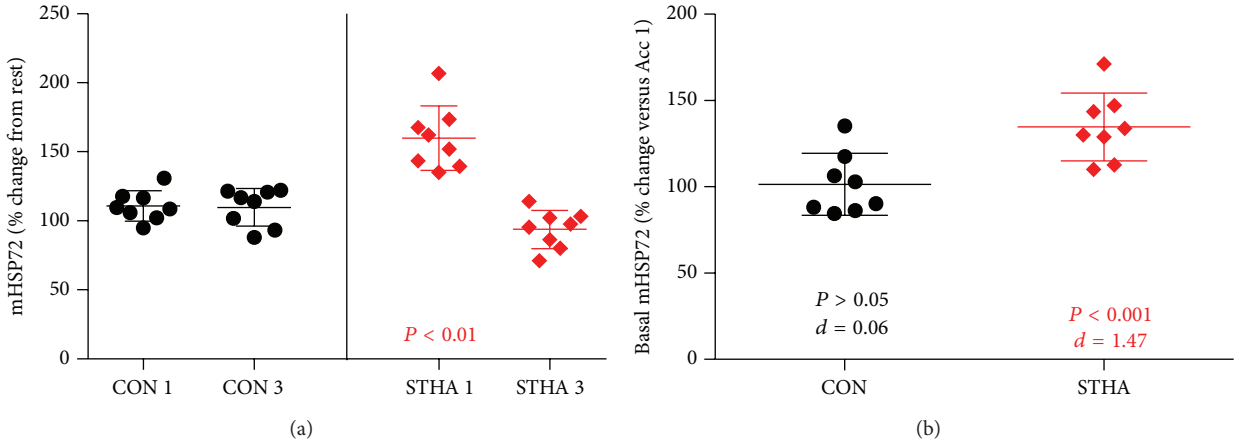


Figure 2: (a) Fold change in mHSP72 compared to resting baseline on intervention days 1 and 3 in the CON group (black circles) and STHA group (red diamonds). Post-HA1 mHSP72 was unaltered in the CON group and elevated in the STHA group ($P < 0.01$). Postexercise mHSP72 expression was attenuated following HA3 compared to postexercise HA1 in the STHA group ($P < 0.05$). (b) Basal mHSP72 remained unchanged throughout the acclimation period in the CON group ($P > 0.05$) and was elevated at baseline on HA3 in the STHA group ($P < 0.001$).

of 8 on the final day of acclimation (58.5±4.2 mins). Mean and peak HR, T_{rec}, T_{skin}, T_{body}, PSI, RPE, and TS were significantly higher during all 3 days of acclimation in STHA compared to the CON group ($P < 0.01$) (Table 1). Mean and peak HR, T_{rec}, T_{skin}, T_{body}, PSI, RPE, and TS did not vary from HA1 to HA3 in either group ($P > 0.05$; Table 1). Sweat rates were unchanged throughout the acclimation period in CON (day 1 = 0.55 ± 0.18 L·hour^{-1}, day 2 = 0.60 ± 0.33 L·hour^{-1}, day 3 = 0.61 ± 0.38 L·hour^{-1}; $P > 0.05$) and were higher in STHA compared to CON ($P < 0.01$). Sweat rate increased throughout the acclimation period in STHA (day 1 = 1.20±0.46 L·hour^{-1}, day 2 = 1.42 ± 0.74 L·hour^{-1}, day 3 = 1.48 ± 0.5 L·hour^{-1}; $P < 0.05$ versus HA1). Baseline plasma volumes were 52.9 ± 2.7% and 54.6 ± 2.9% in CON and STHA, respectively. Resting plasma volume was unchanged throughout acclimation in CON (day 2 = −1.1 ± 5.1%; day 3 = 1.0 ± 4.0%; $P > 0.05$). Plasma volume expansion was evident prior to HA3 in STHA; though this was highly variable (day 2 = 1.8 ± 3.9, day 3 = 4.6 ± 5.7%; $P < 0.05$).

3.2. mHSP72 Responses to the Acclimation Period. Resting mHSP72 did not vary between groups on HA1 ($P > 0.05$). mHSP72 increased immediately following HA1 STHA (58 ± 27%, $P < 0.01$, Figure 2(a)) but not CON (11 ± 11%, $P > 0.05$, Figure 2(a)). Resting mHSP72 was elevated from pre-HA1 to pre-HA3 in STHA (31 ± 23%, $P < 0.001$; $d = 1.47$, Figure 2(b)) and remained unchanged in CON (2 ± 18%, $P > 0.05$; $d = 0.06$, Figure 2(b)). mHSP72 was not elevated from rest following exercise on HA3 in either group ($P > 0.05$, Figure 2(a)). Postexercise mHSP72 on HA3 was lower compared to the postexercise data on HA1 for the STHA group ($P < 0.05$, Figure 2(a)). Regression analysis showed a negative relationship in the STHA group between preexercise expression and the magnitude increase (% change) in mHSP72 on HA1 ($R^2 = -0.66$, $P = 0.014$), which was weakened following HA3 ($R^2 = 0.19$, $P = 0.278$).

3.3. Circulating HSP72 Responses to the Acclimation Period. eHSP72 remained unchanged from rest following exercise

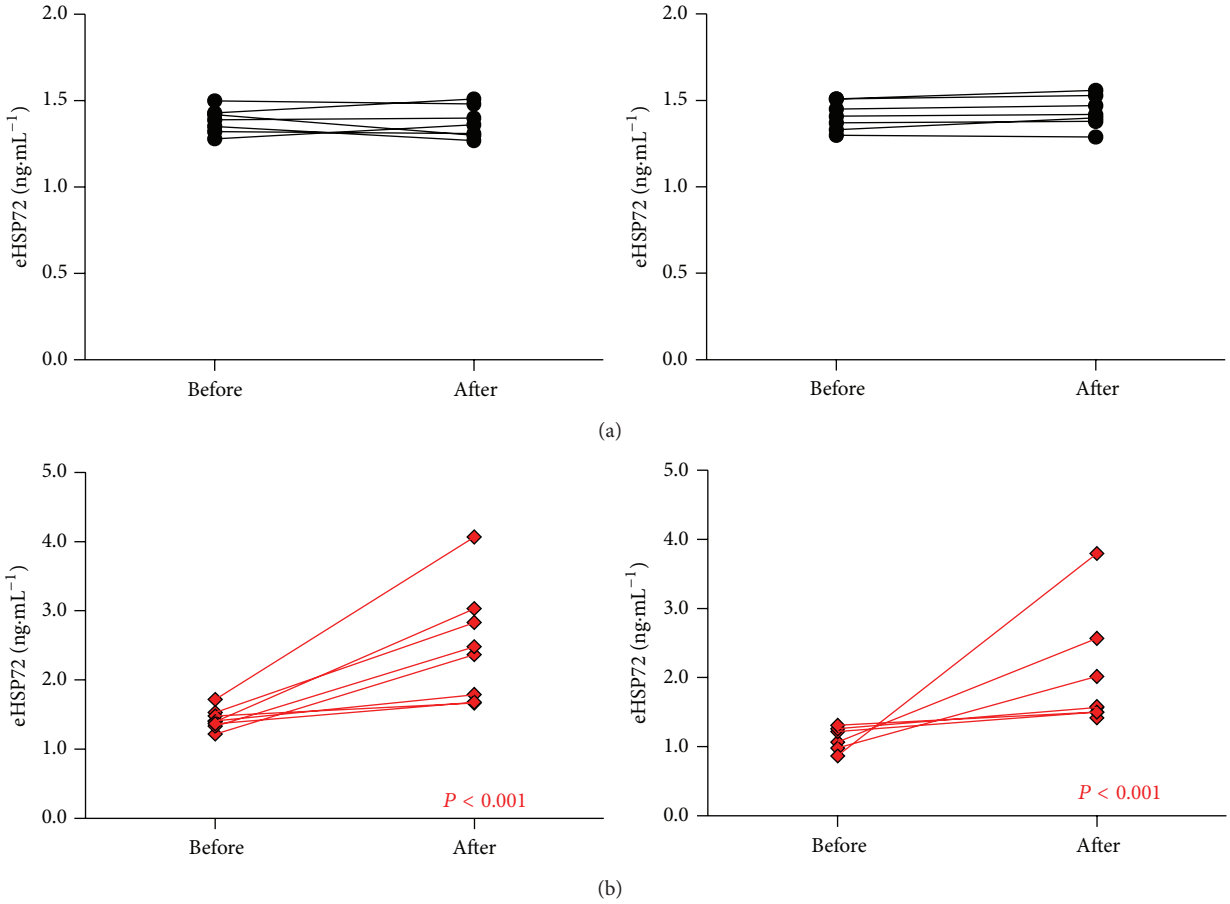


FIGURE 3: The intervention period did not induce any changes in postexercise eHSP72 in CON (black lines (a)). Postexercise eHSP72 was elevated from rest after exercise on HA1 and HA3 in the STHA group ($P < 0.001$, red lines (b)). Lines represent individual data.

on each day of the 3-day protocol in the CON group ($P > 0.05$, Figure 3(a)). eHSP72 increased following exercise on day 1 and day 3 of the acclimation period (day 1: 1.06 ± 0.74 ng·mL^{-1}; day 3: 1.04 ± 1.06 ng·mL^{-1}; $P < 0.001$) in STHA (Figure 3(b)). Resting eHSP72 was lower ($n = 6$) on day 3 of STHA compared to day 1 (day 1: 1.43 ± 0.15 ng·mL^{-1}; day 3: 1.12 ± 0.54 ng·mL^{-1}), although this observation failed to reach statistical significance due to the high intersubject variability ($P > 0.05$; Figure 3(b)).

3.4. Physiological Responses to the Hypoxic Stress Test. Heart rate was reduced in HST2 compared to HST1 ($P = 0.019$); however there was no trial x group interaction ($P > 0.05$). HR was lower in HST2 from HST1 at 20–30 minutes for CON ($P < 0.05$, Figure 4(a)) and 20–60 mins for STHA ($P < 0.05$, Figure 4(b)). SpO_2 was higher between 20 and 30 mins in CON ($P < 0.05$, Figure 4(a)) and throughout exercise in HST2 compared with HST1 in STHA ($P = 0.006$, Figure 4(b)), although no trial x group interaction was observed ($P > 0.05$) (Figure 4). SpO_2 data may have been affected by erroneous measurements during minutes 20 and 30 of HST2 in the CON group. One participant displayed fluctuating and unusually high SpO_2 values in this time due to equipment malfunction, which was immediately corrected once identified and a replacement used. As this occurred during HST2 it was impractical to retest the participant. Removing the spurious data point affects the significance observed for SpO_2 during the CON trial ($P > 0.05$).

3.5. Thermoregulatory Responses to the Hypoxic Stress Test. T_{core} was reduced and T_{skin} elevated during HST2 compared to HST1 for both experimental groups ($P < 0.05$; Figures 5(a1), 5(a2), 5(b1), and 5(b2)) whereas T_{body} did not vary between HST1 and HST2 ($P > 0.05$; Figures 5(c1) and 5(c2)). No trial x group interaction was found for rectal, mean skin, or mean body temperature ($P > 0.05$). Physiological strain was reduced during HST2 compared to HST1 in both groups ($P < 0.01$; Figures 5(d1) and 5(d2)) with no trial x group interaction observed ($P > 0.05$; Table 2).

3.6. Subjective Responses to the HST. RPE ($P = 0.04$) and TS ($P = 0.02$) were lower during HST2 compared to HST1 for both groups in comparison to HST1, with no trial x group interaction being observed ($P = 0.17$).

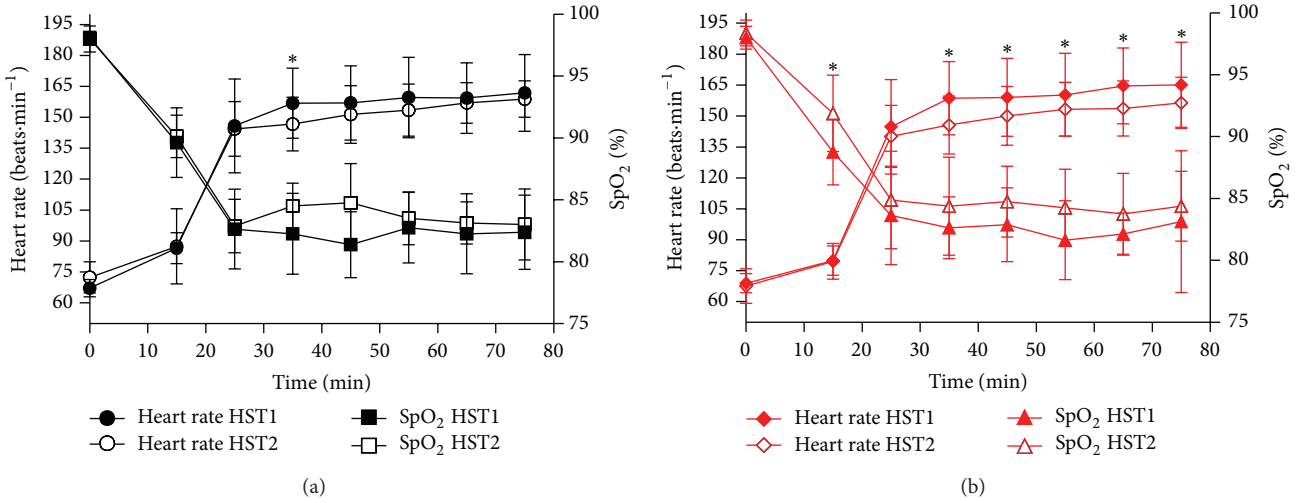


Figure 4: Heart rate was lower and SpO_2 was higher between 20 and 30 minutes of exercise during HST2 in the CON group (a). However when an erroneous value from a participant is removed from the analysis no difference in SpO_2 is present (see inset of (a)). Heart rate was lower and SpO_2 higher in HST2 compared to HST1 for the STHA group throughout the exercise period ($^*P < 0.05$ versus HST1). Data are mean ± SD.

Table 2: Peak and mean exercising (mean ± SD) physiological and thermoregulatory measures for the pre- (HST1) and postacclimation (HST2) hypoxic stress tests for the control (CON) and short-term heat acclimation (STHA) groups.

Measure	Change in NBM (kg)	Peak HR (bts·min^{-1})	Mean HR (bts·min^{-1})	Peak T_{core} (°C)	Mean T_{core} (°C)	Peak T_{skin} (°C)	Mean T_{skin} (°C)	Peak T_{body} (°C)	Mean T_{body} (°C)	Peak PSI (A.U)	Mean PSI (A.U)
CON											
HST1	0.45 ± 0.3	162 ± 19	157 ± 15	38.1 ± 0.4	37.8 ± 0.4	33.5 ± 1.2	32.6 ± 1.0	37.2 ± 0.4	36.8 ± 0.5	6.0 ± 1.2	5.3 ± 1.2
HST2	0.50 ± 0.2	160 ± 8*	154 ± 12*	37.9 ± 0.3*	37.8 ± 0.3*	34.0 ± 1.0*	33.4 ± 1.3*	37.3 ± 0.1	36.9 ± 0.4	5.8 ± 0.8*	4.9 ± 1.1*
STHA											
HST1	0.51 ± 0.2	165 ± 20	159 ± 20	38.1 ± 0.4	37.8 ± 0.4	33.1 ± 0.8	32.4 ± 0.5	37.1 ± 0.4	36.7 ± 0.5	6.4 ± 1.6	5.3 ± 1.5
HST2	0.81 ± 0.2	156 ± 12*	150 ± 14*	37.8 ± 0.3*	37.6 ± 0.3*	33.7 ± 1.3*	33.3 ± 1.1*	37.0 ± 0.3	36.8 ± 0.4	5.6 ± 0.9*	4.8 ± 1.2*

*Difference between HST1 and HST2 ($P < 0.05$).

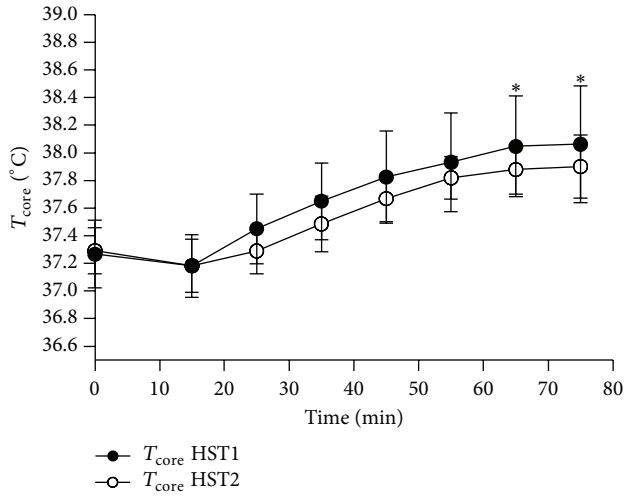
3.7. mHSP72 Responses to Hypoxic Stress Test. The initial HST produced an increase in mHSP72 in CON (34 ± 51%) and STHA (39 ± 37%). This response was not different between groups ($P > 0.05$). Resting mHSP72 was elevated from HST1 to HST2 in STHA (28 ± 26%, $P < 0.05$; $d = 0.94$) and unchanged for CON (3 ± 27%; $d = -0.08$). The mHSP72 response to HST2 was similar to HST1 in the CON group (48 ± 30%). STHA attenuated in post-HST2 mHSP72 expression compared to HST1 (98 ± 12%; $P < 0.05$) and was lower after exercise in STHA compared to CON ($P < 0.05$; $d = -0.45$) (Figure 6). Significant correlations were observed for the preexercise mHSP72 expression and the percentage change in expression following exercise for HST1 in both the CON and STHA groups (Figure 7(a)) and were also present during HST2 for the CON group (Figure 7(b)). This relationship was weakened for HST2 in the STHA group (Figure 7(b)).

3.8. Circulating HSP72 Responses to the Hypoxic Stress Test. eHSP72 increased to a similar magnitude following HST1 and HST2 in the CON group (HST1: 0.35 ± 0.29 ng·mL^{-1}; HST2: 0.55 ± 0.40 ng·mL^{-1}) and STHA group (HST1: 0.51 ± 0.35 ng·mL^{-1}; HST2 0.32 ± 0.34 ng·mL^{-1}; Figure 8). The eHSP72 response to the HST1 was smaller and less variable compared to the response to an acute heat stressor (HA1: 1.06±0.74 ng·mL^{-1}; HST1 0.51±0.35 ng·mL^{-1}).

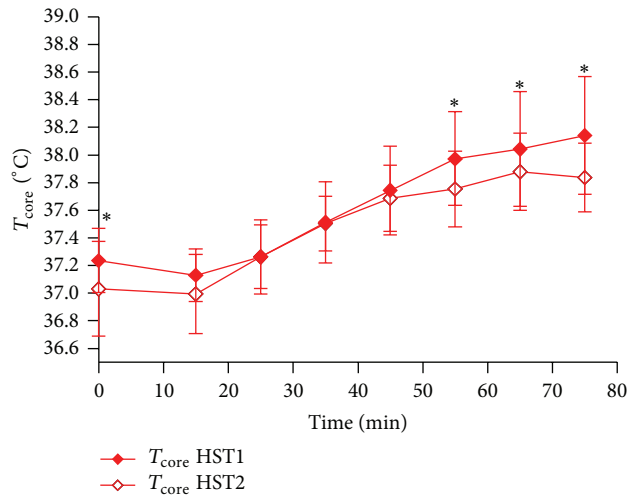
4. Discussion

To the author's knowledge this is the first *in vivo* human study examining the phenomenon of cross acclimation between heat and hypoxic stressors during the initial phase of heat acclimation. The key findings of the study were that 3 daily exercise-heat exposures were sufficient to increase basal mHSP72 stores. The increase in basal mHSP72 observed prior to HA3 was present prior to the onset of HST2 in the STHA group resulting in an attenuation of hypoxia mediated mHSP72 expression after exercise. These results support the experimental hypothesis and indicate that STHA has potential for improving cellular tolerance to acute hypoxic exercise.

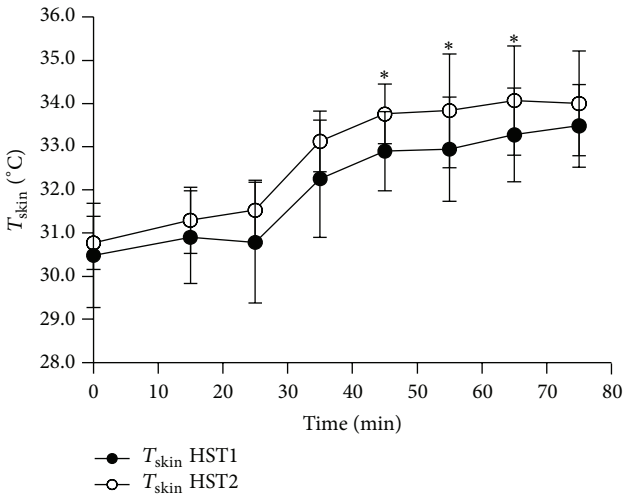
4.1. Attainment of Heat Acclimation. An important methodological aspect of the present study was the capacity of



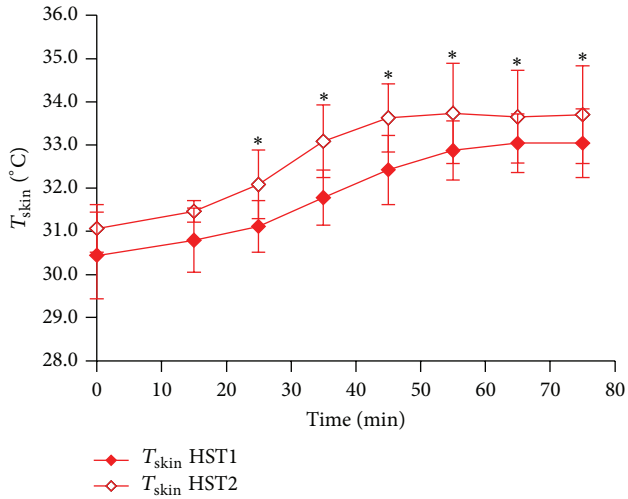
(a1)



(a2)



(b1)



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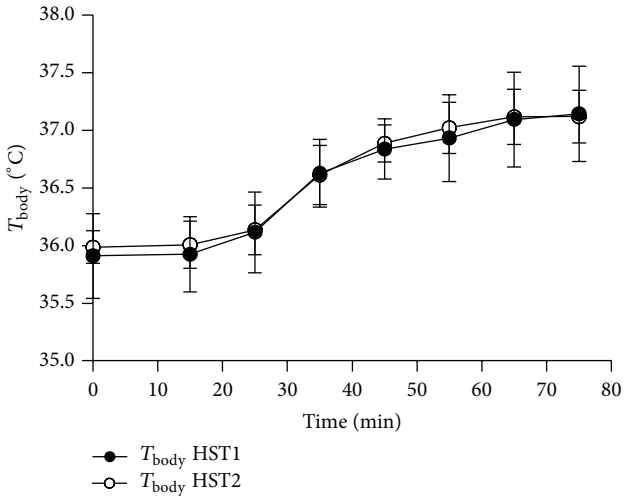
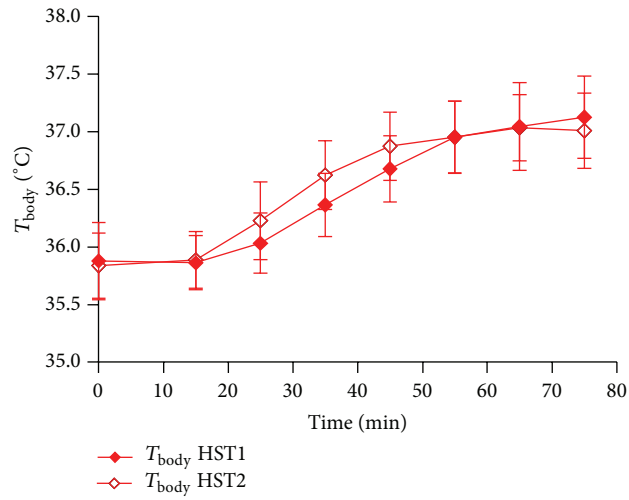


(c1)



(c2)

Figure 5: Continued.

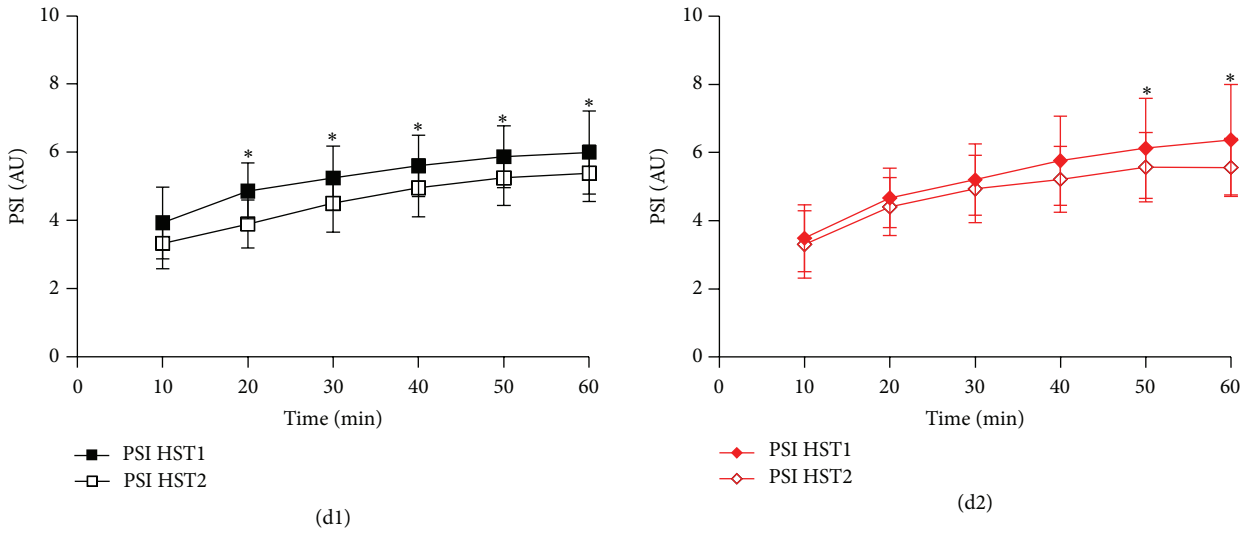


FIGURE 5: Thermoregulatory responses to the HST. T_{core} was lower in HST2 at 50–60 minutes in CON and from 40 minutes in STHA ($P < 0.05$). T_{skin} was elevated during HST2 from 30 to 50 minutes in CON and 20–60 minutes in STHA ($P < 0.05$). T_{body} was unchanged between HST1 and HST2. PSI was reduced from 20 to 60 minutes in CON and at 50 and 60 minutes in STHA ($P < 0.05$).

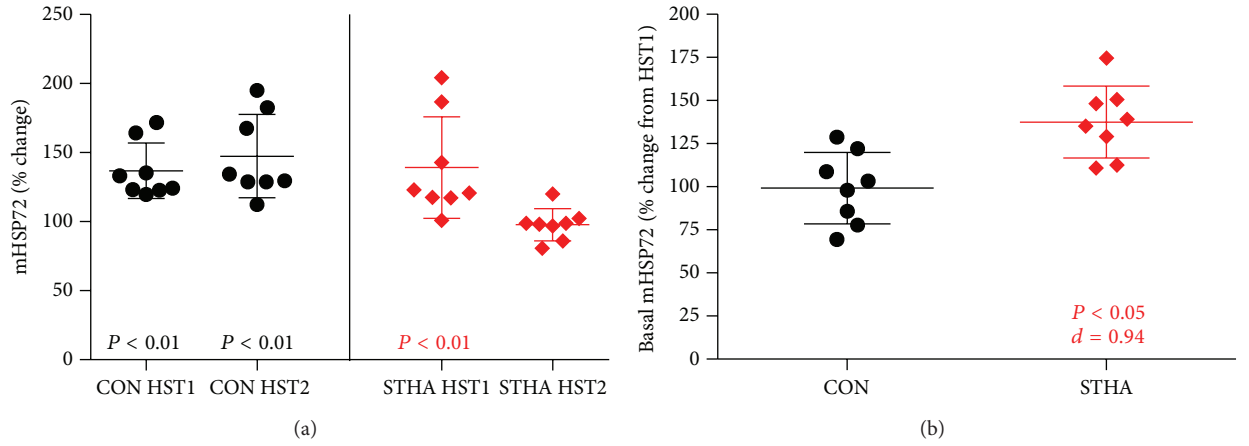


FIGURE 6: (a) Fold change in mHSP72 compared to resting baseline on HST1 and HST2 in the CON group (black circles) and STHA group (red diamonds). Post-HST1 mHSP72 elevated from baseline in both experimental groups ($P < 0.01$). mHSP72 was elevated from rest after HST2 in the CON group ($P < 0.01$) and attenuated in the STHA group. (b) Basal mHSP72 remained unchanged between HST1 and HST2 in the CON group ($P > 0.05$) and was elevated prior to HST2 in the STHA group ($P < 0.05$, $d = 0.94$).

3 days of repeated heat exposures in initiating heat acclimation adaptations. The STHA group displayed the classically described reductions in exercising HR and exercising core temperature, reduced overall physiological strain, and increased sweat rates (Table 1). The magnitudes of peak exercising reductions in the STHA group for HR (~7 beats·min^{-1}), T_{core} (~0.3°C), and PSI (~0.5 A.U) are less than those observed following an identical acclimation protocol in similarly trained participants (~14 beats·min^{-1}; 0.4°C; 1.6 A.U; [31]). Our results are similar to those reported by Marshall et al. ([14]; ~9 beats·min^{-1}, 0.2°C, 0.7 A.U) in participants that were described as heat acclimated following 3 repeated heat exercise exposures. This suggests that our STHA group was in the initial phases of heat acclimation.

4.2. Monocyte and Circulating HSP72 Responses to Heat Acclimation. Prior to the commencement of STHA (7 days after HST1), basal mHSP72 values had returned to those observed before HST1 in both groups. This is experimentally important, as the magnitude of HSP72 response to a stressful insult has been shown to be proportional to its basal content prior to stressful insults [32]. mHSP72 was increased from baseline following the initial day of heat exposure in the STHA group as previously observed following acute heat exposure (Figure 2(a); [33]). The mHSP72 response occurred to a similar magnitude when sampled at similar time points to those reported in other studies [33, 34]. After 2 acclimation days resting mHSP72 remained elevated (30 ± 23%; $d = 1.47$, Figure 2(b)) compared to values recorded at rest on day 1

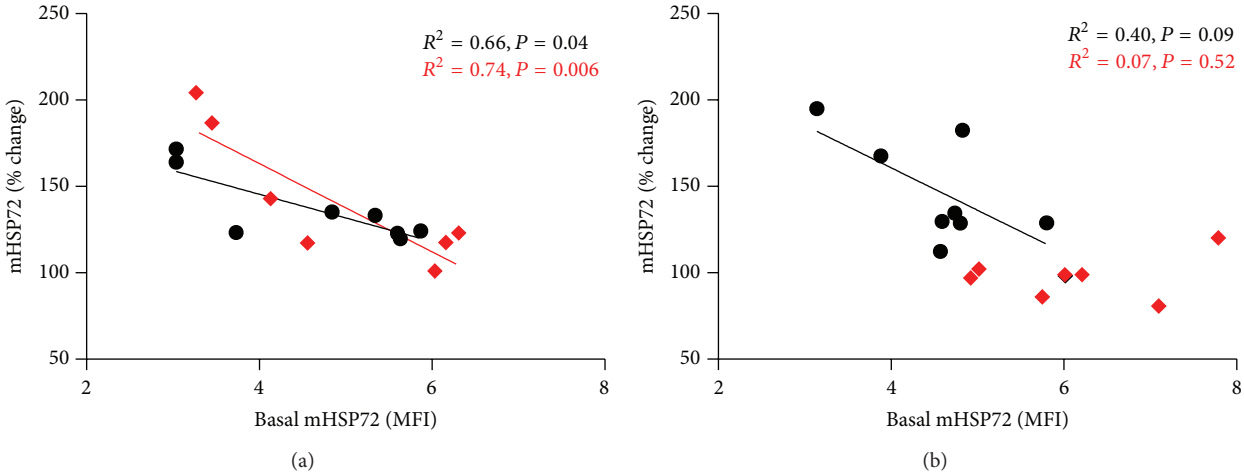


Figure 7: Regression analysis between preexercise monocyte expression of mHSP72 and the fold change (%) in mHSP72 following HST1 (a) and HST2 (b). Black circles denote CON and red diamonds denote STHA. Prior to HST1 the percentage change in mHSP72 after exercise had an inverse relationship with basal mHSP72. This feature was present in both CON (black circles) and STHA (red diamonds). After the intervention period the inverse relationship was still present in the CON (b), but no longer a feature of the STHA group, possibly as a result of the increase in basal mHSP72 observed prior to onset of HST2 (Figure 6(b)).

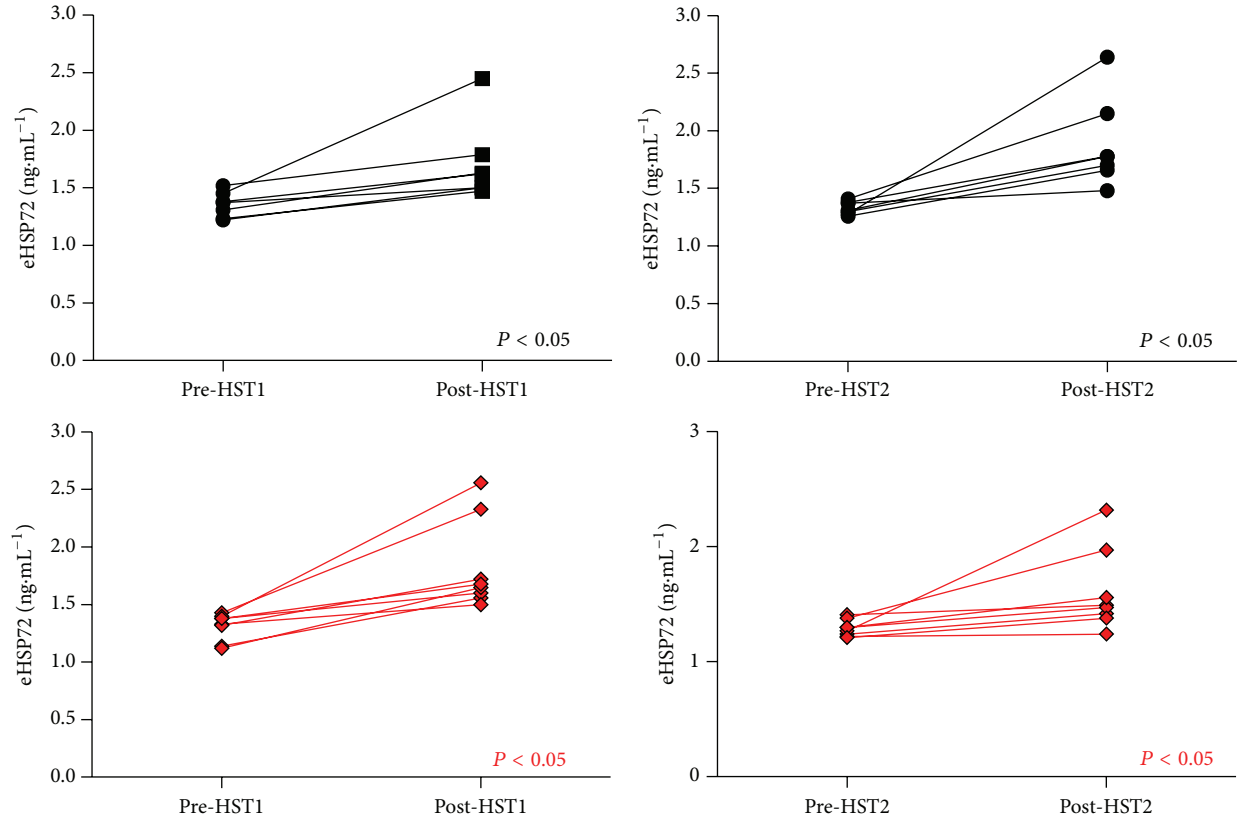


Figure 8: eHSP72 before and immediately after HST1 and HST2. Lines represent individual participants. Postexercise eHSP72 was elevated after exercise in both groups following each HST.

of the acclimation period. This is similar to data reported in previous studies. For example, a 40% increase in basal lymphocyte HSP72 has been observed after 4 days of walking for 90 minutes in 33°C, 30–50% humidity [35], and increases of ~30% in mHSP72 MFI were observed 24 hours after 60 minutes of running in hot conditions (60 minutes at 90% of lactate threshold velocity, 28°C; [33]). A negative relationship between basal mHSP72 and the magnitude of postexercise

change was observed in the current study on the first day of the HA period ($r = -0.81$, $P = 0.014$). This is in line with the accepted inverse relationship between basal mHSP72 and its induction via a stressor [32]. Following 3 days of exercising heat exposure this relationship was weakened ($r = -0.44$, $P = 0.28$). The increase in basal levels during the present investigation blunted the mHSP72 response following HA3 (94 ± 14%, Figure 2(a)), which is an accepted characteristic in the shift towards a heat acclimated state [1, 15, 36] and indicative of increased cellular tolerance [6, 36].

Resting eHSP72 has been shown to decrease following the initial 2 days [2], 5 days [37], and 11 days [36] of exercise-heat acclimation. Our data appear to follow this trend (Figure 3(b)) but did not reach statistical significance. Two participants in the heat group recorded levels below the detection level of the assay at rest on HA3. It is worth noting that these participants displayed the largest postexercise changes in eHSP72 immediately after HA3. This follows the observed trend in intracellular mHSP72 responses in that greater magnitudes in expression are observed in those with lower basal values. It is unclear whether a similar relationship exists for eHSP72 release. Postexercise eHSP72 was still significantly increased from rest on day 3, though the magnitude of this increase was smaller. The inhibition of eHSP72 release/production was also observed following a 5-day HA protocol, but only in participants that displayed classical signs of heat acclimation (reduced exercise HR and T_{core}; [37]). A similar response was observed after a heat stress test (90 minutes running at 50% $\dot{V}O_{2peak}$ using a controlled hyperthermia protocol) after 11 days of heat acclimation [36]. The release of eHSP72 has been shown to be both intensity and duration dependent [13] and also requires a minimum level of external stress to induce its appearance/release [38]. The 3-day STHA period in this present study reduced the level of both thermal and cardiovascular strain, as evidenced by the reduced exercising HR and T_{rec}, (Table 1); thus it is possible that the external conditions experienced by participants on HA3 were no longer sufficient to activate a similar response in eHSP72. That the CON group displayed minimal changes to these variables indicates that it is reasonable to conclude that the heat load and the consequent increase in physiological strain experienced by the STHA group were of sufficient magnitude to induce a shift towards the acclimated phenotype.

4.3. Monocyte HSP72 Responses to the Hypoxic Stress Test. Cross-tolerance between heat acclimation and oxygen deprivation stressors is well documented within animal models [39, 40]. In accordance with previous research [3, 8, 41], mHSP72 increased following the first acute hypoxia exposure in both CON (33 ± 37%; Figure 6(a)) and STHA (39 ± 17%; Figure 6(a)). A similar magnitude in mHSP72 response was observed following an identical hypoxic challenge within our laboratory [29]. It is likely that the increased oxidative stress associated with acute hypoxia and the subsequent damage to membrane structures and proteins, while activating apoptotic pathways, act as stimuli for HSP72 induction during hypoxia [3, 42, 43]. That the postexercise values reported in this present study are lower than those reported by an acute-resting intervention [3, 8] is likely to be due to the different participant characteristics and large interindividual variation in the mHSP72 response to stressors.

The STHA induced increase in mHSP72 persisted for at least 48 hours after the final HA session in the STHA group. Basal mHSP72 was elevated prior to HST2 (28 ± 26%) when compared to the preexercise levels before HST1 (Figure 6(b)). This prolonged elevation in mHSP72 after removal from repeated daily stress exposures has been observed 48 hours after 10 consecutive daily, 75-minute passive exposures to hypoxia in healthy humans [41]. The authors observed increases in mHSP72 of ≈30% per day for the first 5 days of daily repeated hypoxic exposure, decreasing to 16% per day for the final 5 days, representing a plateau in the response of mHSP72. Thus a total ≈200% increase in mHSP72 over the 10-day period, which remained elevated 48 hours after the final exposure (≈225% [41]). However during the initial 3 days of the hypoxic acclimation period, mHSP72 was increased by ≈50% from baseline. The magnitude of mHSP72 induction following the early stages of repeated hypoxic exposures is not dissimilar to that seen in the current experiment as a response to repeated exercising heat exposure (≈30% increase in baseline on day 3). It is worth noting that participants in the current investigation began to show a blunting in the mHSP72 on the third day of acclimation, whereas Taylor et al. [41] demonstrated continual, modest increases in mHSP72 following passive hypoxic exposures on days 4, 5, and 10. This indicates that the internal strain placed on participants in the present investigation may have reached an earlier ceiling for the level of strain required to produce further mHSP72. Increased basal HSP72 is a well-defined characteristic of both acquired thermotolerance [35, 44] and improved cellular tolerance to repeated hypoxic exposures [8, 41] in humans. Thus it is not surprising that a stressor that invokes the HSR and leads to the subsequent increase in basal mHSP72 would lead to improved cellular tolerance to a second, novel stressor, in this instance acute hypoxia.

The mHSP72 response to the HST was attenuated after STHA with a large and significant effect observed in the STHA group ($d = -0.45$, $P < 0.05$; Figure 6(a)). The mechanism by which an increase in basal levels of mHSP72 may inhibit its own expression is related to HSP72 binding to heat shock transcription factor 1 (HSF1). In unstressed cells HSF1 is bound to HSP72. Under stressful conditions HSP72 binds to denatured proteins, freeing HSF1. HSF1 trimerises and relocates to the nucleus where it binds to the heat shock element (HSE), initiating transcription of HSP72. When sufficient HSP72 has been produced to deal with the rigors of the stressor, HSP72 rebinds to the HSF and halts further transcription [4]. It is possible that STHA induced increases in mHSP72 elevate the cellular stress required to induce further HSF1 activation. The induction of mHSP72 via a STHA period was sufficient to allow the cells to cope with the hypoxic challenge, maintaining normal cell function and homeostasis. The authors do not suggest whole body preconditioning and cellular tolerance has been conferred from the initial phase of acclimation studied and subsequent elevations in one marker of cellular stress. Without parallel

measures in skeletal muscle, the whole body responses during the initial phase of acclimation cannot be fully explored, and thus this response requires further investigation. The inclusion of a normothermic-exercise control group allowed for the effects of exercise and heat to be separated, which has been a design issue with other studies investigating the heat shock response in humans. Exercise in the absence of an external heat stress led to small nonsignificant (10%; Figure 2(a)) increases in mHSP72, similar to those previously reported for similar work bouts [45]. It is possible that the increased physiological strain observed in the STHA group, and not the imposition of heat *per se*, drove the cross acclimatory affect [46]. No attempt was made to ensure that each group was achieving similar exercising heart rates; thus the increased exercise intensity in the STHA group may in part have elicited the HSR and alterations in physiological function. The work rate utilized (50% $\dot{V}O_{2peak}$) in the present investigation has been shown to allow participants to remain below the individual anaerobic threshold when exercising in both normothermic and hot (40°C) conditions [47]. In addition, individual $\dot{V}O_{2peak}$ in 40°C conditions has been shown to decline by ~5% compared to $\dot{V}O_{2peak}$ achieved in normothermic conditions in similarly trained individuals compared to those used in the present investigation [47]. Therefore it is likely that the metabolic stress presented by the work intensity and differences in the relative workloads was not significantly different between the conditions, with heat being the mediating factor for observed experimental effects. The inclusion of a hypoxic exercise group in future studies would also allow differences in expression kinetics to be quantified by the two divergent physiological stressors and a further exploration of heat-mediated tolerance to hypoxia.

4.4. Circulating HSP72 Responses to the HST. Plasma HSP72 also increased following the HST in both sets of participants (Figure 8). This is the first study to measure eHSP72 in response to an acute exercising hypoxic exposure in humans. eHSP72 increased significantly after HST1 in each experimental group (Figure 8), whereas normoxic exercise failed to induce any change in this variable in the control group during the intervention period (Figure 3(a)). It is likely that the normoxic exercise challenge failed to invoke a significant endogenous stress in order to stimulate the release of eHSP72 in this group. In contrast to this, the acute exercising heat challenge experienced on HA1 by the heat group produced a significantly greater increase in this biomarker than which was seen in response to the initial HST. This would suggest that the level of thermal strain experienced by this group presented a greater physiological stress than experienced during the level of acute hypoxia studied in this investigation. This is perhaps not surprising, as the rate of core temperature increase and delta change in core temperature have been found to be important external moderators in altering eHSP72 expression [38]. However, while these are important factors invoking a change in circulating levels of this protein, other factors have also been shown to be important. For example, both intensity and duration of exercise affect eHSP72 concentrations when work is performed in thermoneutral conditions [13, 33], with the addition of a thermal stressor increasing this response [2, 14, 48]. Therefore the increase in eHSP72 following HST1 may reflect the increase in relative work intensity. The absolute level of work used (50% normoxic $\dot{V}O_{2peak}$) has been shown in our laboratory, using participants of similar physiological characteristics and training background, to correspond to 78% of hypoxic $\dot{V}O_{2peak}$ [47]. In conditions of matched heat stress (40°C, 50%RH) but differing workloads (60 and 75% $\dot{V}O_{2peak}$), no difference in postexercise eHSP72 was observed, despite markedly different times to exhaustion (60%: 58.9 ± 10.9 minutes; 75%: 27.2 ± 9.0 minutes) [13]. The magnitude of eHSP72 increase following the HST was lower than observed by Fulco et al. [49] who reported an increased eHSP72 of approximately 2 ng·mL^{-1}. In order to focus on the specific effects of hypoxia *per se* on eHSP72, matching both absolute and relative levels of work would be required in both normoxic and hypoxic conditions. This was beyond the scope of this present investigation; thus the response of eHSP72 to acute bouts of moderate hypoxia therefore warrants further investigation.

4.5. Physiological Responses to the HST. It is relatively common for both athletes and military personal to be exposed to moderate altitude and be expected to perform physical tasks without undergoing prior acclimatization. It is well established that, even in the moderate altitude conditions studied herein, exercise performance, psychomotor performance, and cognitive function are reduced [49–51]. Adaptation to altitude requires approximately 14 days of residence, with molecular adaptations serving to improve oxygen delivery to cells as well as maintain the structure and function of cells and organs [6]. However, in scenarios where the rapid deployment of troops is necessary the extended altitude acclimation time frame poses logistical problems. From a practical perspective, interventions which can maintain or improve performance at altitude are therefore of interest. Heat acclimation reduces oxygen uptake, induces glycogen sparing, increases plasma volume, and improves myocardial efficiency and contraction, thereby reducing the stress on the cardiovascular system for a fixed level of work [52–54]. Despite the hematological and respiratory mechanisms of adaptation differing between heat and hypoxia, the increased physiological efficacy that is seen following a period of heat acclimation [55] coupled with the shared heat and hypoxic molecular adaptations of the HSP network may point to a cross acclimation effect being attainable. However experiments that have explored the cellular and molecular responses to preconditioning and cross acclimation interventions have done so without due consideration of the whole body physiological implications arising from any observed adaptation or increased cellular tolerance [8]. This study attempted to determine if any heat acclimation-induced alterations in the cellular stress response elicited measureable improvements in physiological tolerance when exposed to a subsequent, acute exercising hypoxic challenge. The findings of this present investigation point to the possibility that a prior period of exercise-heat stress may be associated with

beneficial physiological outcomes when later exposed to a period of acute hypoxic work. The reduced exercising HR in the STHA group, combined with an elevated SpO_2 (Figure 4), would indicate that this group was more tolerant to the acute hypoxia after the acclimation period. The reduction in SpO_2 may have been related to the reduced body temperature in the STHA producing a leftward shift in the O_2 dissociation curve. These preliminary results indicate that further work examining heat acclimation and hypoxic performance is warranted. The reductions in mean and peak exercising HR of ~9 beats·min^{-1} may indicate an increased capacity for work in these conditions; however follow-up studies involving a hypoxic-adaptation group would allow the efficacy of time matched acclimation protocols to be assessed. Furthermore, a physical performance test before and after intervention would determine whether the reductions in HR and improved cellular tolerance observed herein following STHA can enhance physical performance in hypoxic conditions. It would also be of interest to determine the "decay rate" of the acclimation and cross acclimation affect during both short-term (<5 days) and long-term acclimation protocols in order to optimize the time frame for exposure to secondary stressors, a methodological consideration that was not practicable in the present investigation.

5. Conclusion

In conclusion, STHA consisting of 3 consecutive exercise-heat exposures resulted in increased levels of monocyte HSP72 in humans and affected the expression characteristics of this protein during the acclimation period and in subsequent exposure to acute normobaric hypoxic exercise. The improved capacity of the chaperone system may have attenuated the cellular stress response to subsequent hypoxia and warrants additional investigation. Additionally, small yet significant changes in cardiovascular and thermoregulatory responses to subsequent hypoxia were evident in the STHA compared to CON. These responses also warrant further investigation during different phases of the acclimation process.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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Research Article

Cardiopulmonary Response to Exercise in COPD and Overweight Patients: Relationship between Unloaded Cycling and Maximal Oxygen Uptake Profiles

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Cardiopulmonary response to unloaded cycling may be related to higher workloads. This was assessed in male subjects: 18 healthy sedentary subjects (controls), 14 hypoxemic patients with chronic obstructive pulmonary disease (COPD), and 31 overweight individuals (twelve were hypoxemic). They underwent an incremental exercise up to the maximal oxygen uptake (VO_2max), preceded by a 2 min unloaded cycling period. Oxygen uptake (VO_2), heart rate (HR), minute ventilation (VE), and respiratory frequency (fR) were averaged every 10 s. At the end of unloaded cycling period, HR increase was significantly accentuated in COPD and hypoxemic overweight subjects (resp., $+14 \pm 2$ and $+13 \pm 1.5\ \text{min}^{-1}$, compared to $+7.5 \pm 1.5\ \text{min}^{-1}$ in normoxemic overweight subjects and $+8 \pm 1.8\ \text{min}^{-1}$ in controls). The fR increase was accentuated in all overweight subjects (hypoxemic: $+4.5 \pm 0.8$; normoxemic: $+3.9 \pm 0.7\ \text{min}^{-1}$) compared to controls ($+2.5 \pm 0.8\ \text{min}^{-1}$) and COPDs ($+2.0 \pm 0.7\ \text{min}^{-1}$). The plateau VE increase during unloaded cycling was positively correlated with VE values measured at the ventilatory threshold and VO_2max. Measurement of ventilation during unloaded cycling may serve to predict the ventilatory performance of COPD patients and overweight subjects during an exercise rehabilitation program.

1. Introduction

Predictive values of tests exploring mild physical activity have been already reported. Thus, a strong association was reported between the 6 min walk distance (6 MWD) and peak oxygen uptake (VO_2max) in patients with cardiac and pulmonary disease [1, 2]. Unloaded pedalling represents a reproducible warm-up exercise bout. It can be taken by subjects with low to moderate exercise capacity and allows simultaneously measuring ventilation and oxygen uptake (VO_2). We hypothesized that this mild exercise test may serve to predict the cardiopulmonary responses to heavy exercise.

Cardiopulmonary limitation at peak exercise is well documented in patients with COPD [3–5] and in individuals with overweight [6–10]. Both subjects have risk of pulmonary hyperinflation due to expiratory flow limitation. Moreover, severe overweight often results in hypoxemia. On the other hand, we found no data in the literature on the response of COPD and overweight subjects to unloaded cycling.

Chronic hypoxemia present in patients with COPD and also in numerous subjects with severe overweight may modify their response to unloaded cycling. Indeed, we already showed in healthy humans that experimental normobaric hypoxemia attenuated the ventilatory but not the cardiac response to unloaded cycling [11]. Mimicking overweight condition in healthy subjects also reduced their ventilatory response to submaximal exercise [12, 13]. Based on these data

Table 1: Morphological characteristics, physiological data at rest, and oxygen uptake at the different epochs of exercise. Asterisks denote significant variations compared to controls ($^{*}P < 0.05$; $^{**}P < 0.01$; and $^{***}P < 0.001$). For pulmonary function, values in brackets are the percentage of predicted normal data.

	Controls	COPDs	Overweight subjects	
			Hypoxemic	Normoxemic
Number	18	14	12	19
Age, y	49 ± 6	54 ± 4	55 ± 2	53 ± 3
Body mass index, $kg{\cdot}m^{-2}$	23 ± 1.0	24 ± 1.5	31 ± 2.0**	29.4 ± 2.2*
Forced vital capacity (FVC), lBTPS (% predicted)	4.54 ± 0.39 (101%)	3.56 ± 0.20 (68%)	3.79 ± 0.36* (70%)	4.12 ± 0.50 (97%)
Expiratory reserve volume (ERV), lBTPS	1.37 ± 0.20	1.03 ± 0.15	0.72 ± 0.35*	0.86 ± 0.25
Total lung capacity (TLC), lBTPS (% predicted)	6.72 ± 0.39 (102%)	6.45 ± 0.34 (100%)	5.81 ± 0.36* (75%)	5.92 ± 0.31* (78%)
Forced expiratory volume in 1 s (FEV1), lBTPS (% predicted)	3.89 ± 0.33 (96%)	2.38 ± 0.25** (60%)	3.18 ± 0.23 (81%)	3.27 ± 0.18 (86%)
FEV1/FVC, %	86	67	84	79
PaO_2	85	67***	72*	85
SpO_2, %	99 ± 0.8	95 ± 0.6	96 ± 0.5	99 ± 0.7
Heart rate, min^{-1}	72 ± 3	81 ± 5	69 ± 4	77 ± 3
Minute ventilation (VE), $lBTPS{\cdot}min^{-1}$	11	11	10	13
Respiratory frequency (fR), min^{-1}	16	18	15	17
Oxygen uptake (VO_2), $mlSTPD^{-1}{\cdot}kg^{-1}$ VO_2 rest	4.9 ± 0.2	4.5 ± 0.2	4.2 ± 0.2	4.3 ± 0.3
VO_2 end unloaded cycling	8.1 ± 0.4	8.2 ± 0.6	7.3 ± 0.3	7.4 ± 0.4
VO_2 threshold	21.2 ± 1.0	16.2 ± 0.9**	15.2 ± 1.4**	18.4 ± 0.8*

obtained in situations mimicking hypoxemia or overweight in healthy subjects, we hypothesized that the response to unloaded exercise may differ between patients with hypoxemia or overweight only. This might affect the supposed predictive values of their cardiopulmonary responses to unloaded pedalling.

2. Methods

2.1. Patients. The study population consisted of 63 age-matched male subjects (18 normal sedentary subjects; 14 normal-weight hypoxemic patients with COPD; and 31 individuals with overweight; among them 12 were hypoxemic). Their physical characteristics are shown in Table 1. Control subjects had no medical illness at the time of the study, none were smokers, and none were involved in an exercise training program. COPD was diagnosed according to the criteria of the ATS/ERS task force [14]. The patients had not experienced any respiratory tract infection or any exacerbation of their disease for at least 4 wk before the study and received no oral corticosteroid therapy but all were treated by inhaled corticosteroids (360 μg/day) and cholinergic antagonist (tiotropium bromide, 22.5 μg/day). There was no supplemental oxygen during the week before the protocol. Patients with overweight were not hypertensive and did not receive any medication which could interact with the exercise response. Procedures were carried out with the adequate understanding and written consent of the subjects who all signed the informed consent form. The protocol was approved by our institutional ethics committee.

2.2. Study Design. The subject performed an exercise on an electrically braked cycloergometer (Ergometrics ER 800, Jaeger, Germany) connected to a microcomputer software. The testing protocol consisted in a 5 min rest period followed by a 2 min zero-watt work load period at 1 Hz cycling frequency (unloaded pedaling). Then, the load was increased as a ramp of 20 watt/minute (W/min) until the predicted maximal VO_2 was reached (healthy subjects) or when the patient decided to interrupt the exercise bout (symptom-limited VO_2max). Instantaneous heart rate (HR) and breath-by-breath ventilatory data were averaged every 10 s. Because the entrainment of the breathing frequency by exercise rhythm has been documented [15, 16], we imposed a constant pedaling rate of 60 revolutions per minute (rpm) which

was maintained by all subjects throughout all the exercise challenge.

2.3. Methods. All subjects had measurements of forced vital capacity (FVC), forced expiratory volume in one second (FEV1), and total lung capacity (TLC) with a whole body pressure displacement plethysmograph (MasterLab Jaeger, Bunnik, The Netherlands). Reference values were those proposed by Quanjer [17]. Prior to the experiment, the ear lobe was pretreated with a vasodilator cream. Then, it was incised to sample arterialized blood in 100 μL heparinized capillary tubes. Oxygen (PaO_2) and carbon dioxide ($PaCO_2$) partial pressures and arterial pH (pHa) were measured (Corning-Chiron model 860, Bayer Corporation, East Walpole, MA, USA). Predicted PaO_2 value took into consideration the corresponding $PaCO_2$ level, pHa, and total hemoglobin [18]. At rest, then at determination of the ventilatory threshold (VTh) and VO_2max, blood gas tensions were analyzed. The percutaneous oxygen saturation (SpO_2, %) was continuously measured with a pulse oximeter whose accuracy equals plus or minus two oxygen saturation percentage points between saturations of 70–100% (NPB 40, Nelcor Puritan Bennett, Pleasanton, CA, USA).

The subject wore a face mask forming an air-tight seal over the nose and mouth. Ventilation was measured with a volumetric rotor transducer (Triple V digital volume transducer, Jaeger, Germany). A side port was connected to fast-response differential paramagnetic O_2 and CO_2 analyzers (Jaeger: 90% response time in 100 ms). The software (Oxycon Beta, Jaeger, Germany) computed breath-by-breath data of minute ventilation (VE), respiratory frequency (fR), tidal volume (VT), oxygen uptake (VO_2) and carbon dioxide production (VCO_2), and the ventilatory equivalents for O_2 (VE/VO_2). The ventilatory threshold (VTh) corresponded to the VE and VO_2 values at which the ventilatory equivalents for O_2 (VE/VO_2) exhibited a systematic increase without a concomitant increase in VE/VCO_2 [19]. The heart rate was continuously monitored (Cardiognost Hellige, Stuttgart, Germany) and simultaneously stored with ventilatory data on the computer.

2.4. Data Analyses. Data are presented as mean ± standard error of mean (SEM). Statistical inferences were made by the two-way analysis of variance (ANOVA) for repeated measures at predetermined epochs (rest, 10 s, 30 s, 60 s, and 120 s of unloaded cycling), patients with COPD or overweight subjects versus controls being one factor and time course being the second factor. Comparisons between groups or over time were performed taking into account the difference between each variable measured at a time during unloaded exercise and its baseline value. Correlations between variables were evaluated with Spearman's test. Cardiac and respiratory variables obtained at rest were compared using a stepwise multiple linear regression model to determine an equation that would best predict the cardiopulmonary response during unloaded cycling. The same statistical analysis allowed linking plateau values of variables measured during unloaded cycling with those measured at VTh and VO_2max. The potential coefficients of equation included HR, fR, VT, VE, VO_2, and PaO_2. Significance was set at the 0.05 level.

3. Results

3.1. Baseline Variables. Table 1 shows that subjects with overweight had significant lower total lung capacity (TLC) and expiratory reserve volume (ERV) compared to COPDs and normal-weight normoxemic controls. According to the ATS/ERS task force [14], our COPD patients had moderate airway obstruction ($FEV1/FVC < 70\%$; $50 < FEV1 < 80\%$ predicted), hypoxemia ($58 < PaO_2 < 70$ mmHg; $93 < SpO_2 < 97\%$), and no hypercapnia. Hypoxemic overweight subjects had the highest body mass index and the lowest TLC values. In the four groups (controls, hypoxemic COPDs, normoxemic, or hypoxemic overweight subjects), the resting values of HR, VE, fR, VT, and VO_2 did not differ, allowing the consideration of the absolute variation of each variable during unloaded cycling.

3.2. Influence of Resting Levels of Physiological Variables on the Cardiopulmonary Response to Unloaded Cycling. Multiple regression analysis showed that, among the different variables, only the resting fR was negatively correlated with the plateau fR increase at 120 s of unloaded pedaling (Δ fR, min^{-1} $= -0.42 * \text{fR rest} + 10.20$; $r = 0.521$; $P < 0.01$). Despite the fact that ERV was significantly lower in overweight individuals compared to controls and COPDs, we found no correlation between the reduction of lung volumes and the ventilatory response (VE, VT, and fR) to unloaded cycling.

3.3. Response to Unloaded Cycling. At the end of the unloaded cycling period, no significant changes in resting level of SpO_2 were measured in controls, COPDs, and overweight subjects. Table 1 reports the VO_2 values measured at the end of the unloaded pedaling period, ventilatory threshold, and maximal workload. Two-minute unloaded cycling period increased VO_2 value by 3.0 (overweight subjects) to 3.6 (controls and COPD), the intergroup differences being not significant. Figure 1 shows the time course of changes in HR and ventilatory variables measured during unloaded cycling. In controls, the changes in HR, VE, and VT were already significant at 10 s and at 30 s for fR. In the other groups, the first significant increase in HR, VE, and VT was only measured at 30 s and, in COPDs, fR increase only occurred at 60 s. In the four groups, a plateau response was obtained at 120 s. HR increase was significantly accentuated in COPD and hypoxemic overweight subjects (resp., $+14 \pm 2$ and $+13 \pm 1.5$ min^{-1}, compared to $+7.5 \pm 1.5$ in normoxemic overweight ones and $+8 \pm 1.8$ min^{-1} in controls). On the other hand, the fR increase was accentuated in all overweight subjects (hypoxemic: $+4.5 \pm 0.8$; normoxemic: $+3.9 \pm 0.7$ min^{-1}) compared to $+2.5 \pm 0.8$ in controls and $+2.0 \pm 0.7$ min^{-1} in COPDs. We noted a gradation of the VE response to unloaded cycling in the four groups (controls: $+5.6 \pm 0.6$ LBTPS·min^{-1}; COPDs: $+6 \pm 0.5$; normoxemic overweight subjects: $+7 \pm 0.4$; hypoxemic overweight subjects: $+8 \pm 0.6$).

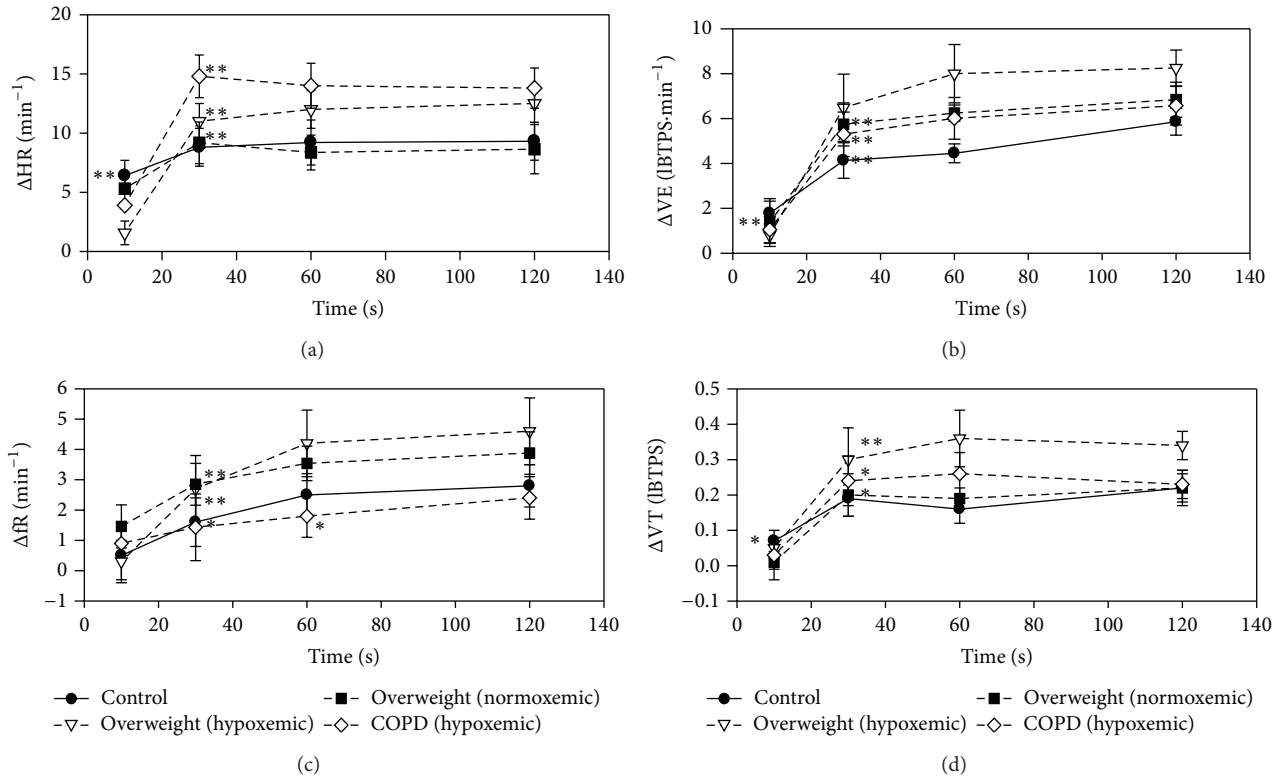


FIGURE 1: The changes in cardiorespiratory variables, related to their corresponding resting levels, during the 2 min period of unloaded cycling exercise in the four groups. Normoxemic and hypoxemic subjects are identified by black and open symbols, respectively. Up to down: heart rate (HR), minute ventilation (VE), respiratory frequency (fR), and tidal volume (VT). Values are mean ± SEM. Asterisks denote the first significant change ($^{*}P < 0.05$ and $^{**}P < 0.01$). All further variations were significant (**).

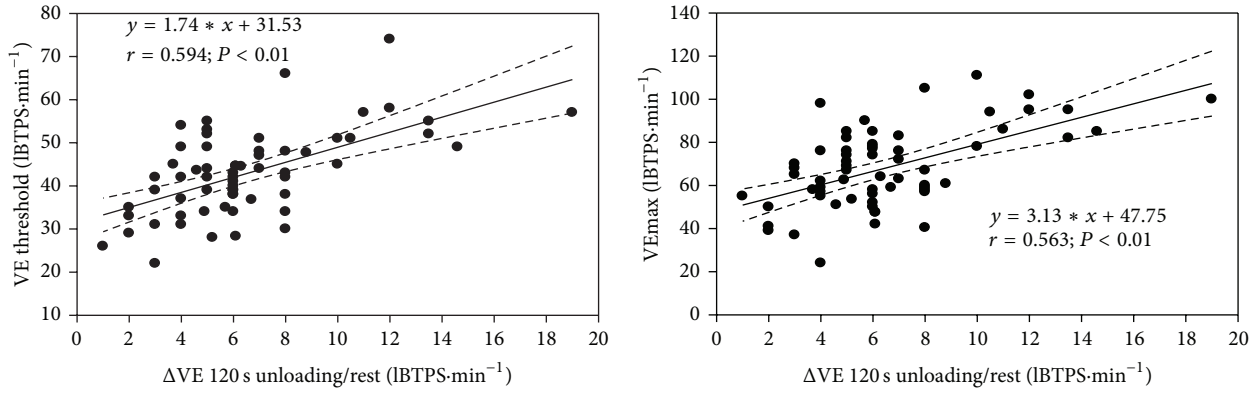
3.4. Influence of the Cardiorespiratory Response to Unloaded Cycling on the Performances during Incremental Maximal Exercise. We found no relationship between HR changes measured at the end of unloaded cycling and HR values measured at determination of the ventilatory threshold and VO_2max. By contrast, the plateau VE increase during unloaded cycling was positively correlated with the ventilation measured at the ventilatory threshold and VO_2max (Figure 2). No correlation was found between the plateau increases in fR and VT during unloaded cycling and their corresponding changes at the ventilatory threshold and VO_2max.

4. Discussion

The present study shows that two factors (hypoxemia and overweight) differently affect the heart rate and ventilatory responses to unloaded cycling. Thus, the plateau HR increase was higher when hypoxemia was present whereas the overweight increased the VE and fR responses. We noted that the plateau VE increase during unloaded cycling was correlated with the magnitude of hyperventilation measured at the ventilatory threshold and maximal workload. On the other hand, the plateau HR increase during unloaded cycling was not correlated to HR changes at the ventilatory threshold and VO_2max. It was already reported that pedaling with no load before exercise did not affect the heart rate increase during incremental exercise [20]. The energy cost of the 2 min unloaded cycling period was commensurate with that measured during walking at 1 km·hr^{-1} on a treadmill [21, 22]. Thus, unloaded cycling elicited a similar response than walking at a low rate on a flat ground.

Our study has evidenced a clear relationship between the resting respiratory frequency and its plateau value during unloaded cycling, the individuals having the lowest respiratory frequency exhibiting the highest response. We already reported in healthy sedentary subjects that the ventilatory response to the activation of different respiratory afferents depends on the breathing pattern at rest. Thus, in resting [23] and exercising subjects [24], the entrainment of the breathing rhythm by high frequency mechanical muscle stimulation was only significant in individuals having the slowest respiratory frequency at rest. The same relationship was also found between the breathing response to transient hypercapnia and the resting breathing pattern [25]. Thus, the role played by respiratory afferents seems to be accentuated in subjects having a slow and ample spontaneous breathing pattern.

The present data in COPDs and also in overweight subjects with chronic hypoxemia partly confirm our previous observations in healthy subjects exposed to acute normobaric hypoxemia [11], an experimental condition which delayed



(a) (b)

Figure 2: Relationships between minute ventilation measured at the ventilatory threshold (VE threshold) and maximal oxygen uptake (VEmax) during an incremental cycling exercise and the maximal VE changes measured at 120 s of unloaded cycling exercise. All subjects were pooled together for this analysis. Regression line with 95% confidence intervals.

the VE and HR responses to unloaded cycling and markedly lowered both the plateau increases in VE and HR. In the present study, all subjects with chronic hypoxemia had delayed VE and HR responses to unloaded cycling but the plateau HR increase was accentuated. We noted that the magnitude of plateau VE increase did not vary in hypoxemic COPDs but only increased in overweight subjects. Thus, the sole common point between the present study and the previous observations was a delayed cardiopulmonary response to unloaded cycling exercise in both acute and chronic hypoxemic conditions. The slowed kinetics of minute ventilation and heart rate changes in COPD patients and also in overweight subjects may be related to the reduction of blood and oxygen supply to limb muscles. Indeed, a limitation of oxygen uptake in the lower limbs during cycling exercise is documented in patients with COPD [26] and the peripheral vascular conductance is reduced in obesity [8]. Animal experiments have revealed that group III-IV muscle afferents, which play key role in the cardiopulmonary response to exercise via the activation of the muscle metaboreflex, are also activated by the increased muscle blood flow at the onset of exercise [27]. Thus, it was tempting to speculate that the limitation of blood and oxygen supply to exercising muscle in COPDs and overweight subjects may alter the muscle sensory pathways, delaying the response to unloaded pedalling. It is well known that the exercise pressor reflex induces cardiovascular adjustments to exercise via increases in sympathetic nerve activity and by withdrawal of parasympathetic nerve activity [28] and the beta-adrenergic and parasympathetic control of HR may be different between our groups.

We reported a significant elevation of the plateau minute ventilation increase during unloaded cycling in all patients with overweight compared to controls, whatever their baseline PaO_2 level. Their ventilatory response differed from controls through a prevailing increase in respiratory frequency. This was mostly present in the hypoxemic group which had also the highest overweight. It is documented that increase during incremental cycling exercise was higher in individuals with upper body adiposity than in lean subjects [29]. This effect was also present in experiments in healthy lean subjects reproducing the overweight-related limitation of chest wall mechanics [7, 8]. The restriction of lung volumes in our overweight subjects was not correlated with the magnitude of their accentuated ventilatory response to unloaded cycling, indicating that spirometric indices are not reliable predictive indicators of loaded breathing at work.

We noted an elevated plateau HR response in our hypoxemic subjects (COPDs and individuals with overweight). This observation is poorly documented in the literature. In COPDs with partial pressure of oxygen in arterial blood around 65 mmHg, Schrijen and coworkers [30] have reported a larger increase in systemic arterial pressure during constant load supine bicycling from loadless to 30 W and they attributed the enhanced circulatory response to the vasoconstrictor effect of hypoxia. Unfortunately, they did not simultaneously measure cardiac variables. Experiments in healthy subjects exposed to normobaric hypoxia showed an accentuated heart rate and activation of muscle sympathetic nerve activity in response to rhythmic handgrip exercise [31, 32]. We supposed that the enhanced heart rate increase during unloaded cycling in our hypoxemic subjects (patients with COPD or overweight) may result from the potentiation by hypoxemia of the exercise-induced sympathetic neural response.

5. Conclusion

The present data indicate a predictive value of the ventilatory but not heart rate response to unloaded cycling on performances at higher workload. The relationship between VE increase during unloaded cycling and VE changes measured at both ventilatory threshold and maximal exercise

power may have some interest to predict the ventilatory performance at work not only in healthy individuals but also in COPD and overweight subjects included in an exercise rehabilitation program.

Conflict of Interests

The authors declare that they have no competing interests.

Acknowledgment

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Research Article

The Effects of Metabolic Work Rate and Ambient Environment on Physiological Tolerance Times While Wearing Explosive and Chemical Personal Protective Equipment

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This study evaluated the physiological tolerance times when wearing explosive and chemical (>35 kg) personal protective equipment (PPE) in simulated environmental extremes across a range of differing work intensities. Twelve healthy males undertook nine trials which involved walking on a treadmill at 2.5, 4, and 5.5 km·h^{-1} in the following environmental conditions, 21, 30, and 37°C wet bulb globe temperature (WBGT). Participants exercised for 60 min or until volitional fatigue, core temperature reached 39°C, or heart rate exceeded 90% of maximum. Tolerance time, core temperature, skin temperature, mean body temperature, heart rate, and body mass loss were measured. Exercise time was reduced in the higher WBGT environments (WBGT37 < WBGT30 < WBGT21; $P < 0.05$) and work intensities ($5.5 < 4 < 2.5$ km·h^{-1}; $P < 0.001$). The majority of trials (85/108; 78.7%) were terminated due to participant's heart rate exceeding 90% of their maximum. A total of eight trials (7.4%) lasted the full duration. Only nine (8.3%) trials were terminated due to volitional fatigue and six (5.6%) due to core temperatures in excess of 39°C. These results demonstrate that physiological tolerance times are influenced by the external environment and workload and that cardiovascular strain is the limiting factor to work tolerance when wearing this heavy multilayered PPE.

1. Introduction

Personal protective equipment (PPE) is required in sporting and occupational settings to protect the wearer from a range of hazards [1]. Unfortunately, PPE may increase the rate of metabolic heat production at rest and during exercise. Concomitant elevations in thermoregulatory and cardiovascular strain during exercise in high ambient temperatures and humidity can lead to progressive increases in body heat content and if left unchecked this may lead to heat related illnesses [2]. In humans, the primary source of heat dissipation during exercise is through increased skin blood flow and sweating [2–4]. Wearing PPE may impede evaporative heat loss through sweating and a condition of uncompensable heat stress may occur [5, 6]. Consequently, information regarding work tolerance and rest cycles is of paramount importance for the health of the wearer in an occupational setting.

Several studies have examined the physiological strain encountered by fire fighters [7], police offices [8, 9], security guards [10, 11], pilots [12], and military personnel [13, 14] where PPE is a necessity. The major focus of this research has been the development of safe occupational guidelines for participants wearing PPE in the workplace. This is of particular importance as symptoms of heat illness ranging from headache to loss of consciousness and even death have previously been reported in emergency first responders and military personnel [15–17]. However, there has been comparatively little research done to represent the physiological strain experienced by explosive ordinance disposal (EOD) technicians.

We have previously examined the physiological effects of wearing EOD PPE in the field [17, 18] and in a controlled laboratory [19] setting. However, in theatre EOD technicians regularly have to don chemical PPE in addition to the EOD

ensemble when the severity or type of threat is unknown. Typically clothing which confers protection from chemical threats is fully encapsulating and impermeable in nature and requires the use of a respirator or self-contained breathing apparatus [1, 5, 6, 20]. In preparation for such operational scenarios, these technicians regularly train and operate while wearing these ensembles in extreme environments. It is well established that, in isolation, multiple layers of protective clothing, load-carriage, and the use of a respirator have a negative effect on ventilatory function, thermoregulation, and exercise tolerance during prolonged submaximal exercise [1, 13, 21]. Multiple clothing layers and load-carriage increase the energy cost of physical activity, apart from the added weight of the clothing per se [22]. Each layer of protective clothing also traps air between the skin and/or other clothing layers, and a microenvironment which serves as an insulator is created [23, 24]. Moreover, chemical protective garments typically have a high water vapour resistance and this further reduces the ability of the wearer to evaporate sweat [1, 25]. Respirators are also known to impair exercise capacity [21] by placing extra stress on the cardiorespiratory system during exercise. Ultimately, these components combine to increase an individual's metabolic rate and reduce their ability to dissipate heat during exercise, and a condition of uncompensable heat stress is created. Taken together, these findings suggest that the addition of chemical PPE to an EOD ensemble may impair thermotolerance and increase the risk of heat related illness.

Thus, the purpose of the present investigation was to examine the physiological tolerance times while wearing chemical and explosive protective clothing concurrently across a range metabolic work rates and ambient environments. Establishing this information has implications for determining safe tolerance times for EOD technicians when required to wear this PPE in various environments.

2. Methods

2.1. Participants. Twelve healthy, recreationally active males [(mean ± SD): age = 24.1 ± 3 years, height = 1.79 ± 0.06 m, body mass = 76.4 ± 8.4 kg, body surface area 2.0 ± 0.1 m^2, sum of eight skinfolds 79.1 ± 31.6 mm, maximal oxygen uptake ($\dot{V}O_{2\,max}$) 56 ± 5 $mL{\cdot}kg^{-1}{\cdot}min^{-1}$, heart rate max 195 ± 9 $beats{\cdot}min^{-1}$] volunteered to participate in the study. The study was approved by the university human research ethics committee and all participants completed an informed consent form and medical history questionnaire. To eliminate the confounding influences of gender on physiological responses to heat stress, only nonacclimatised, nonsmoking males, free from any known cardiovascular, metabolic, and respiratory diseases, were considered. Participants were asked to refrain from vigorous exercise and avoid the consumption of caffeine and alcohol during the 24 hours preceding the laboratory visits.

Participants attended the laboratory on four separate occasions, at the same time of day, separated by a minimum of 7 days. In the initial visit height and nude body mass were recorded and body surface area was subsequently calculated [26]. Skinfold thickness measures were obtained, using Harpenden (John Bull, West Sussex RH15 9LB, UK) callipers, at eight sites (biceps, triceps, subscapular, iliac crest, supraspinale, abdominal, front thigh, and medial calf). $\dot{V}O_{2\,max}$ was determined by indirect calorimetry during a progressive incremental running protocol on a motorised treadmill [27]. Participants were also provided with the opportunity to familiarise to the PPE ensemble by walking around the laboratory and on the treadmill at the speeds to be utilised for the trials.

2.2. Experimental Protocol. In the three remaining laboratory visits participants wore a fully encapsulating NFPA 1994 Class 3 chemical/biological protective garment, including outer gloves and booties (Extended Response Suit, Lion Apparel, Regency Park 5942, South Australia, Australia; 1.35 kg), and a respirator (Promask with a pro2000 PF10 filter; Scott Safety, Lancashire, England; 0.7 kg). The garment was made from trilaminate, a three-layer chemical/biological protective fabric, consisting of a selectively permeable barrier film laminated between outer and inner textiles. A Med-Eng EOD9 suit (Allen Vanguard, Ogdensburg, New York, USA) consisting of a jacket, trousers, groin protection, and a helmet (33.4 kg) was donned over the chemical PPE and respirator. The combined weight of the ensemble was 35.45 kg. Participants' base layer consisted of a t-shirt, shorts, socks, and underwear [28]. Athletic shoes with a soft rubber sole were also worn during testing [28].

During the trials the participants walked on a treadmill, while wearing the PPE, in an environmental chamber (4 × 3 × 2.5 m; length, width, height, resp.). A wet bulb globe temperature (WBGT) of 21, 30, or 37°C was obtained by the following ambient temperatures and relative humidities: 24°C, 50%; 32°C, 60%; and 48°C, 20%; respectively. A simulated wind speed equivalent to ~4.5 $km{\cdot}h^{-1}$ and a radiant heat load (two radiant heaters positioned ~1.3 m from the participant) were incorporated throughout the testing. These environmental conditions were also monitored independently (Quest Temp, Airmet, Australia). During each of these laboratory visits the participant completed three treadmill-walking trials of 2.5, 4, and 5.5 $km{\cdot}h^{-1}$ with a 1% gradient. This equated to an external work rate [29] of ~135, 207, and 307 $W{\cdot}m^{-2}$ for a 76 kg individual with a body surface area of 2.0 m^{-2}. The order of the testing, for both the work rate and the environment, was randomised using a random number generator in a controlled crossover design.

During each trial, standard termination criteria were applied in accordance with the American Society for Testing and Materials guidelines [28]: (1) core body temperature reaching 39.0°C; (2) 60 minutes of exercise; (3) heart rate > 90% of maximum; or (4) fatigue or nausea. Following the attainment of one of the termination criteria, the participant exited the environmental chamber and doffed the EOD protective clothing. Participants were then instructed to rest in an air-conditioned room. In the following recovery period participants were provided with food and fluid to a volume equivalent to 125% of the body mass loss in the preceding trial. This was undertaken to ensure recovery of body mass

and hydration status prior to commencement of subsequent trials. Core temperature and heart rate were monitored and following their return to baseline levels the participant provided a blood sample, had their nude body mass assessed, and commenced donning the EOD protective clothing for the subsequent trial. Three trials were conducted in this manner per testing session.

2.3. Outcome Measures. The primary outcome measure of the current study was physiological tolerance times measured to the nearest 0.5 min. Core temperature was recorded using an ingestible sensor (CorTemp, HQ Inc., Palmetto, FL, USA) swallowed ~6 hours before each trial. This was to allow sufficient time for the sensor to pass from the stomach to the intestines, where the reading of core body temperature is optimal [30]. Weighted mean skin temperature (Tsk) was calculated using four thermochrons (iButton, Maxim Integrated, CA, USA) attached to the back of neck, inferior border of right scapula, dorsal right hand, and proximal third of the right tibia [31]. Mean body temperature was calculated using the equation proposed by Stolwijk and Hardy [32]. Participants also wore a heart rate monitor (Polar Team2, Kempele, Finland) that was attached before entering the environmental chamber. Physiological strain index (PSI) using simultaneous measurements of core temperature and heart rate was calculated using Moran's [33] equation. PSI was rated on a scale of 1–10, with five indicating moderate, seven high, and nine very high physiological strain [33]. Core temperature, skin temperature, mean body temperature, heart rate, and PSI were recorded continuously and averaged over 30 second intervals for data analysis.

Pretrial hydration status was confirmed using urine specific gravity (USG, PAL 10s, ATAGO, Tokyo, Japan) of <1.020. If participants did not meet the above guidelines they were given an additional 500 mL of water to be consumed prior to commencement of the trial. Nude body mass was undertaken following towel drying and measured to the nearest 50 g (Tanita BWB-600, Wedderburn, Australia) before and after each trial. A cannula was inserted in the antecubital fossa for the attainment of venous blood samples in five mL serum separating vacutainers for the determination of serum osmolality using the freezing point depression technique (Osmomat 030, Gonotec, Berlin, Germany) as previously described [19, 34]. Serum osmolality was calculated in duplicate and the coefficient of variation was <1%.

2.4. Statistical Analysis. The data are displayed as mean ± SD unless otherwise stated. Assumption of normal distribution of data was assessed using descriptive methods (skewness, outliers, and distribution plots) and inferential statistics (Shapiro-Wilk test). When the assumption of sphericity was violated, significance was adjusted using the Greenhouse-Geisser method to adjust the degrees of freedom to increase the critical values of the F-ratio. Tolerance times, body mass loss, and the final value recorded for core temperature, skin temperature, mean body temperature, heart rate, and PSI were analysed using a two-way (environment × work intensity) repeated measures analysis of variance (ANOVA). To determine if baseline physiological and hydration indices were similar, pretrial heart rate, mean body temperature, serum osmolality, and body mass were also analysed in a similar manner. The effect of environment, work intensity, and their interaction were tested. Paired t-tests, using a Bonferroni correction, were conducted where significant differences were observed. All data was analysed using SPSS (SPSS version 21.0, SPSS Inc., Chicago, USA). Significance was set *a priori* at the $P < 0.05$ level.

Table 1: Baseline physiological and hydration indices ($n = 12$).

Speed ($km \cdot h^{-1}$)	HR (bpm)	T_{mb} (°C)	Serum osmolality ($mOsmol \cdot kg^{-1}$)	Body mass (kg)
2.5	102 ± 4.7	36.5 ± 0.08	291 ± 1	76.7 ± 2.26
4	103 ± 4.1	36.5 ± 0.06	292 ± 1	76.7 ± 2.29
5.5	99 ± 3.9	36.4 ± 0.08	292 ± 1	76.7 ± 2.26

Values are means ± SEM. HR, heart rate; bpm, beats per minute; T_{mb}, mean body temperature.

3. Results

3.1. Baseline Data. Participants commenced all nine trials from a resting physiological baseline (Table 1), with no significant differences between trials in heart rate ($P = 0.213$), mean body temperature ($P = 0.176$), serum osmolality ($P = 0.407$), or body mass ($P = 0.894$). The mean ± SD (range) duration of rest was 91 ± 18 min (58–155) when multiple trials were performed on the same day.

3.2. Tolerance Times and Termination Criteria. All twelve participants completed all nine trials (total trials: 108) with no serious adverse events recorded. The majority of trials (85/108; 78.7%) were terminated due to participants' heart rate exceeding 90% of their maximum (Table 2). A total of eight trials (7.4%) lasted the full duration of 60 min. Finally, nine (8.3%) trials were terminated due to volitional fatigue and six (5.6%) due to core temperatures in excess of 39°C.

A significant main effect in tolerance time (Figure 1, Table 2) was observed for environment (WBGT37 < WBGT30 < WBGT21; $P < 0.001$; $1 - \beta = 1.0$), work intensity ($5.5 < 4 < 2.5\,km \cdot h^{-1}$; $P < 0.001$; $1 - \beta = 1.0$), and their interaction ($P < 0.001$; $1 - \beta = 0.999$).

3.3. Physiological Data. Work intensity (Table 3) had a significant effect on core temperature ($P < 0.001$; $1 - \beta = 0.992$), skin temperature ($P = 0.002$; $1 - \beta = 0.936$), mean body temperature ($P < 0.001$; $1 - \beta = 0.997$), heart rate ($P = 0.022$; $1 - \beta = 0.682$), and body mass loss ($P < 0.001$; $1 - \beta = 1.0$). Core temperature ($P < 0.01$), skin temperature ($P < 0.05$), and mean body temperature ($P < 0.01$) were lower at the end of the $5.5\,km \cdot h^{-1}$ trials compared to the 2.5 and $4\,km \cdot h^{-1}$ trials. Body mass loss was also greater in the lower work intensities ($5.5 < 4 < 2.5\,km \cdot h^{-1}$; $P < 0.01$). Conversely, despite a trend for an increase in heart rate at the $2.5\,km \cdot h^{-1}$ trials compared to the 4 ($P = 0.055$) and 5.5 ($P = 0.077$) $km \cdot h^{-1}$ trials, no post hoc differences were observed.

Table 2: Tolerance time (mean, range) and termination criteria across the different environmental conditions and work rates ($n = 12$).

WBGT (°C)	Speed (km·h^{-1})	Tolerance time (min)	HR (>90% max)	T_c (>39°C)	Fatigue or nausea	Duration (=60 min)
21	2.5	52.1 (27.5–60)[b,c]	4			8
	4	36.0 (18–53)[b]	8	1	3	
	5.5	20.4 (6.5–39)	11	1		
30	2.5	39.1 (18.5–51.5)[a,b,c]	9	2	1	
	4	27.4 (12–47.5)[b]	11		1	
	5.5	16.9 (9–28.5)	12			
37	2.5	33.5 (13.5–44.5)[a,b,c,d]	8	1	3	
	4	24.7 (9–33)[a,b]	11	1		
	5.5	15.1 (6–25.5)	11		1	

Values are mean (range). Main effect observed for environment (WBGT37 < WBGT30 < WBGT21; $P < 0.001$), work intensity (5.5 < 4 < 2.5 km·h^{-1}; $P < 0.001$), and their interaction ($P < 0.001$). WBGT, wet bulb globe temperature; HR, heart rate; T_c, core temperature. [a]Significantly different to the same speed at WBGT 21°C ($P < 0.05$); [b]significantly different to 5.5 km·h^{-1} at the same environmental condition ($P < 0.05$); [c]significantly different to 4 km·h^{-1} at the same environmental condition ($P < 0.05$); [d]significantly different to the same speed at WBGT 30°C ($P < 0.05$).

Table 3: Physiological data at the cessation of each trial ($n = 12$).

WBGT (°C)	Speed (km·h^{-1})	HR (bpm)	T_c (°C)	T_{sk} (°C)	T_{mb} (°C)	PSI	Body mass loss (%)
21	2.5	164.0 (132–187)[b]	38.3 (37.7–39.0)	37.1 (36.5–38.1)	38.0 (37.5–38.7)	6.7 (4.7–9.2)	1.4 (0.6–2.1)
	4	174.6 (152–187)	38.3 (37.7–39.1)	37.2 (36.8–37.9)	38.1 (37.5–38.9)	7.0 (5.5–8.7)	1.1 (0.5–2.2)
	5.5	178.3 (164–190)	37.9 (37.5–39.0)	36.7 (34.8–38.5)	37.7 (37.2–38.9)	6.6 (5.2–9.0)	0.7 (0.2–1.3)
30	2.5	174.2 (130–186)	38.4 (37.7–39.1)	38.0 (37.4–38.6)	38.3 (37.7–39.0)	7.2 (3.6–9.1)	1.2 (0.6–1.7)
	4	175.0 (135–186)	38.3 (37.6–38.9)	37.9 (37.5–38.5)	38.2 (37.6–38.7)	7.1 (6.0–8.1)	1.0 (0.3–1.5)
	5.5	178.0 (165–188)	37.9 (36.7–38.5)	37.5 (36.7–38.3)	37.8 (36.7–38.5)	6.7 (5.7–8.1)	0.7 (0.3–1.2)
37	2.5	170.7 (113–187)	38.5 (37.9–39.1)	38.5 (38.0–39.2)	38.5 (38.0–39.2)	7.3 (3.6–9.0)	1.2 (0.5–1.7)
	4	178.8 (166–190)	38.2 (37.8–39.3)	38.4 (37.7–39.3)	38.3 (37.8–39.3)	7.2 (5.9–9.5)	0.9 (0.4–1.6)
	5.5	179.2 (166–191)	37.8 (37.4–38.7)	38.0 (36.6–39.4)	37.9 (37.3–38.8)	6.5 (5.4–8.3)	0.6 (0.3–1.1)

Values are mean (range). WBGT, wet bulb globe temperature; HR, heart rate; bpm, beats per minute; T_c, core temperature; T_{sk}, skin temperature; T_{mb}, mean body temperature; PSI, physiological strain index; [b]significantly different to 5.5 km·h^{-1} at the same environmental condition ($P < 0.05$). *Note*. Significant main effects ($P < 0.05$) for work intensity (T_c, T_{sk}, T_{mb} and body mass loss) and environment (T_{sk}, HR, body mass loss) were observed—see Results section for main effect comparisons.

Skin temperature ($P < 0.001$; $1 - \beta = 1.0$), heart rate ($P = 0.022$; $1 - \beta = 0.682$), and body mass loss differed across the three environments. Skin temperature ($P < 0.001$) and body mass loss ($P = 0.027$) were significantly higher in the WBGT21 condition compared to the WBGT37 environment. Skin temperature was also higher ($P = 0.019$) in the WBGT21 condition compared to the WBGT30. Heart rate was higher ($P = 0.02$) in the WBGT21 environment compared to the WBGT37 environment. The ambient environment had no significant effect on core temperature ($P = 0.886$; $1 - \beta = 0.056$), mean body temperature ($P = 0.067$; $1 - \beta = 0.533$), or PSI ($P = 0.519$; $1 - \beta = 0.144$). No other statistically significance differences were observed.

4. Discussion

The current study is the first to examine the physiological effects of wearing explosive and chemical PPE across a range metabolic work rates and ambient environments. The main findings of this study are that (1) physiological work tolerance is significantly influenced by the external environment and workload, (2) despite the short durations of exercise (~24 min), on average moderate to very high levels of physiological strain were experienced by the participants, and (3) cardiovascular, rather than thermoregulatory, strain is the limiting factor to work tolerance when wearing this ensemble.

As anticipated, tolerance time was reduced in the higher ambient environment and work intensities (Table 2; Figure 1) when wearing the EOD and chemical PPE. However, the ambient temperature and vapor pressure had far less impact on physiological tolerance time as the metabolic rate increased. When the metabolic rate exceeds 250 W·m^{-2} or 500 W, as evident in the 5.5 km·h^{-1} trials, the role the environment plays in the rate of heat storage and work tolerance is limited [5]. These data compliment the findings of Cheung and colleagues [5] and demonstrate minimal differences between the tolerance times in the highest work intensity (>300 W·m^{-2}) across the three environments (20.4, 16.9, and 15.1 min in the WBGT21, -30 and -37 environments, resp.; Table 2). In contrast, significant differences were observed in the lower work intensities, especially in the 2.5 km·h^{-1} trials, and tolerance times were greater in the cooler environments (53.1, 39.1, and 33.5 min; Table 2). The actual tolerance times in the WBGT21 environment, when walking at 2.5 km·h^{-1},

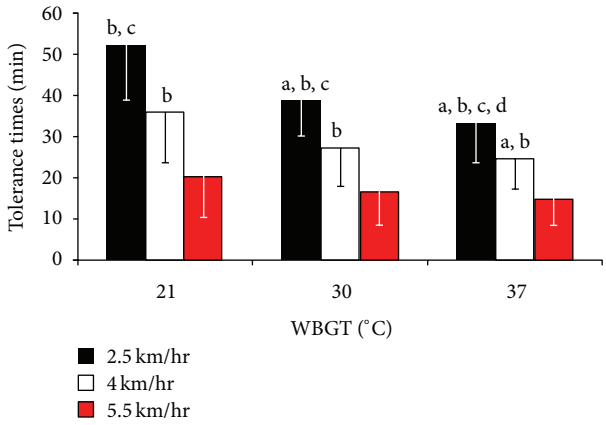

Figure 1: Tolerance time ± SD across the different environmental conditions and work rates ($n = 12$). Main effect observed for environment (WBGT37 < WBGT30 < WBGT21; $P < 0.001$), work intensity (5.5 < 4 < 2.5 km·h^{-1}; $P < 0.001$), and their interaction ($P < 0.001$). [a]Significantly different to the same speed at WBGT 21°C ($P < 0.05$); [b]significantly different to 5.5 km·h^{-1} at the same environmental condition ($P < 0.05$); [c]significantly different to 4 km·h^{-1} at the same environmental condition ($P < 0.05$); [d]significantly different to the same speed at WBGT 30°C ($P < 0.05$).

are likely to be even greater compared to the other conditions as 8 of the 12 participants completed the maximum duration of 60 min (Table 2). These individuals were physically capable of exercising beyond the termination criteria of 60 min; however, work beyond this duration is unlikely when wearing this PPE in the field [17, 18].

The current study is in agreement with previous research findings examining PPE of similar weight [1, 19] and further indicates that cardiovascular strain governs physiological tolerance times regardless of environment or work intensity. Over 78% of the trials in the current study were terminated based on heart rates in excess of 90% of maximum. These near maximal heart rates resulted in moderate to very high levels of physiological strain in almost all trials (Table 3), despite core temperature only reaching 39°C in eight of the 108 trials. The unique finding of this study is that, on average, tolerance times were more than 8 min shorter when wearing the chemical and EOD PPE in comparison to the EOD ensemble in isolation [19]. Although this duration may appear insignificant, it equates to a reduction of more than 20% in exercise tolerance which may be of practical importance for operational success in the field. It is possible to compare these two data sets as the methodologies and the participants were similar between studies. Although a unique group of participants performed both studies, we employed the same methodological protocol and utilised the same environmental conditions (WBGT21, -30, and -37), work rates (2.5, 4, and 5.5 km·h^{-1}), and termination criteria [28]. Moreover, the participant demographics and fitness levels (~57 mL·kg^{-1}·min^{-1}) were similar.

Considering the combined weight of the chemical protective clothing and respirator is minimal (~2 kg); it is unlikely that the additional weight alone was responsible for this reduction in work capacity. For example, Teitlebaum and Goldman [22] have previously demonstrated that metabolic rate increases by only ~3% per layer of clothing. Increased levels of thermal and cardiovascular strain have however been attributed to covering of the head with a helmet [14] or respirator and hood [13] during similar thermotolerance trials. Breathing through a respirator when fully encapsulated also reduces the core temperature that is tolerated at exhaustion by ~0.3°C [13]. This may be related to the effects of breathing through a respirator on the cardiopulmonary system and thermal perception. Respirators, regardless of their make or function [1], typically increase inspiratory and expiratory breathing resistance, decrease maximal voluntary ventilation [21], and reduce $\dot{V}O_{2\,max}$ [35]. Moreover, it is widely known that the head, in particular the forehead, has one of the highest sweat gland densities and usually has a greater sweating response than all other body segments during thermal loading [36, 37]. Consequently, higher mean skin temperatures and greater subjective discomfort are often associated with the use of the mask and breathing through the respirator [13]. This hypothesis is supported in the current study as maximal mean skin temperatures (>39.0°C in some individuals; Table 3) were higher in all trials, despite being 20% shorter in duration, compared to those observed in the EOD ensemble in isolation [19].

It has been postulated that the impact of multiple clothing layers, particularly if associated with extra load carriage, may have greater effects on work tolerance than the added resistance of breathing through a respirator [1, 21]. Regardless of the individual or combined effects of these aforementioned factors, the ensemble utilised in the current study incorporates a plethora of elements that are likely to impair thermotolerance. These include multiple layers of heavy PPE, a helmet, a respirator, and a fully encapsulating suit, examined across a range metabolic work rates and ambient environments. EOD technicians should therefore be cognisant that physiological tolerance times are significantly reduced when a respirator and chemical clothing are added to the EOD PPE.

The findings of this study are limited to young healthy males. Due to physiological differences between males and females [38] and younger and older individuals during exercise in the heat [39], additional research on older and female participants is warranted. The participants in this study also started each trial from a rested physiological state which may not be feasible in the field. Therefore, future research should examine the effects of prior exercise, dehydration, and elevated body temperatures when wearing this type of PPE.

5. Conclusions

In conclusion, this investigation has demonstrated that physiological tolerance times are significantly reduced in higher ambient environments and workloads when wearing explosive and chemical PPE. Secondly, despite the short durations of exercise, high to very high levels of physiological strain were experienced by all participants. Finally, cardiovascular strain is the limiting factor to work tolerance when wearing this heavy, multilayered, and encapsulating PPE.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of the paper.

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Research Article

The Effects of Cold and Lower Body Negative Pressure on Cardiovascular Homeostasis

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Purpose. The purpose of this study is to determine how cold exposure and lower body negative pressure effected cardiovascular variables. *Methods*. Eleven males (20.3 years ± 2.7) underwent two 20-minute exposures to LBNP. During the 2 trials, the subjects were exposed to cold air (10°C) (COLD) and to ambient temperature (23°C) (AMB). The trials consisted of a 100-minute pre-LBNP period followed by a 20-minute exposure to LBNP and then a 15-minute recovery period. Cardiovascular variables were recorded every 30 minutes using bioimpedance. *Results*. When LBNP was applied during the AMB trials, stroke volume immediately decreased. During the COLD trial, there was a five-minute delay before the decrease in stroke volume. Heart rate increased immediately after LBNP initiation during the AMB trials but there was a delay in the increase during the COLD trials. That same pattern was followed with mean arterial blood pressures. Cerebral oxygenation was significantly lower throughout the COLD trial as compared to the AMB trials. Six subjects reported symptoms of syncope or presyncope during the AMB trials but there were no reports of symptoms during the COLD trials. *Conclusion*. From analysis of this data, cold improved the subject's tolerance to LBNP.

1. Introduction

Hemorrhage is a leading cause of death in both civilian and battlefield trauma. Survival rate increases when victims requiring immediate intervention are correctly identified in a mass-casualty situation, but methods of prioritizing casualties based on current triage algorithms are severely limited [1]. Controlled study of acute hemorrhage in humans is not possible; however, it is possible to safely simulate hemorrhage by applying negative pressure to the lower extremities. Specifically, lower body negative pressure (LBNP) sequesters blood from the thorax into dependent regions of the pelvis and legs, effectively decreasing central blood volume in a similar fashion to hemorrhage [1].

It is well established that a thermal stressor elicits physiological responses that alter central blood volume. As environmental temperature decreases or length of exposure increases, the body attempts to maintain thermoregulatory homeostasis via multiple mechanisms [2]. Specifically, the peripheral vasculature will constrict, shunting warmer blood to the core to maintain core temperature, the onset of metabolic heat production, and shivering thermogenesis [2]. When environmental temperature increases, the body will attempt to maintain thermoregulation through peripheral vasodilation and sweating [2].

Optimal management of hemorrhage and controlling the effects of a reduction in central blood volume require recognition and integration of multiple complex physiological responses. The current therapy, which is warming the patient, used in the majority of cases has shown the potential to cause other issues and has been challenged [1, 3].

Numerous studies have looked at the effects that cold has on tolerance of LBNP. The majority have used a reduction in skin temperature and controlled the core temperature to prevent a decrease and then subsequent shivering thermogenesis [4–7].

Table 1: Participant characteristics (M ± SD).

Variable	M ± SD ($N = 11$)
Age (yr)	20.3 ± 2.7
Height (cm)	180.8 ± 5.7
Mass (kg)	81.6 ± 13.4
A_D (m^2)	2.0 ± 0.2
% BF (skinfolds)	8.1 ± 2.3
VO_{2max} ($mL \cdot kg^{-1} \cdot min^{-1}$)	42.7 ± 5.1
Body density	1.1

Note. A_D, body surface area; VO_{2max}, maximal oxygen uptake.

The purpose of this investigation was to determine the effects that decreasing the body's core temperature has on tolerance of exposure to LBNP and the subsequent reduction in central blood volume and whether that response is different from previous studies.

2. Materials and Methods

2.1. Subjects. Eleven Caucasian male subjects between the ages of 18 and 25 years volunteered for this investigation. Only male subjects were used to limit thermoregulatory variables. Participant characteristics are listed in Table 1. All subjects were free of disease and were not taking any medication that will influence cardiovascular or fluid regulation. Health status was determined via a self-reported health history questionnaire. Each subject was provided with and read and signed an informed consent in accordance with the guidelines set forth by the Kent State University Human Subjects Review Board.

2.2. Design. Participants underwent an exposure to LBNP in a cold experimental trial in one visit (COLD) and an exposure to LBNP in an ambient experimental trial (AMB) in another visit. Both trials were staged in the Environmental Chamber (Neslab, Napa, California), Figure 7, in the Kent State Environmental Laboratory. The order was determined in a counterbalanced manner. Participants refrained from exercise and from consuming any alcohol or caffeine 24 hours prior to the start of either trial. The two trials (COLD and AMB) were separated by a minimum of 48 hours so that the effects of prior cold exposure, which was confirmed verbally, would not alter tolerance of temperature or of LBNP with the subsequent trial which was verified verbally prior to the start of the second trial.

2.3. Instrumentation. Prior to the start of the experimental trial, participants were instructed to insert a rectal thermometer (ER 400-12, O.E. Meyer Co., Sandusky, OH) 13 cm past the anal sphincter for continuous measurement of core temperature. Skin thermistors (Model number 409 B, Yellow Springs Instruments, Inc., Yellow Springs, OH) were applied to the individual's body and held in place by waterproof tape (Hy-tape International, Patterson, NY). The thermistors were applied to the right forearm, triceps, chest, thigh, and calf [8]. Skin thermistors were applied and the participants were fitted with a Polar heart rate monitor (Accurex Plus, Polar Electro, Inc., Woodbury, NY) which was used to monitor heart rate. Bioimpedance sensors (Cardiodynamics BioZ, San Diego, CA) [9] were placed on both carotid arteries and the axillary regions so that mean arterial blood pressure and stroke volume were monitored using impedance cardiography. The INVOS system was used for real-time monitoring of changes in regional brain oxygen saturation (rSO_2) of blood flow in the frontal lobe of the brain (Somanetics Corp., Troy, MI) [10]. Subjects were fitted with one sensor on their forehead prior to the start of the experimental trial and it remained there until completion of the trial.

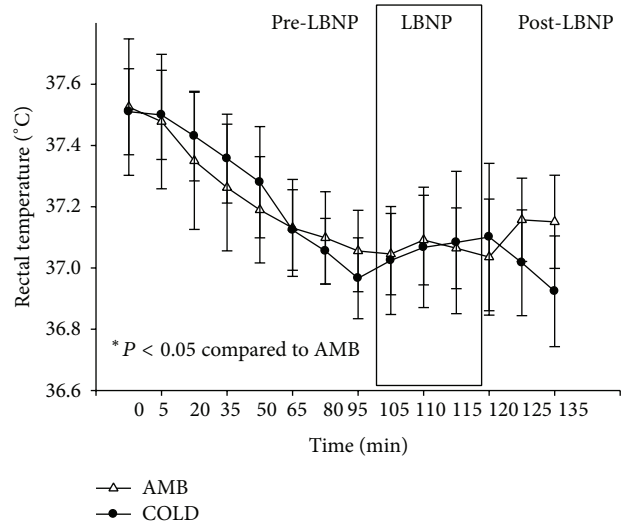

Figure 1: Changes in rectal temperature (°C) (M ± 95% CI) during pre-LBNP, LBNP, and post-LBNP periods.

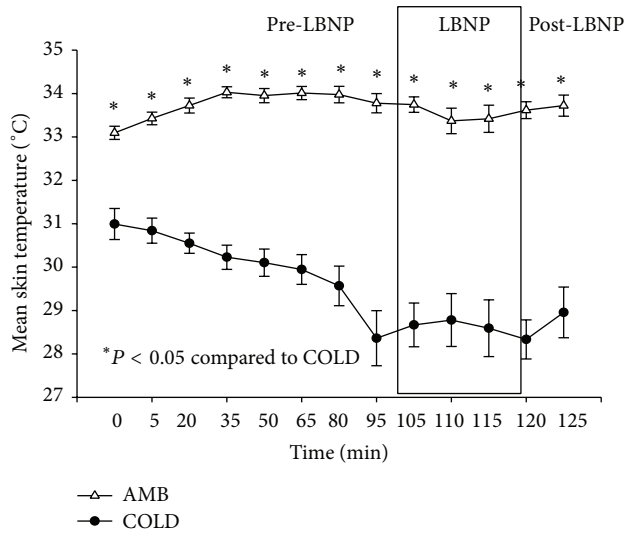

Figure 2: Changes in mean skin temperature (°C) (M ± 95% CI) during pre-LBNP, LBNP, and post-LBNP periods.

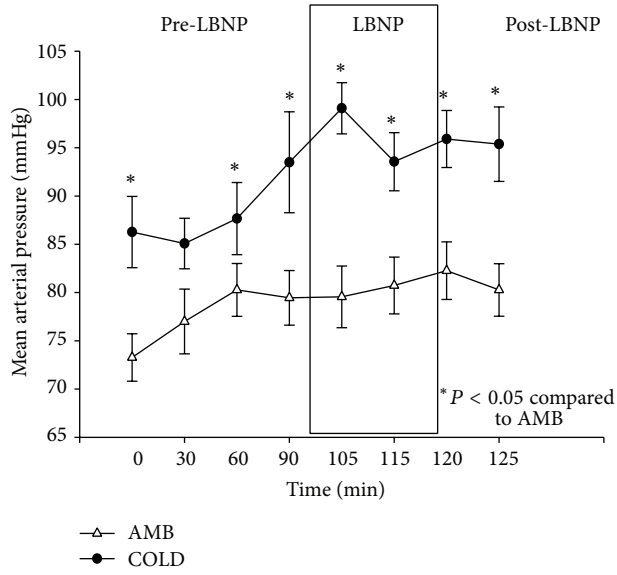

Figure 3: Changes in mean arterial blood pressure (mmHg) (M ± 95% CI) during the pre-LBNP, LBNP, and post-LBNP periods.

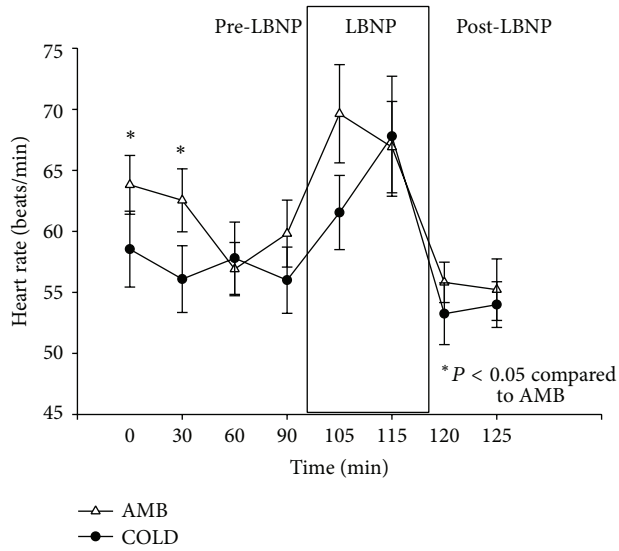

Figure 4: Changes in heart rate (beats/min) (M ± 95% CI) during the pre-LBNP, LBNP, and post-LBNP periods.

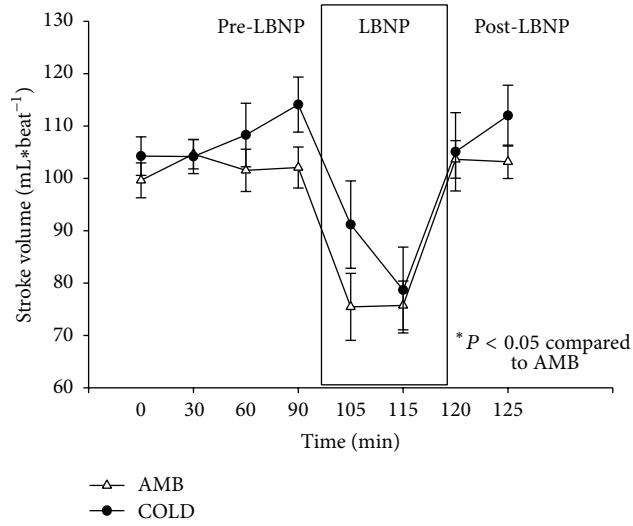

Figure 5: Changes in stroke volume (mL × beat^{-1}) (M ± 95% CI) during the pre-LBNP, LBNP, and post-LBNP periods.

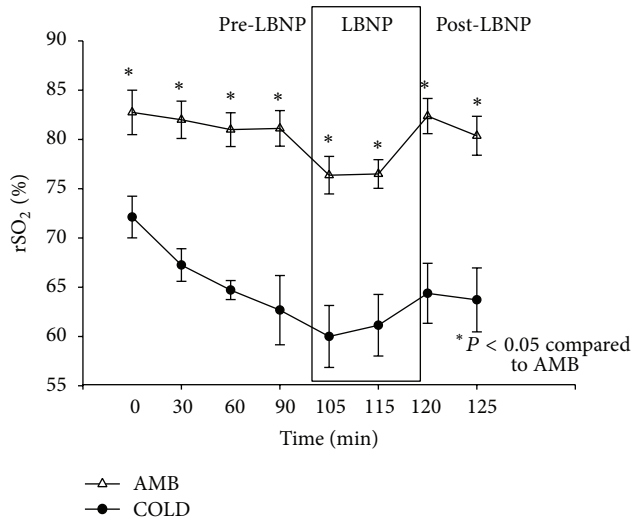

Figure 6: Changes in cerebral oxygenation (%) (M ± 95% CI) during the pre-LBNP, LBNP, and post-LBNP periods.

2.4. Protocol. Subjects were dressed in athletic shorts only for both trials. Subjects positioned themselves in the LBNP box, which is pictured in Figure 8, up to the level of the anterior superior iliac spine. The subject then lied quietly on a table (Hausmann Industries, Northvale, NJ) for the duration of the trial. LBNP was applied at a level of −40 mmHg for both trials. The LBNP box was placed in the chamber prior to cooling and, for both trials, the top of the box was removed for the initial exposure period. This insured that there was not any increase in core temperature due to being sheltered from the environmental stress. The COLD experimental trial consisted of two components: an initial acute cold exposure (ACE) in an environmentally controlled chamber (Neslab, Napa, CA) followed by the LBNP application period. The acute cold exposure was to last for 100 minutes unless the subject's core temperature dropped 1°C at which time the LBNP application was initiated. LBNP application was terminated after 20 minutes or if the subject began experiencing nausea or lost consciousness. The environmental chamber was controlled within ±3°C of 10°C. There was a 15 min recovery period after the trial. The AMB experimental trial took place in the chamber with the temperature controlled within ±5°C of 23°C. LBNP was applied 100 minutes after initiation of the trial and terminated after 20 minutes. There was a 15-minute-recovery period after the AMB trial.

2.5. Data Analysis. Means and standard deviations were calculated for subject characteristics (age, height, mass, body

Figure 7: The exterior of the environmental chamber at the Kent State Environmental Laboratory.

Figure 8: The LBNP box.

surface area, surface area-to-mass ratio, body fat percentages, and VO_2). The study included two treatments, LBNP in ambient temperature and LBNP in cold. A group of male college aged students ($N = 11$) were subjects. All data were analyzed with SPSS software version 17 (Chicago, Ill). Two separate two-way repeated measures analysis of variance (ANOVA) by 10 time-points (0, 5, 20, 35, 50, 65, 80, 95, 110, and 125 minutes) with two conditions (AMB and COLD), were utilized to examine differences in T_{re} and T_{sk}.

Repeated measures analysis of variance was used to determine if there was a difference in dependent variables across time and condition (COLD and AMB). Specifically, a 2×8 repeated measures ANOVA was used to analyze mean arterial blood pressure, heart rate, stroke volume, and regional oxygen saturation. For all comparisons, alpha was set at 0.05.

3. Results

3.1. Rectal and Skin Temperature. Repeated measures ANOVA demonstrated that there was a significant condition by time interaction ($P < 0.05$) for both rectal and skin temperature (Figures 1 and 2). Rectal temperature decreased with time but was not different between conditions. Subjects core temperatures did drop more than 1°C during the COLD trials so all subjects were initially exposed to cold for 100 minutes. However, skin temperature decreased during COLD trial but did not change across time during the AMB trial. Skin temperature was significantly less at each time point for the COLD trial as compared to the AMB trial.

A Shapiro-Wilk's test ($P > 0.05$) [11] showed that the temperature readings were approximately normally distributed with the exception of the readings at one hundred thirty-five minutes of the AMB trials. Partial eta squared was 0.334.

3.2. Mean Arterial Blood Pressure (MAP). MAP increased during the pre-LBNP period for both the COLD and AMB trials. The data for MAP is presented in Figure 3. Repeated measures ANOVA demonstrated a significant condition by time interaction ($P < 0.05$). The average change in pressure was 6.18 mmHg for the AMB trials and 7.91 mmHg for the COLD trials. MAP leveled off after the 60-minute measurement during the AMB trials. There was an average increase of 1.27 mmHg during the LBNP application for the AMB trials and a decrease of 0.45 mmHg during the post-LBNP period.

During the COLD trials, there was an initial increase in MAP (on average, the increase was 5 mmHg) 5 minutes after application of LBNP followed by an average decrease of 6 mmHg before the cessation of LBNP. This was likely due to a further shift in fluids to the core with LBNP. The overall average change in MAP during LBNP application was −1 mmHg on average.

A Shapiro-Wilk's test ($P > 0.05$) [9] showed that mean arterial pressures were approximately normally distributed with the exception of the reading at the sixty-minute mark of the AMB trials. Partial eta squared was 0.290.

3.3. Heart Rate. Heart rate decreased by an average of 4 bpm on average during the pre-LBNP period of the AMB trials and by an average of 1 bpm on average during the COLD trials. The data for heart rate is presented in Figure 4. Heart rate dropped at the 60-minute measurement during the AMB trial before a sharp increase prior to the application of LBNP. Heart rate stayed steady during the pre-LBNP period of the COLD trials. Repeated measures ANOVA demonstrated that there was not a significant condition by time interaction ($P > 0.05$).

A Shapiro-Wilk's test ($P > 0.05$) [9] showed that heart rates were approximately normally distributed. Partial eta squared was 0.148.

3.4. Stroke Volume. During the pre-LBNP period, there was an average increase of 2.45 mL/beat during the AMB trials. The data for stroke volume is presented in Figure 5. There was an average increase of 5 mL/beat during the pre-LBNP of the COLD trials. Repeated measures ANOVA demonstrated that there was not a significant condition by time interaction ($P > 0.05$).

A Shapiro-Wilk's test ($P > 0.05$) [9] showed that stroke volumes were approximately normally distributed with the exception of values taken at the thirty-minute mark and the one hundred fifteen-minute mark during COLD trials. Partial eta squared was 0.196.

3.5. Cerebral Oxygenation. During the pre-LBNP period, there was an average decrease of 1.62% during the AMB trials and an average decrease of 9% during the COLD trials. Application of LBNP caused an average decrease of 4.63% during the AMB trials. All of the AMB readings were significantly higher than the COLD readings. The data for cerebral oxygenation is presented in Figure 6.

During the COLD trials, there was an average decrease of 1%. An average increase of 3.88% was observed during the post-LBNP period of the AMB trials. There was an average increase of 2.57% during the post-LBNP period. There was a significant condition by time interaction ($P < 0.05$).

A Shapiro-Wilk's test ($P > 0.05$) [9] showed that stroke volumes were approximately normally distributed with the exception of the readings at ninety minutes and one hundred twenty-five minutes during the COLD trials. Partial eta squared was 0.333.

4. Discussion

The present investigation was conducted to evaluate the effects of cold exposure and lower body negative pressure (LBNP) on cardiovascular and thermoregulatory homeostasis. The subjects in this study were exposed to cold for 100 minutes prior to exposure to LBNP in an effort to decrease core temperature and evaluate the effects on a simulated hemorrhage. When an individual is exposed to the cold, both central and peripheral mechanisms are utilized so that core temperature may be maintained. In an effort to retain core temperature, shivering thermogenesis, in part, increases metabolic heat production while peripheral vasoconstriction minimizes heat loss [2].

This may be an advantage in treatment of hemorrhage. The resultant reduction in central blood volume from hemorrhage elicits a left and upward shift of the Frank-Starling curve, which leaves the cardiovascular system at risk for collapse with any further reduction in volume [12]. The mechanisms that the body uses in maintenance of core temperature during cold exposure should attenuate this.

Hemorrhage leads typically to a reduction in central blood volume. The response of the body is to maintain perfusion of tissues. In order to do that, blood pressure must be maintained. The data in this investigation indicates that, during a simulated hemorrhage (LBNP) in ambient temperature, there was a decrease in stroke volume, rSO_2, which is indicative of a fluid shift away from the core. The reduction in central blood volume and blood pressure causes compromised perfusion of tissues [4]. Cerebral blood flow velocity was measured in a previous study by Wilson et al. [13]. They used a head-up tilt to create the blood pooling in the lower extremities. They used a tube lined suit for skin surface cooling and found that cerebral blood flow velocity was unchanged when the skin surface was cooled. They also reported that the subjects did not demonstrate symptoms of syncope with the cold exposure. Anecdotally, there were 6 subjects who reported symptoms of syncope during the AMB trials, but there were no episodes of syncopal symptoms during the COLD trials.

A previous study by László et al. [14] suggested that there was no significant increase in MAP with LBNP at −35 mmHg. When the pressure increased to −55 and −65 mmHg, there was an increase in MAP, so they concluded that there was a dose-response relationship with LBNP and the cardiovascular system. The results of the present investigation agree with studies done by Wilson et al. [13] and Keller et al. [7].

Increased afterload shifts the curve down and to the right, which indicates that the subjects were able to maintain perfusion of tissues in spite of a significantly lower rSO_2 that was seen during the COLD trials.

Durand et al. [6] found similar results to this investigation. A significant increase was reported in cumulative stress index which showed improved tolerance to LBNP. Researchers examined different negative pressures for 5 minutes as opposed to this investigation which used one pressure for 20 minutes. The longer exposure time showed the physiological pattern for each variable as a response to LBNP.

Cui et al. [5] examined central venous pressure with lower body negative pressure. Five-minute intervals of LBNP at various pressures were investigated as opposed to one 20-minute interval for this investigation. Results were consistent with this investigation, as Cui et al. found a significant increase in central venous pressure with cold exposure.

In summary, the subjects were able to maintain blood pressure much better in cold temperatures than in ambient one. Cold may have delayed and/or minimized the effects of lower body negative pressure on cardiovascular and thermoregulatory variables. This is in agreement with other studies that used a decrease in skin temperature as its only stressor.

5. Limitations

One limitation of this investigation was that a wide range of subject morphologies was observed. The weight range of our subjects was from 55.8 kg to 100.24 kg. Subjects with a similar height and weight may show trends of cardiovascular and thermoregulatory variables. This would be due to similar total fluid volume for each subject. There was also a wide range of cold tolerance noted. One possible way to address this issue would have been to pretest each individual and determine their tolerance of LBNP prior to the trials. Another possible limitation of this investigation was that oxygen saturation in the periphery was not monitored and compared to brain oxygen saturation. The significant main effect for condition may have been due to vessel constriction secondary to the cold response or it may have been due to the inability of the NIRS unit to read the true values due to vessel constriction.

6. Conclusions

This investigation found that cold altered the physiological response of the body to LBNP. This may be an indication that cold may delay and/or minimize the onset of shock by maintaining blood pressure for a longer period. This may provide more time for an individual to receive more advanced

interventions. Further investigation is warranted to provide further support for this.

Abbreviations

LBNP: Lower body negative pressure
COLD: Cold trial
AMB: Ambient trial
rSO_2: Regional oxygen saturation.

Disclosure

Research was performed in the Applied and Environmental Physiology Laboratory at Kent State University in Kent, Ohio. This research was presented at the Annual Meeting of the American College of Sports Medicine, 2012, in San Francisco, CA.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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Clinical Study

Exhaustive Exercise Attenuates the Neurovascular Coupling by Blunting the Pressor Response to Visual Stimulation

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Neurovascular coupling (NVC) is assessed as an increase response to visual stimulation, and is monitored by blood flow of the posterior cerebral artery (PCA). To investigate whether exhaustive exercise modifies NVC, and more specifically, the relative contributions of vasodilatation in the downstream of PCA and the pressor response on NVC, we measured blood flow velocity in the PCA (PCAv) in 13 males using transcranial Doppler ultrasound flowmetry during a leg-cycle exercise at 75% of maximal heart rate until exhaustion. NVC was estimated as the relative change in PCAv from the mean value obtained during 20-s with the eyes closed to the peak value obtained during 40-s of visual stimulation involving looking at a reversed checkerboard. Conductance index (CI) was calculated by dividing PCAv by mean arterial pressure (MAP) to evaluate the vasodilatation. At exhaustion, PCAv was significantly decreased relative to baseline measurements, and the PCAv response to visual stimulation significantly decreased. Compared to baseline, exhaustive exercise significantly suppressed the increase in MAP to visual stimulation, while the CI response did not significantly change by the exercise. These results suggest that exhaustive exercise attenuates the magnitude of NVC by blunting the pressor response to visual stimulation.

1. Introduction

Neurovascular coupling (NVC) is important to maintain adequate blood flow to working cerebral regions when information processing occurs. NVC has been assessed as the regulation of blood flow to the visual cortex by measuring the increase in blood flow of the posterior cerebral artery (PCA) in response to a given visual stimulation, as evaluated using transcranial Doppler (TCD) ultrasound [1]. PCA is the main artery that supplies blood to the visual cortex, and an increase response in PCAv to visual stimulation has been believed to be induced by regional vasodilatation accompanied with the underlying cortical neural activity [2]. Impaired NVC in the PCA should be avoided to ensure adequate blood flow in working regions.

There is evidence in the literature that suggests that NVC is altered during or by certain physiological conditions. For instance, NVC was reportedly maintained during aerobic exercise at 70% of maximal heart rate (HR) [3], at various intensities of submaximal dynamic exercise [4], as well as during static exercise [5], hypercapnia [6], and orthostatic stress [7], and increased during the cold pressor test [8]. However, previous studies revealed that there was a decrease in NVC during hyperventilation-induced hypocapnia at rest [9]. NVC should be maintained during exhaustive exercise because it is important to support a stable or increased metabolism in the visual cortex against a decrease in the global cerebral blood flow (CBF) due to lower P_aCO_2 via hyperventilation-induced cerebral vasoconstriction [10]. Considering that exhaustive exercise has been associated with a hypocapnic environment, it is interesting to inquire how exhaustive exercise affects NVC. While it has been illustrated that NVC is maintained during submaximal exercise, it is currently unknown how exhaustive exercise affects NVC.

We hypothesized that NVC might be altered during exhaustive exercise because of two factors related to possible

mechanisms of NVC: hypocapnia and a changed mean arterial pressure (MAP). Hypocapnia, which occurs during high-intensity exercise, induces vasoconstriction in the cerebral vessels, decreasing blood flow velocity in the PCA (PCAv), and consequently inhibits NVC [9].

A change in MAP during exhaustive exercise is another important factor that may decrease NVC. Our previous study indicated that the pressor response to visual stimulation plays a role in NVC, as well as local vasodilatation at rest and during static exercise, and that an increase in the pressor response to visual stimulation preserved NVC against a blunted vasodilatory response during static handgrip exercise at 30% maximum voluntary contraction [5]. It was suggested that NVC is maintained during exercises via contributions by both the pressor response and vasodilatation, calculated from PCAv and MAP, in response to visual stimulation. Thus, it is uncertain which of these two factors has a greater impact on NVC.

The purpose of the present study was to investigate the effects of the pressor and regional vasodilatory responses to visual stimulation on NVC during exhaustive exercise. In this study, we measured PCAv and pressor responses to visual stimulation during a leg-cycle exercise at 75% of maximal HR, which was performed until exhaustion. We previously found a blunting of the pressor response to visual stimulation during high-intensity dynamic exercise [4]. Based on this data, we hypothesized that exhaustive exercise would reduce baseline PCAv and attenuate the magnitude of the response in the PCAv to visual stimulation. In addition, we aimed to confirm the role of the MAP on NVC.

2. Materials and Methods

2.1. Subjects. Thirteen healthy, active, nonsedentary males (age 21 ± 0.8 years (mean ± SE), height 173 ± 1 cm, body mass 64 ± 2 kg) participated in this study. All of the subjects were nonsmokers, normotensive, and free from any known autonomic dysfunction and cardiovascular disease and were not taking any medications. The Ethics Committee of the Institute of Health Science, Kyushu University, Japan, approved the experimental protocol, and all subjects provided written informed consent to participate prior to the commencement of the study. Every protocol used conformed to the Declaration of Helsinki. Each subject visited the laboratory before taking part in the experiments for familiarization with the techniques and procedures of the protocol. Subjects who normally wore glasses took them off before the experiment.

2.2. Protocol. The subjects arrived at the laboratory after having abstained from caffeinated beverages and strenuous exercise for 6 h, and from eating for at least 2 h before the experiment; they had not experienced sleep loss the previous night. All studies were performed in a darkened and quiet room at an ambient temperature of 22°C. The individual target work rate at 75% of their maximal heart rate (136 ± 6 W) was determined using an incremental cycle ergometer test at least seven days prior to the experiment. After a 2 min resting period, the subjects began cycling at half of the target work rate. After 1 minute, the exercise intensity was increased to the target work rate. This exhaustive exercise was continued until subjects could no longer maintain a pedaling cadence of 60 rpm. Following the exercise test, subjects pedaled at 20 W for cooling down for 1 minute prior to a 2 min recovery period. Subjects received visual stimulation for 2 min at resting baseline and recovery and every 5 min after the onset of exercise until exhaustion.

2.3. Measurements. Blood pressure (BP), HR, and PCAv were continuously recorded throughout the trial. End-tidal carbon dioxide partial pressure ($P_{ET}CO_2$) and NVC data were obtained at resting and recovery and every 5 min during exercise. External ear temperature was recorded before and after the exhaustive exercise.

Beat-by-beat BP was monitored with a continuous finger photoplethysmography device attached to the left middle finger (Finometer, Finapres Medical Systems, Amsterdam, The Netherlands). HR was measured continuously using a standard electrocardiogram (ECG; MEG2100, Nihon-Kohden, Tokyo, Japan). These analogue signals were sampled at 1 kHz using an A/D converter (PowerLab 8/30, ADInstruments, Colorado Springs, CO, USA). Minute-by-minute HR and MAP were calculated from the ECG and BP recordings. $P_{ET}CO_2$ and tidal volume were monitored with a gas analyzer (AE-310 s, Minato Medical Science, Tokyo, Japan), and the P_aCO_2 was estimated [11]. External ear temperature was measured using an infrared ear thermometer (MC-510, Omron Healthcare, Kyoto, Japan), which detects the tympanic temperature with infrared rays irradiated toward the tympanum from the entrance to the ear canal.

Mean PCAv was obtained by transcranial ultrasonography (WAKI, Atys Medical, St-Genis-Laval, France). A 2 MHz Doppler probe was placed at the right temporal window and fixed using a headband. The vessel was identified by TCD ultrasonography according to standard criteria [12]. Middle cerebral artery (MCA) was insonated at a depth of 50–60 mm with a standard hand probe, followed by insonation of the PCA at a depth of 60–70 mm. To confirm the accurate insonation of the PCA, we performed ipsilateral carotid compression, which increased PCAv and decreased MCAv [13]. The length of the sample volume was set at 6 mm.

2.4. Visual Stimulation. The visual stimulus was comprised of two repetitions: a 20 s eyes closed and 40 s visual stimulation period, using a reversed checkerboard. Subjects were seated 0.5 m from the front of a 24-inch flat computer screen (visual angle of 25°). During the stimulation period, subjects were asked to gaze at a small red spot at the center of the computer screen. During the eyes closed period, the screen was masked and the subjects were asked to close their eyes. The checkerboard pattern comprised of black and white squares arranged with a spatial frequency of 1.6 cycles/degree. The black-and-white squares were alternated at a frequency of 2 Hz.

2.5. Data Analysis. We estimated NVC as the relative change in the PCAv between the mean value during the 20 s with the eyes closed and the peak response during the 40 s visual stimulus. PCAv response to visual stimulation was averaged

Table 1: The heart rate (HR), mean arterial pressure (MAP), arterial partial pressure of carbon dioxide (P_aCO_2), and the posterior cerebral artery blood flow velocity (PCAv) during the rest, exercise, and recovery periods.

	Rest	Exercise period		Recovery
		5 min	Exhaustion	
HR (bpm)	69.9 ± 2.5	151.9 ± 4.3*	173.4 ± 4.4*	117.8 ± 3.5*
MAP (mmHg)	87.5 ± 4.0	112.9 ± 5.6*	98.3 ± 4.9*	85.4 ± 3.6
P_aCO_2 (mmHg)	37.2 ± 1.1	42.0 ± 1.1*	34.3 ± 0.9*	32.9 ± 1.1*
PCAv (cm/s)	32.2 ± 1.9	36.2 ± 2.6*	27.9 ± 2.1*	26.1 ± 1.5*

The data are presented as the mean ± SE values, * $P < 0.05$ versus rest.

over two repetitions for each individual. The peak velocity in response to visual stimulation was identified, and this was averaged across two repetitions. Conductance index (CI) of the cerebral vessel was calculated by dividing PCAv by MAP at the corresponding time points.

The data were expressed as the mean ± SE values. The effects of time on PCAv, MAP, and CI responses to visual stimulation were examined by a one-way repeated-measures ANOVA. When a significant F value was detected, this was analyzed further against the baseline value using Bonferroni's post hoc test. HR, MAP, P_aCO_2, external ear temperature, PCAv, and CI of the nonstimulation (eyes closed) period during exercise were compared to their resting baseline counterparts using a paired t-test. PCAv, MAP, and CI responses to visual stimulation were compared to the prestimulation (eyes closed) baseline data using a paired t-test. The level of statistical significance was set at $P < 0.05$. All of the statistical analyses were performed with the SPSS software program (PASW statistics 18, SPSS, IL, USA).

3. Results

3.1. Systemic Changes. The duration to exhaustion was 23 ± 2 min (range 13–30 min). HR significantly increased above baseline levels throughout the exercise session ($P < 0.05$; Table 1). Exercise significantly increased the MAP, while it returned to baseline level after the cessation of exercise (Table 1, Figure 1(b)). P_aCO_2 significantly increased at 5 min after the onset of exercise, but significantly decreased at exhaustion and during the recovery period (−7 ± 2 and −11 ± 1%, resp., $P < 0.05$). External ear temperature significantly increased after the exhaustive exercise from baseline (35.8 ± 0.1 and 36.6 ± 0.2°C, resp., $P < 0.05$).

3.2. Prestimulation PCAv during Exhaustive Exercise. Five minutes after the beginning of the exercise, PCAv significantly increased from baseline (11.3 ± 2.8%, $P < 0.05$; Table 1; Figure 1(a)). At exhaustion, it significantly decreased by −13.3 ± 3.7% from baseline and continued to decrease during the recovery period (−18.0 ± 3.5%, $P < 0.05$). CI in the PCA was significantly decreased at 5 min after the beginning of exercise, at exhaustion, and during the recovery period (−16.6 ± 2.4, −23.7 ± 4.9, and −18.5 ± 4.9%, resp.; Figure 1(c)).

3.3. PCAv Response to Visual Stimulation (NVC). NVC, that is, the increase response in PCAv to visual stimulation, was significantly decreased at exhaustion (11.1 ± 1.3%) compared to baseline (15.9 ± 1.5%, $P < 0.05$; Figure 2(a)), whereas it was significantly increased during the recovery period (20.2 ± 1.7%, $P < 0.05$). Conversely, the absolute peak value of PCAv in response to visual stimulation was the same level during the exhaustion and recovery periods ($P > 0.05$; Figure 2(a)). Compared to baseline, exhaustive exercise significantly suppressed the increase response in MAP with visual stimulation from 5.2 ± 1.4 to −0.7 ± 0.8 mmHg ($P < 0.05$; Figure 2(b)), while CI response significantly increased during the recovery period from 11.3 ± 1.7 to 16.9 ± 2.1% ($P < 0.05$; Figure 2(c)).

4. Discussion

The main finding of the present study was that exhaustive exercise attenuated the magnitude of NVC by suppressing the pressor response, without any significant change in the vasodilatory response to visual stimulation. In turn, NVC increased after cessation of the exercise, with an enhanced CI response to visual stimulation. Compared with baseline, the prestimulation PCAv was significantly decreased at exhaustion by cerebral vasoconstriction despite the pressor response to exercise, similar to the blood flow response in the MCA [10].

4.1. Prestimulation PCAv during Exhaustive Exercise. In the present study, at exhaustion, PCAv was lower than the resting baseline by 13%, and this was accompanied by a decrease in CI of the PCA. P_aCO_2 decreased by 7% during exhaustive exercise. These findings indicate that the decrease in PCAv was due to hyperventilation-induced cerebral vasoconstriction in the PCA. Regional vasoconstriction in the PCA may have blunted the effect of an enhanced MAP during exhaustive exercise relative to baseline level. The present results support findings by Ogoh et al. [10] who reported reduced blood flow of MCA and P_aCO_2 during exhaustive exercise.

The decreases in the prestimulation PCAv and CI of the PCA persisted during the recovery period. However, MAP returned to the resting baseline level. Thus, the cerebral vasoconstriction in the PCA must have decreased PCAv. The vasoconstriction may have been induced by the decrease in the P_aCO_2 after exercise.

Submaximal dynamic exercise would increase the CBF mainly by the pressor response with a lack of vasodilatation. In the present study, the prestimulation PCAv was significantly increased at 5 min after the beginning of exercise in spite of a decrease in the CI. The elevation in the MAP apparently overwhelmed the effects of the decrease in the CI. These results are consistent with our previous study indicating that an increase in the prestimulation PCAv was induced by an elevated MAP, but not by a change in the CI of the PCA, during high-intensity exercise [4].

The increase in the PCAv at 5 min after the beginning of exercise was not explained by hypercapnia-induced vasodilatation in the present study. Indeed, P_aCO_2 significantly increased from the resting baseline level to 5 min after beginning the exercise. Nevertheless, CI of the PCA decreased,

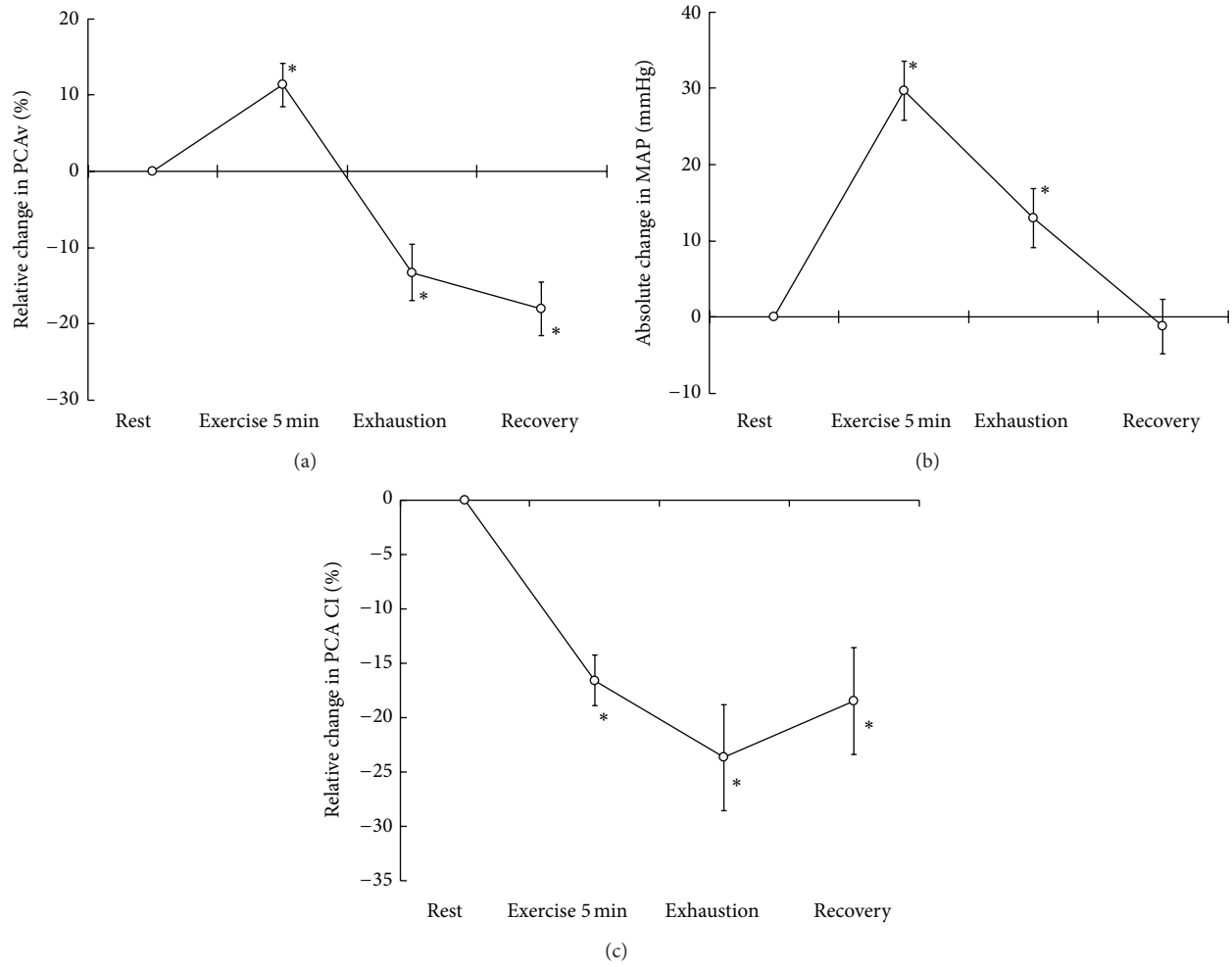

Figure 1: The relative changes in the blood flow velocity of the posterior cerebral artery (PCAv) (a), the absolute changes in the mean arterial pressure (MAP) (b), and the relative changes in the conductance index (CI) (c) from the resting baseline to the exercise and recovery periods. $^{*}P < 0.05$ versus resting baseline.

indicating that there was vasoconstriction. Although a regional difference among the various cerebral arteries during dynamic exercise was identified in a previous study, PCA blood flow responded similarly to MCA blood flow during exercise [14]. These results imply that hypercapnia-induced vasodilatation may be less effective for inducing a CBF response than the exercise-induced pressor response during submaximal dynamic exercise. What impact these factors have on PCA blood flow still remains to be determined [15].

4.2. PCAv Response to Visual Stimulation (NVC). NVC, evaluated as an increase response in the PCAv to a given visual stimulation, was attenuated at exhaustion in the present study. This attenuation was due to suppression in the MAP response to visual stimulation, whereas the increase response in the CI of the PCA to the stimulation was not changed at exhaustion compared to the baseline. This trend is consistent with our previous study indicating a limited contribution of the pressor response to NVC during high-intensity dynamic exercise [4]. The present results suggest that exhaustive exercise has an inhibitory effect on NVC, which is accompanied by a suppression of the pressor response to visual stimulation.

An increase in the eyes closed baseline PCAv during submaximal exercise would not be directly related to NVC, as was shown during static exercise [5]. In the present study, the magnitude of NVC was maintained at 5 min after the beginning of exercise, although PCAv when the subjects had their eyes closed was increased by the exercise-induced pressor response. Increases in both the MAP and CI of the PCA in response to visual stimulation were observed, in agreement with our previous study reporting the effect of submaximal dynamic exercise [4].

In the present study, the elevation of MAP associated with visual stimulation contributed to an increase in the PCAv, except for at exhaustion, supporting the contribution of the MAP to NVC. In the classic concept, a regulation of blood flow in the working cerebral regions is mainly achieved by regional vasodilatation associated with the local metabolic demand in the brain [2]. Conversely, we previously suggested that the pressor response to visual stimulation also had a role

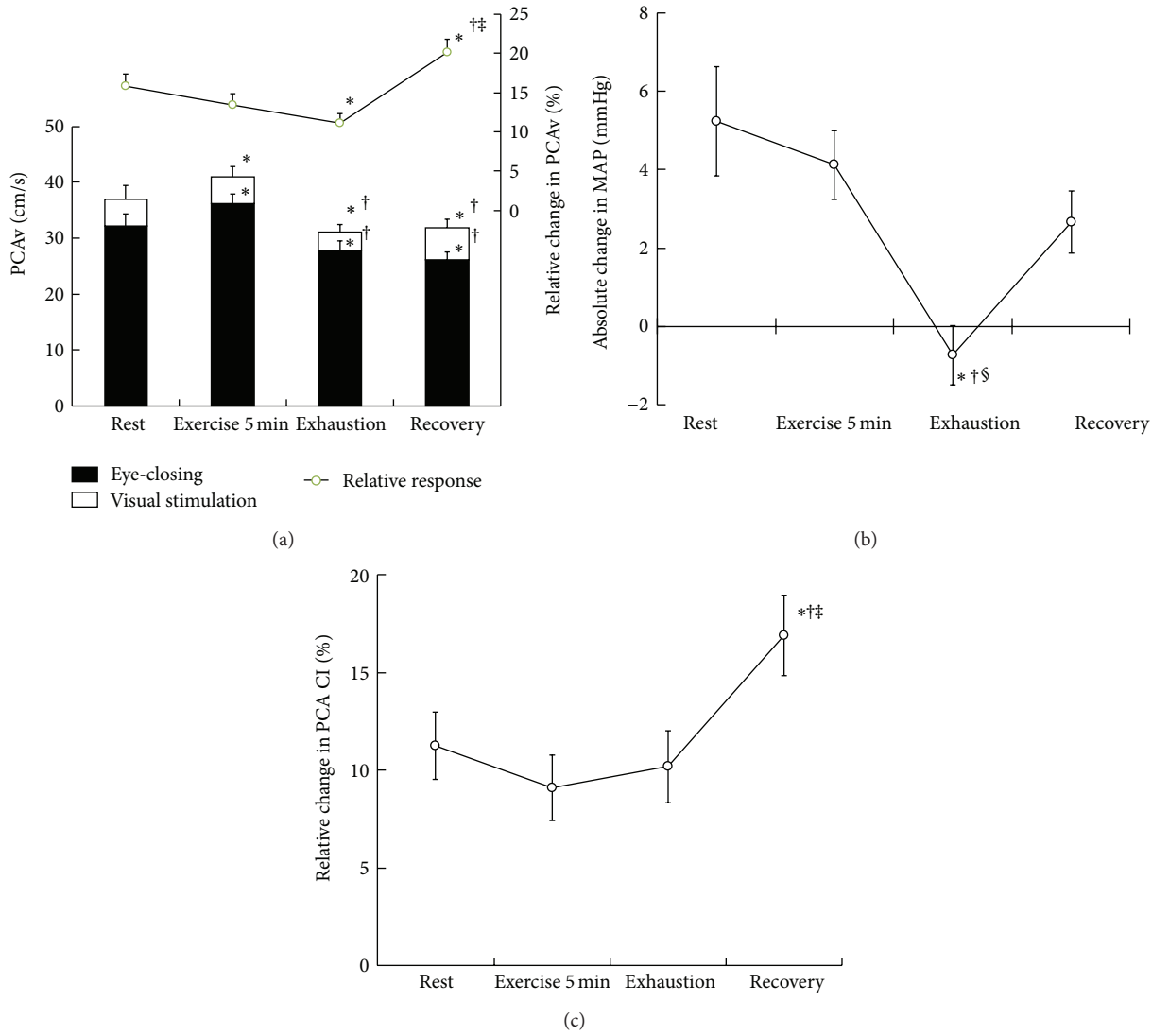

Figure 2: The PCAv during the eyes closed baseline (solid bars) and the increases by the visual stimulation (open bars) and the relative changes in the PCAv (solid line) (a), the absolute changes in the MAP (b), and the relative changes in the CI in response to visual stimulation (c). $^{*}P < 0.05$ versus resting baseline; $^{\dagger}P < 0.05$ versus 5 min of exercise; $^{\ddagger}P < 0.05$ versus exhaustion; $^{\S}P < 0.05$ versus recovery.

for accomplishing NVC [4, 5]. The present study supports our previous results.

On the other hand, the role of an elevated MAP on the increase in the PCAv induced by visual stimulation was abolished at exhaustion, in spite of a maintained increase in the CI of the PCA. External ear temperature was increased after the exhaustive exercise. The suppression of the pressor response to visual stimulation at exhaustion may be due to hyperthermia-induced peripheral vasodilatation to redistribute the blood flow to the skin, as reported in previous studies [16–18].

Cerebral vasoconstriction with hyperventilation-induced hypocapnia during exhaustive exercise attenuated NVC, in line with a previous investigation showing an inhibition of NVC in response to visual stimulation during hyperventilation-induced hypocapnia at rest [9]. Based on the present results, P_aCO_2 was significantly decreased at exhaustion, which consequently attenuated the magnitude of NVC, with a decrease in the prestimulation PCAv. Conversely, according to this finding, NVC was not inhibited although the reduction in the P_aCO_2 continued during the recovery period. The increased vasodilatory response to visual stimulation overwhelmed the effect of the decreased prestimulation PCAv related to cerebral vasoconstriction in order to preserve NVC during the recovery.

NVC increased after exhaustive exercise, whereas PCAv with the eyes closed decreased relative to baseline level at recovery. This increase in NVC can be explained by the increased CI response, because the regional vasodilation increased. On the other hand, we have no clear explanation for the stable peak value and decreased baseline. It is possible that, after exhaustive exercise, the baseline metabolic demand might decrease according to the decreased ability or demand for visual processing. In turn, once a visual stimulus is given,

the metabolic demand might increase as needed, consequently leading to exaggerated vasodilatation to compensate for the decreased baseline to increase the blood flow to a stable peak.

4.3. Technical Considerations. TCD ultrasound flowmetry has a potential limitation. This technique is based on a premise condition that a diameter of the target cerebral blood vessel is being constant. Conversely, physiological stimuli reportedly have no effect on the diameter of MCA [19]. Although there is no available data demonstrating that a diameter of PCA kept relatively unchanged during any disturbances, a change in PCAv by means of TCD ultrasonography reflected its blood flow volume.

5. Conclusions

Exhaustive exercise decreases the prestimulation PCAv and attenuates the magnitude of NVC. The inhibition of NVC at exhaustion would be mainly due to the suppression of the pressor response to visual stimulation. The present findings support our original hypothesis that the contributions of both pressor and vasodilatory responses to visual stimulation play a role in maintaining the NVC at rest and during exercise.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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Research Article

Postexercise Impact of Ice-Cold Water Bath on the Oxidant-Antioxidant Balance in Healthy Men

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The aim of the study was to determine the effect of a 5 min head-out ice-cold water bath on the oxidant-antioxidant balance in response to exercise. The crossover study included the subjects ($n = 24$; aged 28.7 ± 7.3 years) who performed two identical stationary cycling bouts for 30 min and recovered for 10 min at room temperature (RT = 20°C; session 1) or in a pool with ice-cold water (ICW = 3°C, 5 min immersion; session 2). The concentration of thiobarbituric acid reactive substances (TBARS) in blood plasma (TBARSpl) and erythrocytes (TBARSer) and the erythrocytic activity of superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx) were measured three times during each of the two study sessions: before the exercise (baseline) and 20 and 40 min after the appropriate recovery session. Lower concentration of TBARSpl 40 min after postexercise recovery in ICW was revealed as compared with that after recovery at RT ($P < 0.05$). Moreover, a statistically significant postexercise increase in the TBARSpl and TBARSer concentrations was found ($P < 0.01$ and $P < 0.05$, resp.). A short-term ice-cold water bath decreases postexercise lipid peroxidation.

1. Introduction

Low temperatures can induce different effects in the human body depending on the value, as well as duration and type of exposure. The effects of moderate but long-term cold during winter season (meaning daily temperature below 0°C for several months), found in people living in high latitude environments (circumpolar residents), are definitely negative. Among the mildest effects are unpleasant sensations and thermal discomfort. The more serious effects are decreased physical and mental performance resulting in an increased risk of accidents and injuries, as well as an increase in morbidity or even mortality at very low temperatures [1]. However, exposure to cold is widely used in sports medicine for accelerating recovery and improving sports performance. This is due to the positive effects obtained by using extremely low or moderately low temperatures at an adequately short period [2]. The most popular methods of cold therapy in sports, due to their anti-inflammatory/antiedematous and analgesic properties, are ice compresses (crushed ice packs), cold-water immersion (CWI) baths (8–10°C for 4-5 min), and whole-body cryotherapy (WBC) sessions (the vapor of liquid synthetic air at a temperature between –100 and –160°C for maximum 3 min) [2]. Specifically, CWI and ice compresses have been effectively employed for this purpose because of their effectiveness and low expense [2–4]. Another type of cold stimulation, ice-cold water (ICW) bathing, also known as winter swimming in countries where waters freeze during wintertime, has similar properties as the aforementioned cold therapies [5]. Therefore, it is used as a treatment method in rheumatic diseases [5]. Usually, during ICW baths, water temperature is 0–4°C, air temperature is below or a few degrees above 0°C, and duration is between tens of seconds and a few minutes [5–7]. Although ICW baths affect the human organism similarly as the already mentioned cold therapies and are much cheaper than, for example, WBC

sessions, no exact protocol has proven better than the other [8, 9]. Moreover, the literature indicates that ICW baths may not even offer any real benefit and, in fact, may increase postexercise muscle soreness the day after intense exercise [10]. Therefore, the rationale for using ice-cold water baths to assist the recovery of performance and the determination of their optimal protocols require further studies. Moreover, studies of the effects of low temperatures on the oxidant-antioxidant balance related to recovery/performance have been conducted only with the use of WBC [2, 11–13]. The authors emphasize that WBC sessions modulate that balance in a way that improves sports performance [11–13]. Therefore, in the studied subjects' blood, the concentration of thiobarbituric acid reactive substances (TBARS) and the activity of superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx) were measured. TBARS are markers of the lipid peroxidation process [11], while the three enzymes are the most important antioxidant agents and markers of an increased generation of reactive oxygen species (ROS) in the human organism [12]. TBARS and the mentioned enzymes have been accepted by many authors as markers of the disturbance in the oxidant-antioxidant balance induced by exercise and environmental extremes [12–15].

The aim of the study was to investigate the effect of a 5 min ice-cold water bath on the postexercise oxidant-antioxidant balance in healthy men.

Table 1: Characteristics of the study subjects. Values are given as mean ± SD.

Subjects' number	24
Age (years)	28.7 ± 7.3
BM (body mass, kg)	79.8 ± 10.2
BH (body height, cm)	181.9 ± 6.9
BF (body fat, %)	14.2 ± 4.6
TBW (total body water, %)	62.8 ± 3.3
MM (muscle mass, %)	44.9 ± 2.6
BMI (body mass index, kg/m)	24.1 ± 2.5
BS (body surface, m^2)	2.0 ± 0.1
IPAQ (level of physical activity, MET·min/wk)	2574.3 ± 1000.6
$VO_{2\,max}$[1] (maximum oxygen consumption, mL/kg/min)	42.1 ± 4.7
Borg CR10[1] (rating of perceived exertion scale)	3.7 ± 1.1
$VO_{2\,max}$[2] (maximum oxygen consumption, mL/kg/min)	44.3 ± 5.9
Borg CR10[2] (rating of perceived exertion scale)*	3.5 ± 0.9

[1]Session 1 (no ice-cold water bath).
[2]Session 2 (ice-cold water bath).
*No statistically significant differences between VO_{2max} and Borg CR10 in both sessions.

2. Materials and Methods

2.1. Subjects. 24 healthy men aged 28.7±7.3 years volunteered for the study. The subjects had never performed winter swimming or bathing in ice-cold water before the study. The subjects did not change their eating habits or physical activity immediately before or during the study period. The subjects' characteristics are presented in Table 1. The levels of physical activity and aerobic capacity were similar for all subjects.

The study was approved by the Bioethics Committee at Nicolaus Copernicus University, Ludwik Rydygier Collegium Medicum in Bydgoszcz (Poland) (number KB 657/2012). The subjects were informed about the study aims and the potential risks associated with the study and signed informed consent forms.

2.2. Study Overview. The study was divided into 2 sessions with several subjects per week for 1.5 months (a total of 24 subjects). On Mondays (session 1), the subjects performed stationary cycling for 30 min (single submaximal physical exertion), whereupon they recovered in a gym in a sitting position for 10 min (room temperature, RT, 20°C). On Fridays (session 2), the subjects performed the same single 30 min exercise bout and then recovered in a small swimming pool with ice-cold water (ICW, a single 5 min immersion at 3°C; total time: 10 min including undressing). The study subjects were dressed only in swimming trunks and immersed their whole bodies except their heads.

The study was of a crossover design; that is, the subjects were randomly assigned either to RT or ICW conditions for session 1, while for session 2 the same subjects switched their previous recovery conditions. The temperatures were measured with an accuracy of 0.2°C. Moreover, the temperature of ice-cold water was achieved and remained consistent using crushed ice.

During both sessions, blood for laboratory assays was taken from the basilic vein to 9 mL "Vacuette EDTA" tubes 3 times: directly before the cycling bout (baseline), as well as 20 min and 40 min directly after the recovery at RT or in ICW. The laboratory analyses were conducted no later than a few hours after the samples had been taken. During that time, blood was transported to the laboratory in a refrigerator at 4°C and the subsequent centrifugation of the samples in the laboratory was performed at the same temperature.

2.3. Outcomes. An analysis of the body composition of the study subjects, using the Tanita body composition analyzer (Tanita BC 418 MA Corporation, Japan) and the Bioelectric Impedance Analysis (BIA) technique, was performed. Selected indexes are presented in the paper: body mass (BM, kg), the percentage of body fat (BF, %), body height (BH, cm), total body water (TBW, %), muscle mass (MM, kg), body mass index (BMI, kg/m^2), and body surface (BS, m^2) (Table 1).

For rating perceived exertion (RPE), the Borg Category-Ratio-10 (CR10) scale was used [16]. The first rate "0" means "no exertion at all." In turn, the last rate on the scale, marked as "10," means "extremely strong" effort. There is also an exertion rate over 10, marked as "∗." This is an exertion that makes the subject "unable to continue" an exercise bout.

The RPE scale was used in both study sessions after both 30 min exercise bouts (Table 1). The subjects assessed the exercise bouts as "moderate/somewhat hard" [16].

The International Physical Activity Questionnaire (IPAQ) was used to assess the level of physical activity of the subjects during the last 7 days before the study [17]. The IPAQ is expressed in MET·min/week. One MET = 3.5 mL O_2/min/kg and represents the baseline oxygen consumption. The level of physical activity of the study subjects was moderate (2574.3 ± 1000.6; Table 1). This means that the subjects met at least one of the following 3 criteria: (i) 3 or more days of vigorous activity of at least 20 minutes per day, (ii) 5 or more days of moderate-intensity activity or walking of at least 30 minutes per day, and (iii) 5 or more days of any combination of walking, moderate-intensity, or high-intensity activities achieving a minimum of at least 600 MET·min per week [17].

In order to determine the aerobic fitness of the study subjects, the maximum oxygen consumption ($VO_{2\,max}$) was assessed directly before the 30 min exercise bouts using the physical working capacity-170 (PWC_{170}) test as an indirect method [18, 19]. The PWC_{170} index means the load (watts, W) for which heart rate (HR) amounts to 170 beats per minute (bpm). The basis for determining the PWC_{170} index is the 10 min standard cycle ergometer test during which the load in the second half is increased in such a way not to exceed the HR of 170 bpm. The PWC_{170} index was determined using the following linear function: $PWC_{170} = P_1 + (P_2 - P_1)/(170 - HR_1)(HR_2 - HR_1)$, where $P_{1,2}$ = loads of the first and the second half of the test and $HR_{1,2}$ = heart rates during the first and the second half of the test [18, 19]. The value of PWC_{170} index is significantly correlated with the value of $VO_{2\,max}$; therefore, the $VO_{2\,max}$ variables of all the subjects were calculated using the values of PWC_{170} test according to the Astrand-Ryhming nomogram [20]. The values of $VO_{2\,max}$ are presented in Table 1. They demonstrate that the aerobic fitness of the study subjects was average [20]. There is also a linear relationship between the power/load of exertion and HR between 120 bpm and 170 bpm for men aged 19 to 40; therefore, intensities and loads of the 30 min submaximal exercise bouts were determined individually for each subject using the PWC_{170} index via a recalculation to the PWC_{140} index [18, 19]. Analogously, the PWC_{140} index means a load generating the HR of 140 bpm. The mean power of the first exercise bout (session 1) was 153.3 ± 41.2 W to 78.8 ± 17.8% of maximum heart rate (HR_{max}) and the mean power of the second exercise bout (session 2) was 169.4 ± 49.5 W to 79.5 ± 7.5% of HR_{max} (HR_{max} = 205 − (age/2)). There were no statistically significant differences between the loads, HRs, $VO_{2\,max}$ values, and Borg CR10 variables; thus, the two exercise bouts were the same. However, all of these parameters were determined separately for each of the two exercise trials to ensure that they are the same, although the subjects were asked to maintain their eating habits, physical activity, and other lifestyle factors immediately before and during the study period. Earlier, the authors' own pilot studies (not published) revealed that some volunteers at an age similar to that of the subjects in this study were unable to finish the 30 min exercise bout defined by the PWC_{170} index; therefore, the PWC_{140} index was used.

Blood samples were centrifuged for 10 min at 6000 ×g at 4°C. After the centrifugation, the upper layer (plasma) was removed. Subsequently, the isolated cells were washed three times with a phosphate-buffered saline (PBS) solution at a ratio of 1 : 3 with a simultaneous centrifugation of the sample after each wash (6000 ×g/10 min). During the removal of the supernatant, the top layer of white blood cells and platelets was removed as well. Monitoring of the presence of protein in the supernatant was conducted with a 20% aqueous solution of sulfosalicylic acid, and when the reaction was negative, the washed red blood cells were mixed with a PBS solution in such way to obtain erythrocytic suspension with 50% of hematocrit index. A total of five tests were done per one blood sample: one per blood plasma (TBARSpl) and four per erythrocytic suspension (TBARSer, CAT, SOD, and GPx).

The TBARS concentration was determined in both the blood plasma (TBARSpl) and erythrocytes (TBARSer) using the spectrophotometric method by Buege and Aust [21], which was modified by Esterbauer and Cheeseman [22]. To 0.5 mL of erythrocytic suspension or plasma, 4.5 mL of reaction mixture containing 0.375% thiobarbituric acid (TBA) and 15% trichloroacetic acid (TCA) in 0.25 N HCl was added. Thus, the reaction volume was 5 mL. Such prepared samples were subsequently incubated at 100°C for 20 min to optimize the conditions for the reaction of malondialdehyde (MDA) with TBA. The identification of TBARS in blood samples was achieved via the measurement of extinction at a wavelength of 532 nm versus the baseline sample after a preceding centrifugation (2000 ×g/15 min/4°C). Lipid peroxidation is expressed in the method by the level of TBA-MDA colored compounds, since MDA is the main component of TBARS that reacts with TBA. Other TBA-positive complexes would have to be present in the sample at extremely high concentrations to interfere significantly with the TBA-MDA compounds, as the wavelength of 532 nm is the maximum of absorption for TBA combined with MDA. Moreover, to exclude lipid peroxidation during the assay, an antioxidant, butylated hydroxytoluene (BHT), was added to the sample prior to TCA precipitation. The existence of EDTA in the sample from the Vacuette tube additionally protected against this phenomenon [21, 22]. The assay range was found to be 7×10^{-2}–120 nmol/mL of plasma and 10–180 nmol MDA/g of hemoglobin (Hb). The concentrations of TBARS were expressed in 10^{-2} nmol MDA/mL of plasma (TBARSpl) and nmol MDA/g of Hb (TBARSer). The CAT activity was estimated via the Beers and Sizer method [23]. The principle of the method is based on a decrease in the absorbance (λ = 240 nm) of a hydrogen peroxide (H_2O_2) solution. H_2O_2 is decomposed by the enzyme; thus, the decrease in absorbance is directly proportional to the CAT activity which was expressed in 10^4 international units per g of hemoglobin (10^4 IU/g Hb) [23]. The detection limits (DL) were between 15×10^4 and 150×10^4 IU/g Hb. The GPx activity was determined in accordance with the method described by Paglia and Valentine [24]. The method is based on the decomposition of H_2O_2 by GPx with the simultaneous oxidation of reduced glutathione. Oxidized glutathione is then reduced in a reaction catalyzed by glutathione reductase. A coenzyme

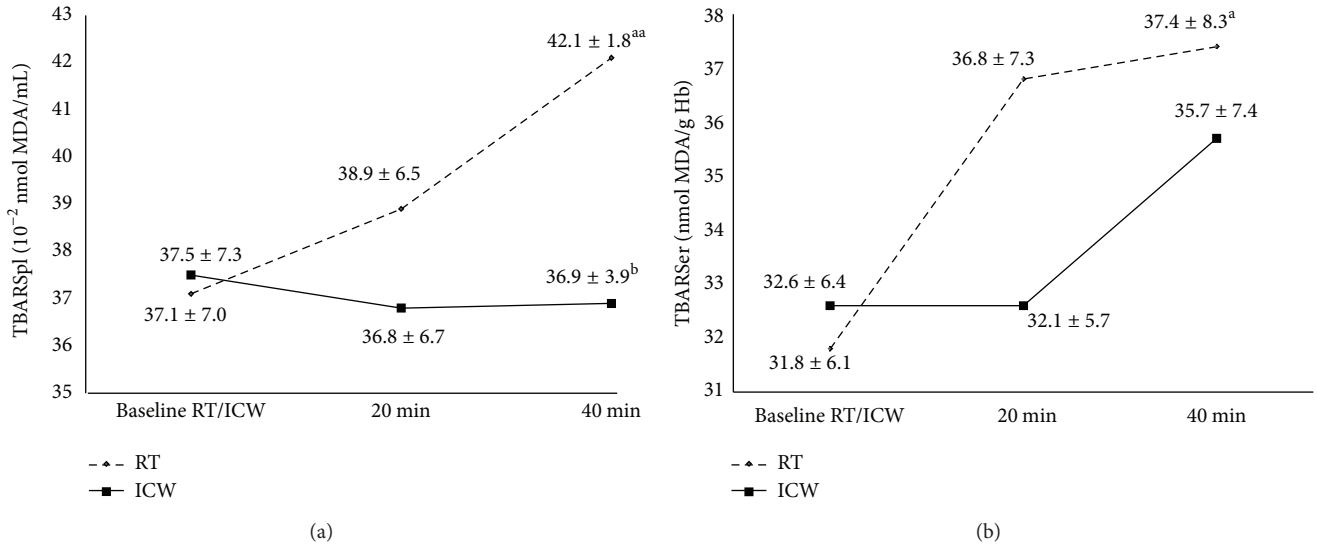

Figure 1: The TBARS concentration in blood plasma (TBARSpl) and erythrocytes (TBARSer) 20 and 40 min after recovery at room temperature (RT, 20°C) or in ice-cold water (ICW, 3°C) preceded by a 30 min aerobic exercise bout in healthy men. Statistically significant differences: versus baseline ($^{a}P < 0.05$, $^{aa}P < 0.01$) and versus 40 min after the recovery at RT ($^{b}P < 0.05$). All data are shown as mean ± SD. TBARS, thiobarbituric acid reactive substances; MDA, malondialdehyde.

in this reaction, reduced nicotinamide adenine dinucleotide phosphate (NADPH), is converted into an oxidized form ($NADP^+$) and induces a change in the absorbance of light (λ = 340 nm) [24]. Since hydrogen peroxide is a catalase substrate as well, sodium azide (Na_3N) was added to block this enzyme [24]. The GPx activity was expressed as U/g Hb and the sensitivity of the assay, expressed as DL, ranged from 2 to 36 U/g Hb. The SOD activity was measured using the Misra and Fridovich method [25]. The method is based on the inhibition of autoxidation of adrenaline to adrenochrome by the enzyme in alkaline environment, which induces a change in the extinction of the solution (λ = 480 nm) [25]. The SOD activity was expressed in U/g Hb and the DL were found to be 350–2050 U/g Hb.

2.4. Statistical Methods. The experimental data are shown as means ± standard deviations (SD) and were statistically analyzed using the analysis of variance (ANOVA) with post hoc analysis (Tukey's range test). Conformity to the normal distribution was determined on the basis of the Shapiro-Wilk test. The equality of variances was assessed using Levene's test. A P value of less than 0.05 was considered as a statistically significant difference.

3. Results

In the study, it was found that the TBARSpl concentration observed 40 min after the ICW recovery was lower by 14.1% than that observed when the subjects recovered at RT ($P < 0.05$; Figure 1(a)). However, the concentration of TBARSpl 20 min after the recovery in ICW versus 20 min after the recovery at RT did not differ in a statistically significant manner. Moreover, no statistically significant changes were found in the TBARSer concentration after the ICW recovery as compared with the RT recovery. No changes in either TBARSpl or TBARSer concentration were revealed after the ICW recovery versus their baseline levels ($P > 0.05$). On the other hand, after the exercise followed by the RT recovery, the levels of these lipid peroxidation products versus the baseline values increased in both blood plasma and erythrocytes ($P < 0.01$ and $P < 0.05$, resp.; Figure 1).

The only significant change in the antioxidant enzyme activities was a 24.4% increase in the GPx activity 40 min after the exercise/RT recovery as compared with the 20 min timepoint following this combination. Thus, the GPx activity after the exercise and RT recovery combination, as well as the exercise/ICW recovery combination, did not change compared to the baseline values ($P < 0.05$, Figure 2(c)). Moreover, the changes in the GPx activity in the entire experiment were similar between the RT and ICW recovery. Similar relationships of changes in the CAT activity between those two recovery types were also revealed. However, a tendency towards increase in the CAT activity 40 min after the exercise/ICW recovery as compared with the baseline was stronger than after the exercise/RT recovery ($P > 0.05$; Figure 2(a)). The SOD activity increased insignificantly 20 min after both recovery types compared to the baseline; however, after 40 min it decreased in the case of ICW recovery intervention and increased in the case of RT recovery intervention versus the 20 min timepoint ($P > 0.05$; Figure 2(b)).

The baseline values of oxidative stress parameters did not differ in a statistically significant manner between both study sessions/recovery types.

4. Discussion

The combination of the 30 min aerobic exercise and the ICW recovery may indicate that ice-cold water bath alleviates the

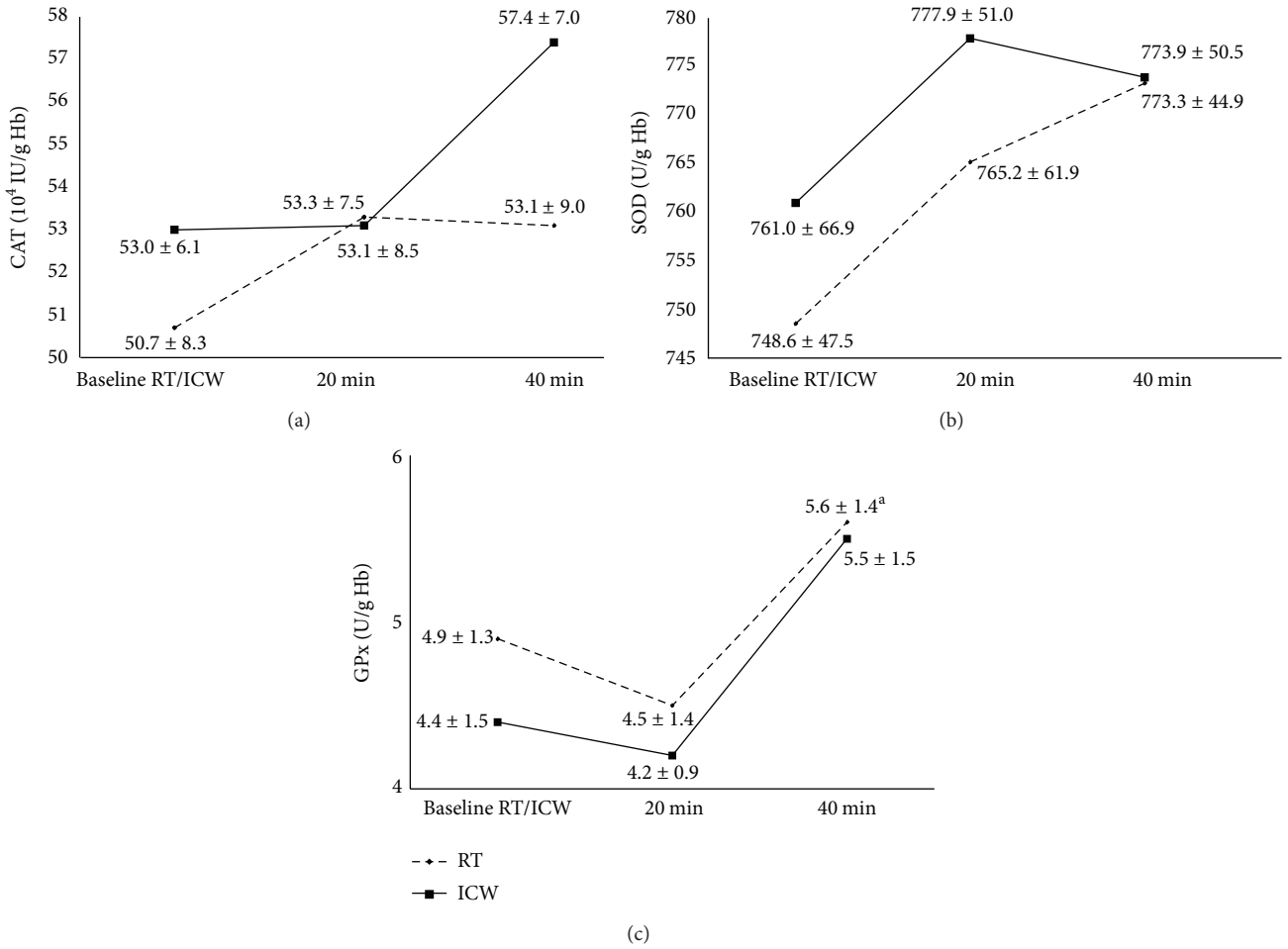

Figure 2: Erythrocytic antioxidant enzymes activities (CAT, SOD, and GPx) 20 and 40 min after recovery at room temperature (RT, 20°C) or in ice-cold water (ICW, 3°C) preceded by a 30 min aerobic exercise bout in healthy men. [a]$P < 0.05$, difference versus 20 min after the recovery at RT. No statistically significant differences were found in the CAT and SOD activities. All data are shown as mean ± SD. CAT, catalase; SOD, superoxide dismutase; GPx, glutathione peroxidase.

lipid peroxidation process enhanced due to the exercise in healthy male subjects. The TBARSpl concentration 40 min after the exercise directly followed by the recovery in ICW was lower in comparison with the same timepoint in the exercise/RT recovery combination ($P < 0.05$). Moreover, there were no statistically significant changes in the TBARSpl and TBARSer concentrations after the exercise/ICW recovery compared to the baseline values, but the changes were observed after the exercise/RT recovery ($P < 0.01$ and $P < 0.05$, resp.; Figure 1). Probably, this could be the result of a decrease in the oxidative damage of lipids in blood plasma and the sarcolemma of skeletal muscles in the course of the exercise/ICW recovery intervention because, no doubt, ROS production has a strong positive correlation with intense exercise [13]. Ice-cold water bath, similarly to WBC, could also improve the mechanism of TBARS elimination: the catabolism of MDA (the major component of TBARS) in the liver [13]. However, Siems et al. revealed that even a single short-term immersion in ice-cold water (temperature between 1°C and 4°C) during winter swimming (air temperature between −1 and 5°C) is a source of ROS [6]. The authors reported that this ice-cold water exposure caused an increase in the oxidized glutathione (GSSG) concentration with a simultaneous decrease in the reduced glutathione (GSH) concentration in erythrocytes, as well as a significant decrease in the plasma uric acid concentration in winter swimmers versus healthy volunteers (control group) who had never performed winter swimming before that study [6]. It should also be mentioned that the baseline SOD and CAT activities were higher in the erythrocytes of the winter swimmers than in the volunteers from the control group [6]. The authors postulated that regularly repeated ice-cold water baths can help the human organism to adapt to oxidative stress; therefore, the baseline activities of the enzymes were higher in the winter swimmers [6]. They confirmed these findings also in their other study [26]. Thus, the increased activities of antioxidant enzymes prove increased ROS concentrations; however, this occurrence can also be profitable as it could constitute a stimulating change, not a damaging one [6, 26]. However, in the study, the decreased concentration of TBARSpl after exercise/ICW recovery versus exercise/RT recovery was not accompanied by a simultaneous increase in the antioxidant

enzymes activities in such comparison ($P > 0.05$). Only a tendency for lower CAT activity 40 min after the exercise followed by the RT recovery than, at the same timepoint, after the exercise associated with ICW recovery was found ($P > 0.05$; Figure 2(a)). Other groups also described the adaption resulting from preexposure to lower doses of ROS. One of the groups revealed no changes in the activity of selected lysosomal enzymes in winter swimmers compared to non-winter swimmers after a single sauna bath [5], a source of ROS *per se* [27]. The authors explained the changes by the hardening of the organisms of winter swimmers, since the leakage of lysosomal enzymes may be induced by oxygen radical-mediated lipid peroxidation [5]. Therefore, the improved stability of lysosomal membranes probably resulted from an increase in the antioxidant capacity in winter swimmers [5]. The improvement of the performance of antioxidant defense in healthy people after long-term winter swimming was also found by Lubkowska et al. [15]. The authors demonstrated that a 5-month season of winter swimming improved antioxidant abilities in healthy men ($n = 15$) and, consequently, the subjects were more resistant to destabilizing effect of the WBC procedures [15]. However, in this study, ice-cold water bath was accompanied by aerobic exercise and the existence of exercise-induced oxidative stress was also revealed. It was found that, 40 min after the recovery at RT preceded by the 30 min aerobic exercise bout that is 70 min after the exercise bout, the TBARSpl concentration was significantly higher as compared with the baseline value ($P < 0.01$; Figure 1(a)). Similarly, TBARSer concentration changed in the subjects ($P < 0.05$). 40 min after the exercise/RT recovery the TBARSer concentration was higher by 17.6% in comparison with the baseline ($P < 0.05$; Figure 1(b)). An increase in the generation of ROS induced by physical exercise was also indicated by the increase in the GPx activity in the subjects' erythrocytes 40 min after the exercise/RT recovery as compared with the 20 min timepoint ($P < 0.05$; Figure 2(c)). However, the erythrocytic SOD and CAT activities did not change at all in response to the physical exercise, that is, both 20 and 40 min after the exercise bout followed by the RT recovery, as compared with the baseline ($P > 0.05$; Figures 2(a) and 2(b)). Moreover, the GPx activities after both the exercise/RT recovery and exercise/ICW recovery combinations did not change compared to the baseline values ($P > 0.05$). Therefore, the intensity of the exercise bout used in the study was probably not sufficient to cause strong oxidative stress. This confirms the subjects' assessment of the exercise bout as "moderate/somewhat hard" (Borg CR10; Table 1) [16]. The lack of statistically significant increases in the activities of these three enzymes 40 min after the exercise bout and recovery intervention, as compared to the baseline values, could also be caused by the fact that these enzymes are indicators of the first stage of ROS generation; however, the secondary indicators, TBARS, definitely increased [28]. Postexercise concentrations of ROS may be so high as to cause radical-mediated microinjuries of muscle fibers and connective tissues (articular cartilages, ligaments) which result in muscle pain, prolongation of recovery, and, consequently, a decrease in sports performance [29]. Thus, it seems reasonable to decrease the exercise-induced oxidative stress in order to improve performance, for example, via antioxidant supplementation [30]. However, there are also reports that indicate a completely opposite effect in the alleviation of the postexercise ROS levels [30]. It results from the fact that ROS are necessary for the proper muscle contraction during both rest and physical exercise [29]. Therefore, the literature recommends an adequate intake of antioxidants and balanced diet remains as the best approach to maintain the optimal antioxidant status in exercising individuals [30]. WBC is a procedure which balances the physiological effects of exercise and treats exercise-induced injuries [2]. The literature describes its effect as oxidant/antioxidant. It was found that a single session of WBC increased the antioxidant enzyme activities and lipid peroxidation in healthy untrained volunteers [12]. Another study in similar subjects showed that 10-day cryotherapy with one WBC session a day induced an increase in the total antioxidant status and the SOD activity [31]. Thus, WBC as an oxidant stimulates and, therefore, improves the antioxidant capacity in healthy untrained subjects. In turn, in elite athletes, WBC sessions used as pretraining stimulation during multiday training camps caused reverse effects: a decrease in both lipid peroxidation and the antioxidant enzyme activities [12–14]. Nevertheless, WBC is increasingly used in professional sports to facilitate the postinjury rehabilitation by reducing oxidative stress, inflammatory reactions, and pain in response to intense exercise [2, 12–14]. Thus, these inconsistent effects of WBC on the oxidant-antioxidant balance in humans probably help to maintain the most optimal ROS concentrations for human health and fitness, that is, during both rest and exercise. Similarly, the inconsistent results of the action of ice-cold water bath on the postexercise balance of the oxidation-reduction processes found in the study may suggest that bathing could also be successfully used for such purposes.

In conclusion, ice-cold water bath alleviated the level of lipid peroxidation after 30 min aerobic exercise on a cycloergometer in healthy men ($P < 0.05$). However, the rationale for the postexercise use of the 5 min head-out ice-cold water bath (3°C) for improving performance is still unknown, since, in this study, only a one-time procedure was used and the effect during long-term training bouts accompanied by ICW baths was not determined. Moreover, there were no changes in the antioxidant enzyme activities after the exercise bout accompanied by the recovery interventions compared to the baseline, as well as between the two types of postexercise recovery interventions. The temperature of ice-cold water might also be too low. Furthermore, in the literature, the long-term association of ICW baths with intense exercise is described inconsistently [9, 10]. Definitely, further studies are needed.

5. Conclusion

A 30 min aerobic exercise bout increases the generation of reactive oxygen species; therefore, it can disturb the oxidant-antioxidant balance in healthy men. However, a 5 min ice-cold water bath (3°C) decreases postexercise lipid peroxidation.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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Research Article

Single Whole-Body Cryostimulation Procedure versus Single Dry Sauna Bath: Comparison of Oxidative Impact on Healthy Male Volunteers

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Exposure to extreme heat and cold is one of the environmental factors whose action is precisely based on the mechanisms involving free radicals. Fluctuations in ambient temperature are among the agents that toughen the human organism. The goal of the study was to evaluate the impact of extremely high (dry sauna, DS) and low (whole-body cryostimulation, WBC) environmental temperatures on the oxidant-antioxidant equilibrium in the blood of healthy male subjects. The subjects performed a single DS bath ($n = 10$; 26.2 ± 4.6 years) and a single WBC procedure ($n = 15$; 27.5 ± 3.1 years). In the subjects' blood taken immediately before and 20 min after the interventions, the activity of superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx) and the concentration of thiobarbituric acid reactive substances in erythrocytes (TBARSer) and blood plasma (TBARSpl) were determined. Single WBC and DS procedures induced an increase in the activity of SOD and GPx, as well as SOD and CAT, respectively. The SOD activity was higher after WBC than after DS. Extremely high and low temperatures probably induce the formation of reactive oxygen species in the organisms of healthy men and, therefore, disturb the oxidant-antioxidant balance.

1. Introduction

All aerobic organisms constantly resist and counteract the harmful effects of reactive oxygen species (ROS). Free radicals including oxygen free radicals (OFR) are by-products of incomplete reduction of oxygen molecule [1]. They are produced in organisms either as by-products of metabolism or as a response to the action of external agents such as environmental factors. The factors may act in different ways but the main mechanism of their action is related with an increased generation of ROS. As regards biological aspects, stress reaction induced by ROS is manifested by structural and chemical changes of organic substances and, in consequence, disorders of tissue metabolism. It may also trigger adaptive mechanisms, including improvements in the antioxidant capacity [2]. Aerobic organisms have developed a variety of mechanisms of antioxidant defence. The mechanisms protect the integrity of the organisms against excessive concentrations of ROS, that is, in the course of oxidative stress. Thus, the antioxidant defence system is responsible for the protection against damage caused by free radicals. Any substance that defends a substrate from oxidative damage is called an antioxidant. Undoubtedly, superoxide dismutase (SOD), glutathione peroxidase (GPx), and catalase (CAT) are the enzymes that play a major role in the enzymatic scavenging of ROS. In addition to the antioxidant enzymes, nonenzymatic scavengers, present in cell membranes and extracellular fluids, play an equally important role. Among the nonenzymatic free radical scavengers are proteins, albumins, and the so-called low-molecular weight antioxidants, such as ascorbic acid, glutathione, and α-tocopherol. Thus, the occurrence of oxidative stress is a sign of inefficiency of the antioxidant defence system [3].

Temperature is one of the environmental factors that causes an increased generation of ROS in the human organism. Both very low and very high temperatures are able

to temper human body. Tempering is precisely based on the mechanisms involving free radicals [4–7]. One of the methods that use this low-temperature effect is whole-body cryostimulation (WBC). The method involves remaining for 1–3 min in the so-called cryo-chamber at an extremely low temperature (from −160°C to −120°C) [8]. The stimulation, combined with intense kinesiotherapy, has special recognition in the treatment of inflammatory arthritis syndromes (i.e., ankylosing spondylitis, AS, and rheumatoid arthritis, RA), fibromyalgia, multiple sclerosis, osteoporosis, overweight, and obesity, as well as in treating sport injuries and in postexercise recovery [7]. The other method, sauna bath, is a curing procedure involving surface application of extremely high temperature in an insulated wooden room, heated to between +45°C and +120°C (depending on the type of sauna), for 5–20 min. The procedure is repeated one to three times for better results. Sauna baths are often used as a supplementary treatment in patients suffering from cardiovascular diseases, depression, and respiratory diseases, especially chronic obstructive pulmonary disease (COPD), as well as from diseases of the musculoskeletal system (usually in fibromyalgia, but also in RA) [9, 10]. This method is also a source of ROS [6, 11]. Nonetheless, sauna is predominantly used for wellness and physical recovery after workout [12, 13]. Currently, the most popular type of sauna is the Finnish sauna, also called dry sauna (DS) [9].

The aim of the study was to investigate the effect of single WBC and DS sessions on the oxidant-antioxidant equilibrium in healthy men who volunteered for the study. In the subjects' blood, the activity of CAT, SOD, and GPx and the concentration of thiobarbituric acid reactive substances (TBARS) were determined.

2. Material and Methods

2.1. Participants. Healthy men, volunteers, performed a single DS bath ($n = 10$) and a single WBC procedure ($n = 15$). The subjects' average age was 26.2 ± 4.6 years in the DS group and 27.5 ± 3.1 years in the WBC group. All the men who volunteered for the study had never used sauna bathing or cryostimulation before the study period. No subject suffered from obesity or cardiovascular or pulmonary diseases. Every subject exercised moderate physical activity and their diet was varied with no supplementation. Additional features of the subjects are shown in Table 1. Moreover, the subjects were informed about the goal of the study and signed the consent forms. The study was approved by the Bioethics Committee at Nicolaus Copernicus University, Ludwik Rydygier Collegium Medicum in Bydgoszcz (nos. KB189/2012 and KB657/2012).

2.2. Study Design. The single WBC treatment (−120°C, 2 min) was carried out at the Olympic Preparation Centre (OPC) in Walcz, Poland. The subjects who entered the cryo-chamber wore shorts, socks, gloves, and headbands for ear protection. Wooden clogs were used as a protection from foot frostbites, whereas the lungs were protected by a surgical face mask (with "double gauze pad layer"). The subjects were informed about the contraindications of WBC (hypertension, circulatory failure) and the necessity of taking inhalations two times shorter than exhalations.

Table 1: Basic characteristics of the participants from both study groups. The values are expressed as means ± standard deviations.

	WBC	DS
Number of participants	15	10
Age (years)	27.5 ± 3.1	26.2 ± 4.6
Body height (cm)	179.9 ± 7.0	180.4 ± 7.2
Body weight (kg)	71.6 ± 3.6	82.2 ± 13.6
Body mass index (BMI, kg/m^2)	23.1 ± 1.3	27.0 ± 6.4
Body surface are (BSA, m^2)	1.9 ± 0.1	2.0 ± 0.2
Smokers	6.67%	20%

The single DS bath was performed at the Sport Factory Fitness Centre, Bydgoszcz, Poland, at +90°C, 10% of relative air humidity, for 40 minutes. The DS bath was divided into 3 consecutive exposures. Each of these exposures lasted for 10 minutes plus approximately 10 minutes for cooling the body with a cold shower and a rehydration between the trials. The subjects entered the sauna in pool slippers.

Blood taken from the basilic vein into test tubes before and 20 min after the DS or WBC sessions was subsequently used for the assays of selected oxidative stress parameters.

2.3. TBARS Measurement. The thiobarbituric acid reactive substances were determined in both the subjects' plasma (TBARSpl) and erythrocytes (TBARSer) using the method described by Buege and Aust [14] and modified by Esterbauer and Cheeseman [15]. TBARS, as lipid peroxidation products containing mainly malondialdehyde (MDA), were identified using thiobarbituric acid (TBA). To the hemolysate or 0.5 mL of plasma, 4.5 mL of reactive mixture containing 0.375% of TBA and 15% of trichloroacetic acid (TCA) in 0.25 M HCl was added. In order to prevent the formation of lipid peroxidation products, 0.01% solution of 3.5-diisobutyl-4-hydroxytoluene (BHT), an inhibitor of lipid peroxidation, was added into the test tubes during the reaction. The samples were incubated for 20 minutes in hot water (+100°C), cooled down, and centrifuged at 2000 ×g for 15 minutes at +4°C. The absorbance of the samples (supernatant) was measured at the wavelength of 532 nm. The TBARSer concentration was expressed in nmol MDA per g of haemoglobin and the TBARSpl was expressed in nmol MDA per mL of blood plasma.

2.4. SOD, CAT, and GPx Measurement. The activity of antioxidant enzymes was determined in the subjects' erythrocytes. The CAT activity was determined using the Beers and Sizer method [16]. The method is based on a decrease in the absorbance of a hydrogen peroxide (H_2O_2) solution. H_2O_2 is decomposed by the enzyme, so the decrease in the absorbance is directly proportional to its activity. The CAT activity was expressed in 10^4 IU per g of haemoglobin.

The GPx activity was determined according to the Paglia and Valentine method [17]. The principle of the method is based on the decomposition of H_2O_2 by GPx with the

Table 2: The values of oxidative stress parameters in the blood of healthy men 20 min after the whole-body cryostimulation (WBC) procedure and the dry sauna (DS) bath. The results are expressed as means ± standard deviations.

	WBC		DS	
	Baseline	After WBC	Baseline	After DS
TBARSpl [nmol MDA/mL]	0.57 ± 0.08	0.59 ± 0.08	0.34 ± 0.05^{aaa}	0.35 ± 0.06^{bbb}
TBARSer [nmol MDA/g Hb]	36.6 ± 13.2	41.1 ± 17.8	18.2 ± 4.1^{aaa}	17.6 ± 5.0^{bbb}
SOD [U/g Hb]	791.1 ± 192.5	1034.5 ± 112.6^{aaa}	773.2 ± 124.3	880.2 ± 121.3^{bbc}
CAT [10^4 IU/g Hb]	74.6 ± 20.6	67.9 ± 11.2	59.8 ± 16.3	66.2 ± 16.0^{cc}
GPx [U/g Hb]	17.8 ± 9.7	25.0 ± 11.5^{aa}	5.9 ± 1.9^{aaa}	7.4 ± 3.3^{bbb}

^{a}Difference versus WBC baseline value ($^{aa}P < 0.01$, $^{aaa}P < 0.001$); ^{b}difference versus post-WBC value ($^{bb}P < 0.01$, $^{bbb}P < 0.001$); ^{c}difference versus DS baseline value ($^{c}P < 0.05$, $^{cc}P < 0.01$). TBARSer, thiobarbituric acid reactive substances in erythrocytes; TBARSpl, thiobarbituric acid reactive substances in blood plasma; SOD, superoxide dismutase; CAT, catalase; GPx, glutathione peroxidase.

simultaneous oxidation of reduced glutathione. Oxidized glutathione is then reduced in a reaction catalysed by glutathione reductase. The role of a coenzyme in this reaction is played by reduced nicotinamide adenine dinucleotide phosphate (NADPH), which is converted into an oxidized form and induces a change in the absorbance of the light. The GPx activity was expressed in U per g of haemoglobin.

The SOD activity was assayed using the method by Misra and Fridovich [18]. The method is based on an inhibition of adrenaline oxidation to adrenochrome in alkaline environment, which induces a change in the extinction of the solution. The activity of SOD was expressed in U per g of haemoglobin.

2.5. Statistical Analysis. The obtained results are presented as means ± standard deviations. Differences between means were tested using the Student's t-test for dependent and independent variables. Before running the t-test, the model assumptions were also verified using the Kolmogorov-Smirnov test for normality and the χ^2-test to assess the equinumerosity of the groups. Differences at a significance level of $P < 0.05$ were assumed as statistically significant.

3. Results

In the WBC group after the procedure, the SOD and GPx activities significantly increased ($P < 0.001$ and $P < 0.01$, resp.). The increase in the SOD activity in the subjects' erythrocytes after the WBC procedure amounted to 30.8% and the GPx activity amounted to 40.4% (Table 2). Furthermore, a negative Pearson's correlation coefficient (r) between the GPx activity and the TBARSer concentration after the WBC procedure was revealed (Figure 1). Twenty minutes after the WBC procedure, a positive correlation coefficient between the concentrations of TBARSer and TBARSpl and a negative correlation coefficient between the GPx activity and the TBARSpl concentration were found ($P < 0.01$ and $P < 0.05$, resp.; Figure 2).

In the DS group, the sauna bath induced a statistically significant increase in the SOD and CAT activities. After the DS procedure, the SOD activity increased by 13.8% ($P < 0.05$) and the CAT activity increased by 10.7% ($P < 0.01$; Table 2). Moreover, very high positive linear correlation coefficients

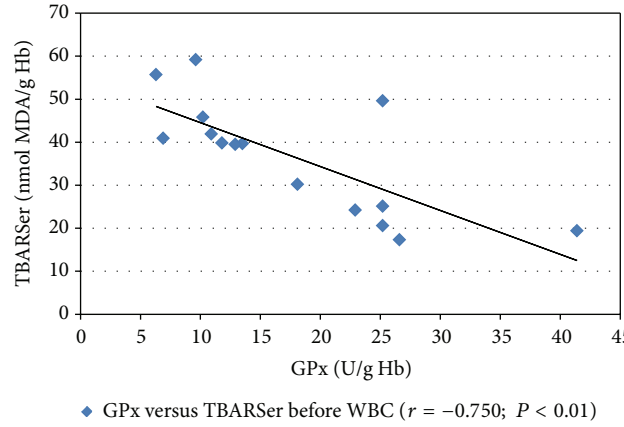

Figure 1: Pearson's correlation coefficient (r) between the TBARSer concentration and the GPx activity in the erythrocytes of healthy male subjects directly before the whole-body cryostimulation (WBC) treatment. TBARSer, thiobarbituric acid reactive substances in erythrocytes; GPx, glutathione peroxidase.

between the activity of SOD and CAT, both directly before and 20 min after the DS bath, were observed ($P < 0.05$ and $P < 0.01$, resp.; Figure 3).

Both study groups differed in a statistically significant manner in oxidative stress parameters (Table 2). Before (baseline) and after the procedures, the TBARSpl, TBARSer, and GPx levels were higher in the participants subjected to WBC compared to the DS-treated subjects ($P < 0.001$). The SOD activity after the WBC procedure was higher by 17.5% compared to the activity measured after the DS bath ($P < 0.01$); however, the baseline values of the SOD activity did not differ ($P > 0.05$). There were no differences between the two groups with reference to the CAT activity ($P > 0.05$).

4. Discussion

The changes in the oxidative stress parameters in the WBC group reported in this paper are similar to the changes reported by Wozniak et al. [8] who studied 10 healthy men subjected to a single WBC procedure (−120°C for 3 min). The authors showed a lack of changes in the plasma and erythrocytic TBARS concentrations ($P > 0.05$) and an

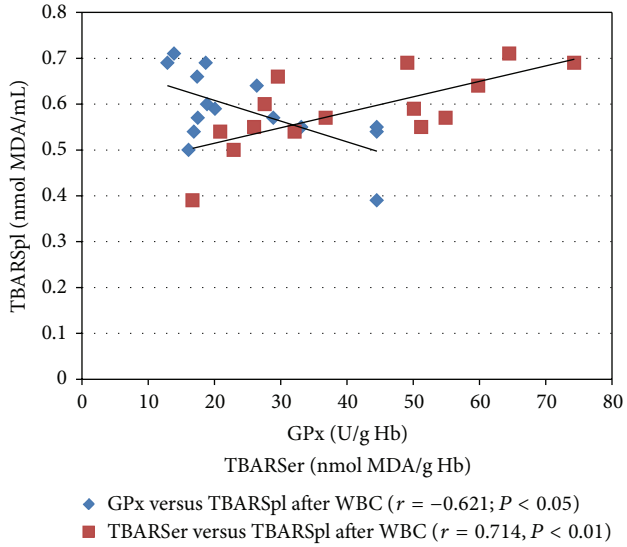

Figure 2: Pearson's correlation coefficient (r) between the concentrations of TBARSer and TBARSpl, as well as between the GPx activity and the TBARSpl concentration in the blood of healthy male subjects 20 min after the whole-body cryostimulation (WBC) treatment. TBARSer, thiobarbituric acid reactive substances in erythrocytes; TBARSpl, thiobarbituric acid reactive substances in blood plasma; GPx, erythrocytic glutathione peroxidase.

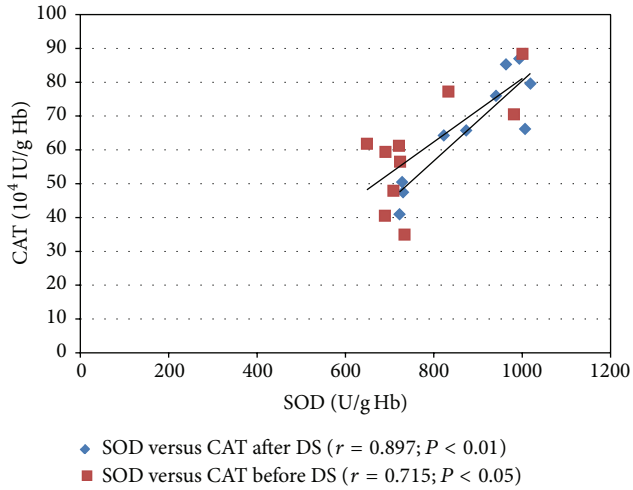

Figure 3: Pearson's correlation coefficient (r) between the activity of SOD and CAT in the erythrocytes of healthy male subjects directly before and 20 min after the dry sauna (DS) bath. SOD, superoxide dismutase; CAT, catalase.

increase in the erythrocytic SOD ($P < 0.001$) and GPx ($P < 0.01$) activities 20 min after the procedure [8]. Similarly, no statistically significant differences in the TBARSpl and TBARSer concentrations were found in another study in the blood of 12 men immediately after a single WBC procedure (–120°C, 2 min) [5]. Moreover, the authors demonstrated that the plasma concentrations of vitamins A and E in the subjects did not change either ($P > 0.05$) [5]. Another study revealed a statistically significant decrease in the total oxidative status (TOS), but also in the total antioxidative status (TAS) 30 min after a single WBC procedure (–130°C, 3 min) in the blood of 15 clinically healthy men aged 21 [19]. Inconsistencies in the obtained data may probably be due to the reduced blood supply to deep tissues, which could be confirmed by the most recent findings regarding WBC by Selfe et al. [20]. The authors demonstrated a decrease in both deoxyhaemoglobin and oxyhaemoglobin for vastus lateralis a few minutes after the WBC procedure (–135°C, 1–3 min) in fourteen elite rugby players [20]. Moreover, Lubkowska et al. revealed no differences in TAS and TOS the day after the WBC procedure as compared with the initial values [19]. All the above mentioned data suggest that a single WBC procedure slightly disturbs the oxidant-antioxidant balance in healthy male subjects but is not sufficient to induce oxidative stress. Furthermore, in the paper, statistically significant high negative r-Pearson's correlations between the erythrocytic GPx activity and the TBARSer concentration before the WBC procedure, as well as between the GPx activity and the TBARSpl concentration after the procedure, were observed (Figures 1 and 2). The correlations, together with the changes in the SOD and GPx activities, as well as the TBARS concentrations, point additionally to the primary generation of ROS. Many papers confirm that WBC treatment has a prooxidative effect that results in adaptive changes [7, 8, 21–23]. Therefore, those references, along with the results of this paper, indicate that cryostimulation induces an increase in the production of ROS in humans which is insufficient to cause oxidative stress but sufficient to trigger beneficial antioxidant processes.

Dry sauna was found to have a similar effect. The increase in the SOD ($P < 0.05$) and CAT activities ($P < 0.01$) after a single DS bath reported in this paper proves the increase in the concentrations of superoxide anion ($O_2^{\bullet -}$) and hydrogen peroxide (H_2O_2) in the subjects' blood. Joint involvement of SOD and CAT in the scavenging of ROS was confirmed by the very high positive linear correlation coefficients (Figure 3). Sutkowy et al. [6] also found a statistically significant increase in the SOD activity induced by dry sauna bath. In seven healthy men exposed to an extremely high temperature (+90°C) three times for 10 minutes, the SOD activity increased both 15 and 60 minutes after the bath as compared with control (measurement performed directly before the bath; $P < 0.05$) [6]. A disturbance in the oxidant-antioxidant balance and adaptive changes after dry sauna bath in healthy young men were also investigated by Zinchuk and Zhadko [11]. After the sauna bath at the start of the experiment ($n = 13$), a statistically significant increase in the concentrations of conjugated dienes (CD), malondialdehyde (MDA), and Schiff bases in both the subjects' blood plasma and erythrocytes was observed, along with a simultaneous decrease in the erythrocytic CAT activity and the plasma concentration of vitamin E. At the end of the experiment ($n = 11$), an adaptive effect was observed: the men became accustomed to the extremely high temperature. After the sauna bath, a postexposure increase was observed only in the erythrocytic MDA and plasma Schiff bases concentrations,

while a decrease was observed in the erythrocytic CAT activity. The authors also revealed an increase in the concentration of total nitrite after the sauna bath both at the start and at the end of the experiment. The authors explained the results by an increase in the nitric oxide (NO) concentration [11]. However, it is unclear why the results obtained by Zinchuk and Zhadko [11] are not consistent with normal distribution and what is meant by "the start of the course" and "the end of the course." The authors only reported that the whole experiment consisted of 20 sauna baths once a week for 5 months. Each bath consisted of two exposures to +85–+90°C, 10–15% of relative air humidity, 5–10 min, followed by cooling down at room temperature (+20–+21°C) for 5 min. Other authors also take into account a stress effect of sauna baths in the context of increased ROS generation and the resulting antioxidant response and adaptation of the human organism [4, 24, 25].

To sum up, the literature describes sauna baths and whole-body cryostimulation as agents that disturb the oxidant-antioxidant balance slightly towards oxidation. Therefore, the effect allows a gradual adaptation and hardening of the organism in the long-term use. The presented study also revealed this effect, but, due to rather small study groups, further studies are necessary to confirm these findings.

5. Conclusions

A single whole-body cryostimulation procedure and a single dry sauna bath probably induce the formation of reactive oxygen species in the organisms of healthy men and, therefore, disturb the oxidant-antioxidant balance.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

Acknowledgments

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Research Article

Physiological and Selective Attention Demands during an International Rally Motor Sport Event

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Purpose. To monitor physiological and attention responses of drivers and codrivers during a World Rally Championship (WRC) event. *Methods*. Observational data were collected from ten male drivers/codrivers on heart rate (HR), core body (T_{core}) and skin temperature (T_{sk}), hydration status (urine osmolality), fluid intake (self-report), and visual and auditory selective attention (performance tests). Measures were taken pre-, mid-, and postcompetition day and also during the precompetition reconnaissance. *Results*. In ambient temperatures of 20.1°C (in-car peak 33.9°C) mean (SD) peak HR and T_{core} were significantly elevated ($P < 0.05$) during rally compared to reconnaissance (166 (17) versus 111 (16) beats·min^{-1} and 38.5 (0.4) versus 37.6 (0.2)°C, resp.). Values during competitive stages were substantially higher in drivers. High urine osmolality was indicated in some drivers within competition. Attention was maintained during the event but was significantly lower prerally, though with considerable individual variation. *Conclusions*. Environmental and physical demands during rally competition produced significant physiological responses. Challenges to thermoregulation, hydration status, and cognitive function need to be addressed to minimise potentially negative effects on performance and safety.

1. Introduction

Competitive motor sport requires high levels of physical and cognitive performance whilst experiencing significant environmental, physical, and psychological demands. These include physical work against considerable gravitational and physical forces in confined spaces; climatic conditions; compulsory safety clothing (fire-protective under-clothing, overalls, balaclavas, helmets, boots, and gloves); proximity to hot engines; pressure to succeed; significance of winning; fear of failure; competitors; observation by spectators and media; and increased information load and distraction. Determining the impact of the demands is important to assist maintaining or enhancing physiological and psychological components of performance and to maximize safety and competition outcome. Relevance of such study extends beyond motor sport to other domains (military, police, and emergency services) where skilled performers with good fitness complete cognitively challenging performances in clothing and conditions that contribute to demands.

Heart rates ranging 130–200 beats·min^{-1} have been recorded across a range of different motor sport disciplines, conditions and simulated versus real competition scenarios [1–5]. Previously, higher heart rates in competition were interpreted as primarily "psychoemotional" in nature [5]; however measurement [3] and estimation [6] of energy expenditure as 5–13 times higher than resting (METS) illustrate that drivers must work hard to perform accurate physical and cognitive tasks under physiological conditions similar to some other sports. Furthermore, in some motor sports competition duration can be considerably longer, spanning multiple days, which increases the probability that central and peripheral fatigue will impact performance.

The effects of heat and dehydration, which in real life setting are usually combined, have been the subject of much research in military [7], occupational [8], and sports settings [9–12]. Current understanding about dehydration, hyperthermia and performance has evolved from "critical threshold" theories, since research has shown performers successfully enduring higher temperatures and greater dehydration

during real competitive events [9–12]. For example, ironman triathletes and sailing crews have experienced average core temperatures of 38.1°C (23°C ambient) and 39.2°C (32°C ambient), respectively, during performances with some individual values in excess of 40°C without incident [13, 14]. However, evidence from endurance performances with less cognitive processing demands and little constraint on clothing cannot be directly compared to motor sport. Although equivocal, recent evidence from controlled laboratory studies suggests even moderate levels of dehydration (1-2% body mass) can have a significant negative impact on cognitive function [15, 16] with less effect from hyperthermia alone. Roberts and Cole [17] found that 5 minutes exercise wearing police body armour produced changes in cognitive function (decreased executive and increased nonexecutive). However, the student participants were completing unfamiliar activities and tasks in which they had no specialized ability or training. Consequently findings do not accurately reflect how expert performers may be able to maintain complex performances where training and skill may offset performance decrements by focusing resources on critical aspects and utilizing specific strategies.

Currently, laboratory research that has sought to isolate distinct effects of heat and dehydration does not have the ecological validity necessary to transfer knowledge to real world applications which requires a more applied approach to research. Morley and colleagues [8] found that 50–120 minutes of exercise, wearing firefighters' protective equipment in 33–35°C heat, produced a rapid rise in core body temperature and a delayed drop in cognitive performance an hour or more following exercise. Interestingly, whilst cognitive tasks completed through audio recordings partially resemble firefighters' cognitive demands of radio communications, the participants were a mix of firefighters and other volunteers. The experience and motivations of volunteer samples are radically different to individuals involved in military operations, emergency response or professional competitive sport, and this may significantly impact on performance capacity.

Thus, the current study aimed to characterize some of the changes in physiological (heart rate, thermoregulation and hydration) and selective attention markers observed in a group of drivers and codrivers during a high-pressure, international motor sport event—a round of the World Rally Championship (WRC). It was hypothesised that there would be significant elevations in heart rate, skin and core temperature, during rally compared to reconnaissance. Furthermore, it was hypothesised that there would be significant differences in urine osmolality and selective attention within and between rally and reconnaissance.

2. Method

2.1. Participants. All ten male subelite performers (5 driver/codriver pairs; Mean (SD) age, 30 (9) years; Height, 1.76 (0.05) m; Mass, 75.9 (11.2) kg) selected onto an international driver development programme (Pirelli Star Driver) signed informed consent to participate. The study was approved by the Research Ethics Subcommittee of the School of Education, University of Edinburgh. Medical questionnaires were screened for contraindications associated with ingested core temperature sensors [18]. Permission was provided by the medical delegate for WRC from the world governing body (Federation Internationale de l'Automobile) and the event Chief Medical Officer.

2.2. Rally Event. Rally is a classification of motor sport involving driver and codriver (navigator) driving a series of stages in the fastest cumulative time, on a variety of surfaces. Cars, resembling showroom models, are turbo-charged, stripped down for weight and strengthened to withstand high speeds (0–100 $km \cdot h^{-1}$ in <3 s; peak > 200 $km \cdot h^{-1}$) and impacts of corners and jumps. This study took place during one round of The World Rally Championship (WRC), the premier competition comprising normally 13 events per year, in locations around the world in sometimes extreme climates. The event in Portugal spanned six days, including two days of reconnaissance making pace notes driving the route at restricted speeds in a normal road car without any safety clothing or helmets, followed by an interval day with mechanical testing and media commitments. Competitive driving then lasted two and a half days with 18 timed "special stages" (ranging 1 to >40 km each), separated by hundreds of kilometres driving on open, public roads at legal speeds. Cars completed approximately 3 or more stages consecutively before returning for short (30 minute) servicing. In WRC winning margins have been as little as 0.1 seconds after three days and several hundred stage kilometres, so enhancing any relevant performance factor could potentially influence outcome. Note that access to participants was completely restricted to one reconnaissance and one competition day within this single event and potential experimental disruption to performance had to be minimised. This was one event of only 6 that this development program entailed, and these drivers had very minimal precompetition testing days in the specific and very expensive rally cars, further adding to the pressure of the event.

Continuous measurement of heart rate (HR), core (T_{core}) and skin temperature (T_{sk}) was completed during one reconnaissance and one competition day. Participants were also assessed at early morning (pre), during (mid) and at the end of the day (post) for urine osmolality (U_{osm}), selective attention and self-reported fluid intake (volume and content).

2.3. Heart Rate, Core and Skin Temperatures. A lightweight (total ~ 80 g) ambulatory monitoring system (Equivital EQ01, Hidalgo Ltd., UK) was used to record (1 Hz) T_{core} (from ingestible pill sensor—VitalSense, Mini Mitter, Philips Respironics, The Netherlands), and T_{sk} (°C) and HR (beats·min^{-1}) (from skin thermistor and sensors in elasticated chest strap). Pill sensors were ingested 3 hours before recording to ensure good reliability and validity of measurement was obtained [18].

Peak in-car temperature was measured using lightweight battery operated thermometers (Model 810–210, Electronic Temperature Instruments Ltd., UK; range −50 to 70°C, accuracy ±1°C, resolution ±0.1°C). Competition restrictions precluded more sophisticated in-car measurement.

Table 1: Summary data (peak and mean) during reconnaissance and rally, for heart rate (HR, including values during the competitive special stages (SS) only), core temperature (T_{core}), and skin temperature (T_{sk}). Data are shown as means (SD) for the whole group (in bold) and also separately for drivers and codrivers.

	Rally			Reconnaissance		
	Group ($n = 8$)	Drivers ($n = 4$)	Codrivers ($n = 4$)	Group ($n = 10$)	Drivers ($n = 5$)	Codrivers ($n = 5$)
SS HR (beats·min^{-1})	**133 (24)**	154 (12)	113 (12)	—	—	—
Mean HR (beats·min^{-1})	**109*** **(8)**	113 (11)	105 (11)	**82 (12)**	80 (16)	84 (16)
Peak HR (beats·min^{-1})	**166*** **(17)**	177 (18)	155 (18)	**111 (16)**	106 (19)	116 (19)
Mean T_{core} (°C)	**37.9*** **(0.3)**	38.1 (0.4)	37.8 (0.4)	**37.3 (0.2)**	37.3 (0.3)	37.4 (0.3)
Peak T_{core} (°C)	**38.5*** **(0.4)**	38.8 (0.4)	38.2 (0.4)	**37.6 (0.2)**	37.7 (0.3)	37.6 (0.3)
Mean T_{sk} (°C)	**33.9**$^{\#}$ **(0.5)**	33.7 (0.4)	34.1 (0.4)	**35.0 (0.6)**	35.0 (0.7)	35.0 (0.7)
Peak T_{sk} (°C)	**36.7**$^{\#}$ **(0.6)**	37.0 (0.2)	36.4 (0.2)	**38.1 (1.4)**	38.0 (1.7)	38.3 (1.7)

*Significantly greater than reconnaissance values ($P = 0.012$ to 0.018).
$^{\#}$Significantly lower than reconnaissance values ($P = 0.012$ to 0.017).

2.4. Urine Osmolality. With limited access to participants during the days of monitoring and no immediate access prior to the event, hydration markers such as changes in body mass and blood markers were excluded. Therefore hydration status was estimated from urine osmolality using the refractive index, a recognised field-based measurement [19, 20]. Midstream samples (~20 mL) from pre- (first morning), mid-, and postreconnaissance and rally days were analysed using a portable refractometer (Osmocheck, Vitech Scientific Limited, West Sussex, UK).

2.5. Selective Attention. Two subtests of the Test of Everyday Attention [21], used previously to assess attentional processing changes in military survival environment [22], were selected as they correspond specifically to some of the performance demands of rally (listening to audio communications and map reading) and did not require specific language proficiency or educational attainment: *Map search*—assessed visual selective attention requiring a 120 second search for 80 target symbols distributed on a coloured map. *Elevator counting with distraction*—assessed auditory-verbal working memory requiring participants to mentally count target tones whilst ignoring distracting tones. To minimise practice and learning effects participants were familiarised with tests the day before the first measurement. Three equivalent versions of both subtests were used and repeated tests with the same version were more than 76 h apart.

2.6. Statistical Analysis. Data was recorded for 10 participants during the reconnaissance and 8 participants on the rally. Data from one participant, taking nonprescription medication for cold-symptoms, was omitted from urine analysis, and data from one participant unable to discriminate tonal differences was omitted from auditory attention scores. Descriptive statistics (Mean (SD) and peak values) were calculated across 6 h on reconnaissance day and 9 h on rally day. Values were also calculated for HR during the rally special stages. Comparison between drivers and codrivers was restricted to qualitative interpretation of the descriptive statistics because of small subgroup size.

Mean and peak values for HR, T_{core} and T_{sk} during the rally and reconnaissance were not normally distributed and were compared using Wilcoxon matched pairs test. Effect sizes for Wilcoxon matched pairs test were calculated as r by dividing Z score by number of observations. A one-way ANOVA with repeated measures was conducted over the time-points for U_{osm} and attention scores, with Bonferroni adjusted post hoc comparisons. Statistical significance was set a priori at alpha <0.05. Effect sizes were calculated using partial eta squared (partial η^2) square-rooted to give correlation coefficients (r) [23]. Comparison for effect sizes were made in line with Hopkins [23]; 0.1–0.3 as small, 0.3–0.5 as moderate, 0.5–0.7 as large and 0.7–0.9 as very large.

3. Results

3.1. Conditions. Mean ambient temperature during the reconnaissance was 19.4°C (peak 23°C, mean relative humidity 70%) and during the rally was 20.1°C (peak 24.2°C, smean relative humidity 67%). Cabin temperatures peaked at 36.9°C and 33.9°C in reconnaissance and rally, respectively. Reconnaissance driving involved 124.1 km of special stages at normal speeds (capped at 90 km·h^{-1}) and 125.7 km of road-driving. Rally driving involved 135.1 km (6 stages) at competitive speeds and 300.1 km of road-driving. The stages were very similar in distance (range 20.2–22.7 km), each lasting approximately 15 minutes in duration.

3.2. Heart Rate, Core and Skin Temperatures. Average and peak HR and T_{core} were significantly higher during the rally than the reconnaissance ($P = 0.012$ to 0.018, $r = 0.59$ to 0.63), with drivers higher than codrivers during the rally but not the reconnaissance (Table 1). The 6 special stages elicited the highest responses in all drivers (Figure 1). Considerable intra- and interindividual variation occurred for T_{sk}, with peak and average reconnaissance values significantly higher

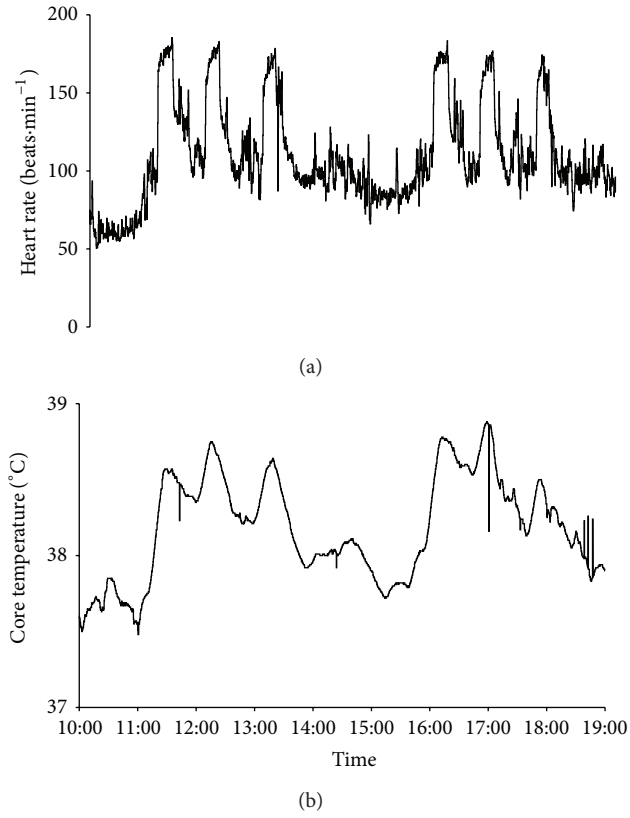

Figure 1: An example profile of heart rate (a) and core body temperature (b) for a representative driver during a single day of rally competition, with the six competitive special stages clearly visible in both profiles.

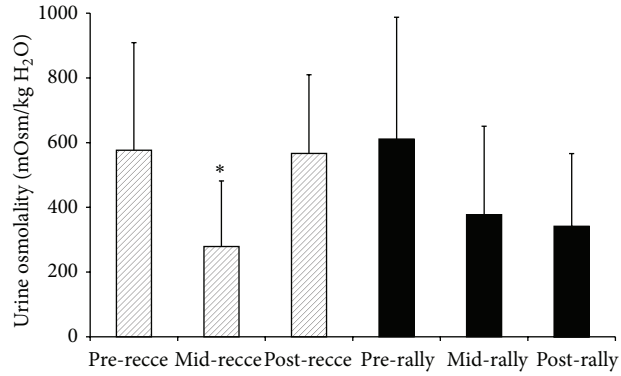

Figure 2: Mean (SD) values for urine osmolality of drivers and codrivers for the first morning sample (pre), during the day (mid) and at the end of one day of the reconnaissance (recce-hashed boxes) and then one day of the rally competition (solid black boxes). *Significantly lower than postreconnaissance ($P = 0.01$).

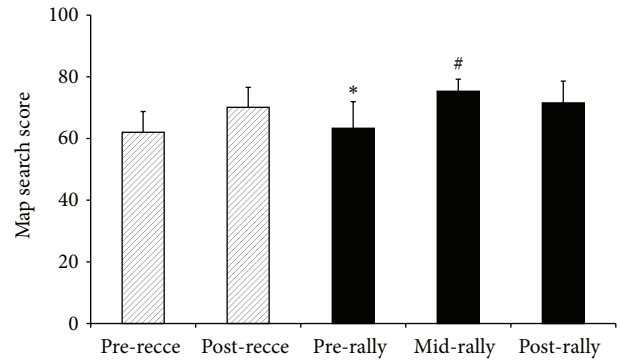

Figure 3: Mean (SD) values for scores of drivers and codrivers in the map search test of selective attention at the start of the day (pre), during the day (mid) and at the end of one day of the reconnaissance (recce-hashed boxes) and then one day of the rally competition (solid black boxes). *Significantly lower than postreconnaissance ($P = 0.007$), mid- ($P = 0.002$) and postrally ($P = 0.01$); #significantly higher than pre- ($P = 0.002$) and postreconnaissance ($P = 0.01$).

than rally ($P = 0.012$ and 0.017, $r = 0.63$ and 0.6, resp.) with little evidence of consistent differences between drivers and codrivers (Table 1).

3.3. Urine Osmolality and Fluid. U_{osm} data showed a significant main effect of time ($P = 0.01$, $r = 0.62$), although with large within participant (across time points) and between participant variations (Figure 2). The only significant increase was from mid- to postreconnaissance ($P = 0.01$), with other changes not significant ($P = 0.44$–1.0). Descriptive analysis shows 42% of driver samples at >700 mOsmol·kgH_2O^{-1} (including two morning samples from the same participant >1000 mOsmol·kgH_2O^{-1}) compared to just 9% of codriver samples. Contrast case analysis across all measurements for the driver subgroup showed that the individual performer with highest U_{osm} drank the least volume on reconnaissance (2 L) and second least on rally (5.5 L) compared to the rest of the driver subgroup, and less than the whole group average for each day. In contrast the driver with lowest U_{osm} drank the second highest amount on reconnaissance (3.15 L) and most on rally (7.5 L) for the driver subgroup. Fluid intake for the whole group ranged from 1.75–4.75 L during the reconnaissance (8 h—equivalent to 0.39 L·h^{-1}) and from 3–8.5 L during the rally (11 h—equivalent to 0.54 L·h^{-1}).

3.4. Selective Attention. Attention scores on the map search task (Figure 3) showed a significant main effect of time ($P < 0.001$, $r = 0.85$), with lowest attention scores prereconnaissance and prerally although no significant difference between them ($P > 0.99$). Prerally attention was significantly lower than midrally ($P = 0.002$), postrally ($P = 0.01$) and postreconnaissance performance ($P = 0.007$). The highest attention level was midrally, significantly higher than both reconnaissance day performances ($P = 0.002$ and 0.01), but not significantly different to postrally ($P = 0.401$). There was no significant main effect of time on the auditory working memory task ($P = 0.719$, $r = 0.26$). This measure showed a tendency towards a ceiling effect.

4. Discussion

This field based investigation found important information about the physiological and cognitive responses of skilled

performers operating under demanding conditions with high stakes. The key findings were that, as hypothesised, larger elevations in HR and T_{core} were recorded in response to competitive driving compared to noncompetitive, with values greater than would be predicted from simulated rally driving [2]. Drivers were also higher than codrivers. Consistent with the hypotheses there were significant changes in urine osmolality and attention. The driver subgroup produced more, high osmolality urine samples than codrivers, suggesting that they could be prone to dehydration. Specific case analysis found logical correspondence between urine osmolality and fluid intake. Attention scores were significantly lower at the start of the rally day than at other times during the day, but were not significantly different to the same time on the noncompetitive reconnaissance day.

Continuous measurement of HR and T_{core} provided accurate data on some of the physiological demands of rally at a major WRC event. Subgroup comparison revealed driver HR values were higher than codrivers during the competitive special stages as expected (Table 1, Figure 1), although across the whole day, mean values were similar. Importantly, driver HR values were significantly greater than during the reconnaissance, reflecting the additional psychophysiological demands of driving rally cars at high speeds, under competitive pressure and wearing protective clothing. For codrivers, there is additional incompetition cognitive load and arousal compared to reconnaissance, but only a small additional cardiovascular demand to maintain timing of pace-notes and body-position during high-speed turns, braking and jumps, evidenced by higher HR values in the special stages. The competitive stage driver HR values (Table 1), with peaks as high as 90% HR_{max}, are consistent with reported ranges in other driver studies [2–5]. The HR values, higher than previously published data for rally drivers during simulated driving in a heat chamber (mean 134 beats·min^{-1} at 50°C) [2], emphasize the need for research in real performance environments. Existing data on HR and energy expenditure suggest that although stages in rally are relatively short and intermittent, fatigue may occur during long, consecutive days which adds to the challenge of continually producing high level performance.

Rally T_{core} values in this study were significantly elevated compared to the noncompetitive reconnaissance conditions and slightly higher in drivers than codrivers (Table 1, Figure 1), with peak values during competitive stages similar to values reported in endurance competitions including yacht racing [14] and Ironman triathlon [13]. The competitors were successfully thermoregulating (e.g., T_{core} was recovering between stages with no accumulating increase) and avoiding hyperthermia, despite combined effects of radiant engine heat, physical exertion, and regulation safety clothing. Rally T_{core} values were slightly lower than previously reported for V8 drivers [4], probably due to the lower ambient (20.1°C versus 33.3°C) and in-car (33.9°C versus 48.6°C) temperatures. Ambient temperatures above 40°C are not unusual at some WRC events and it is logical to predict that T_{core} would be significantly higher for drivers in such events. With cumulative effects over multiple days drivers may be exposed to uncompensable heat storage and risk of hyperthermia with potential consequences for performance and safety [24, 25]. In addition the motivations involved in high level competition may override normal thresholds of comfort, as has been observed in motor sport [24]. Below extreme hyperthermia the potential consequences of high T_{core} on sports performance are currently unknown [9, 12–14, 25], but it would be expected that participants would at least experience increased perceived intensity and effort. Higher T_{sk} values during the reconnaissance (Table 1), without protective clothing or competitive driving forces, raises doubt on the accuracy of measuring T_{sk} from a single thermistor located in a chest-strap. Multiple site measurement is recommended [26], though was not possible during this rally.

Although it was not possible to definitively establish that performers were hypohydrated, given constraints of the study context and with a lack of an agreed gold standard for monitoring hydration status [19, 20, 27], some drivers exhibited U_{osm} values suggesting that they were competing in a potentially hypohydrated state (>700 mOsmol·kgH_2O^{-1}) [20]. In contrast, codrivers appeared to maintain euhydration better, presumably reflecting the reduced thermoregulatory challenge and possibly also better hydration strategies. One driver had U_{osm} first-morning values that were very high (>1000 mOsmol·kgH_2O^{-1}), these samples being most stable and reliable [20, 27] and interestingly this was also the driver who was consuming the least fluid during both days of monitoring. The driver consuming the most fluids maintained euhydration throughout, which underlines the importance of effective hydration strategies. Performers drank mostly water between stages and at service area where it was provided. The majority drank sports drinks containing electrolytes in-car, which could increase palatability, absorption, and prevent hyponatremia, under hot conditions that promote prolonged or excessive sweating, and limited food consumption. Fluid intakes reported here were similar to recommendations for marathon runners (0.4–1.0 L·h^{-1}) [20], but there was high variability of drinking response and strategies, which is not surprising given interindividual variation in preferences [20, 28].

Prerally attention scores were significantly lower than mid- or postrally scores. Whilst this finding is consistent with the group differences in observed hydration states, care must be taken in interpreting this association given the reduced accuracy of urine osmolality in samples other than first-morning [20, 27]. However this pattern is consistent with literature on the impact of dehydration on cognitive function which has been found to occur at levels of dehydration equivalent to about 2% body mass loss [29, 30]. Low scores were found at the start of both days, compared to later day measures, and this can be explained by circadian rhythm led variation in cognitive performances [31] together with the enhancing effects of exercise on cognition [32]. It is noteworthy that despite the inevitable raised motivations for performers on the day of major competition, prerally attention performance was no better than prereconnaissance levels. Whilst the attention measures used in the current study had face validity with regard to task, and were robust to use

in an extreme environment, the audio task may have been susceptible to a ceiling effect. Research on high performing individuals needs to address or avoid this limitation.

There are several practical applications that can be derived from the findings of this study that can be applied to rally performers and to individuals in comparable settings where they must combine motor skills and cognitive processing to complete tasks in demanding environments and with significant psychological pressure. The physiological demand, especially for drivers, shown in this study by elevated heart rate, emphasizes the need for good standards of physical fitness to prepare performers to meet demands effectively, reduce perceived effort, and enhance tolerance. Under relatively benign ambient temperature the effect of protective clothing and an increase in workload increased T_{core} significantly. In situations where ambient temperature is higher and there is greater restriction on fluid intake to aid cooling, the changes on T_{core} may be larger and reach levels where performance and safety might be compromised. Therefore specific actions to avoid unnecessary heat exposure and accumulation, and active engagement in cooling strategies, may become increasingly important. Low attention at the start of the rally day despite motivation may result in detrimental cognitive processing. Morning team briefings are common in rally sport and other situations that require shared understanding (police, military, and medicine) when attention of performers is compromised by time of day (or other factors such as fatigue). Therefore, attempts should be made to offset this decrement (e.g., exercise) or to facilitate processing (mnemonics, cues, and reduced information load).

This study is the first to our knowledge to publish field based group data on rally driver/codriver responses, providing a contrast between reconnaissance and rally days. The wireless monitoring systems and access to performers through the duration of the rally provide a rare opportunity to examine the responses in real life context. The physiological responses and cognitive performance of performers under extreme conditions is of interest for both applied and research perspectives. Practical application can assist performers to maintain standards and safety despite additional challenges of the environment. For researchers, understanding what happens in real performance contexts is critical to ensure that concepts and ideas formulated in controlled research situations with volunteer, mostly student participants, are truly representative and have adequate ecological validity.

5. Conclusions

This study has demonstrated the extent of heart rate elevation within competitive rally driving providing a clear indication of both the peak level (in special stages) and the prolonged lower level elevation across a performance day. Temperature also rose but the elevation was easily tolerable in the ambient temperature during this study. The performers reported a very wide range of fluid intake and urine osmolality despite operating in the same team and receiving the same education on this issue. This reflects the difficulty of ensuring that scientific knowledge is applied effectively to ensure optimal performance state. Cognitive performance followed a pattern consistent with circadian rhythm and was not increased at the start of the event, even though this was of very high importance. Understanding these responses and providing information and strategies to minimise any detrimental impact would be beneficial to those involved in a range of high performance situations.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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Research Article

Changes in Biochemical, Strength, Flexibility, and Aerobic Capacity Parameters after a 1700 km Ultraendurance Cycling Race

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The purpose of the present research was to study the organic response after ultraendurance cycling race. Selected biochemical, leg strength, flexibility, and aerobic capacity parameters were analyzed in 6 subjects 5 days before and 5 days after completing a 1700 km ultraendurance cycling race. After the race, participants presented a significant decrease in Hb (167.8 ± 9.5 versus 141.6 ± 15.7 mg/dL), strength (29.4 ± 2.7 versus 25.5 ± 3.7 cm in a countermovement jump), and oxygen uptake and heart rate at ventilatory threshold (1957.0 ± 458.4 versus 1755.2 ± 281.5 mL/kg/min and 140.0 ± 9.7 versus 130.8 ± 8.3 bpm, resp.). Testosterone presented a decrease tendency (4.2 ± 2.5 versus 3.9 ± 2.6 ng/L) in opposition to the increase tendency of cortisol and ammonium parameters. Transferrin and iron levels presented high values related to an overstimulation of the liver, a normal renal function, a tendency to decrease flexibility, and an increase in aerobic capacity, finding a tendency to increase the absolute maximal oxygen uptake (37.2 ±2.4 versus 38.7 ± 1.8 mL/min) in contrast to previous studies conducted with subjects with similar age. These results can be used to program training interventions, recovery times between probes, and nutritional and/or ergonomic strategies in ultraendurance events.

1. Introduction

Previous studies have examined the organic response in endurance running, swimming, triathlon, kayaking and cycling races [1–4]. In recent years, more athletes have become involved in ultraendurance races, such as the ironman triathlon, the 100 km race, and longer races performed in various days [1, 3–5]. Actually, strenuous physical activities are becoming increasingly popular around the world. It is known how ultraendurance events produce an increase in muscle and protein breakdown [4, 5], an increase in the catabolic state of the organism [5], an increase in the erythropoiesis to compensate the exercise-induced haemolysis [6], and an increase in triglycerides consumption [5], have no effect on renal function [1], and are performed with a blood lactate concentration lower than the anaerobic threshold [7].

Moreover in the line of research related to endurance probes, several studies have shown that endurance training produces interference with strength production [8, 9]. Then ultraendurance events could negatively affect strength production capabilities of athlete's muscles. It is also known that an inverse relation between the flexibility and endurance capabilities exists, producing endurance training a decrease on athlete's flexibility manifestations [10]. Related to cardiovascular and endurance performance factors, numerous studies showed that high intensity and low volume training produce higher adaptations in these parameters than low intensity and high volume efforts [11, 12]. Efforts similar than in ultraendurance probes performed in various days.

Therefore, the effect of endurance and ultraendurance events in the athlete's physiological response has been widely studied in one or few days' probes, but the effect of longer

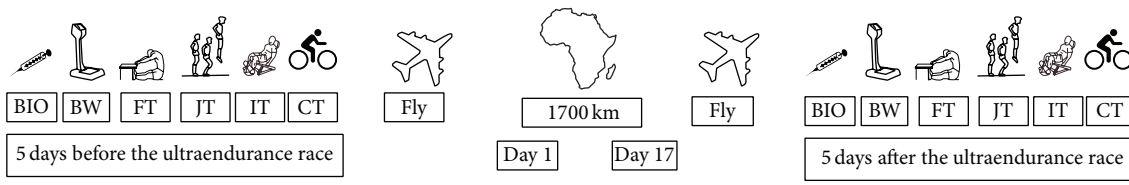

Figure 1: Experimental procedure. BIO: biochemical analysis; BW: body weight analysis; FT: flexibility test; JT: jumps test; IT: isokinetic strength test; CT: cycling test.

ultraendurance events on the athlete's organic response is not known, especially in extreme environmental conditions such as high temperature and humidity. Additionally, to the best of our knowledge, the effect on muscular capacities as strength and flexibility and athlete's cardiovascular function have not been researched in ultraendurance events. For this reason the aim of the present research was to study modifications in selected biochemical, strength, flexibility, and aerobic capacity parameters after completing a 1700 km ultraendurance cycling race. It was hypothesized that an ultraendurance event would alter biochemical markers and would not affect flexibility, strength, and aerobic capacity parameters, since previous studies reported significant changes in biochemical markers after ultraendurance events and secondly only training programs produced changes in flexibility, strength, and aerobic capacity parameters, not only an ultraendurance race.

2. Materials and Methods

2.1. Experimental Approach to the Problem. A descriptive study was performed. Pre-post changes were analyzed in selected biochemical, strength, flexibility, and aerobic capacity parameters five days before and five days after completing a 1700 km ultraendurance cycling event in order to confirm the study hypothesis. Figure 1 represents the experimental procedure of the present study. The dependent study variables were body mass; sit and reach value; biochemical: blood urea, creatinine, sodium, potassium chloride, lactate dehydrogenase (LDH), CK, iron, ammonium, testosterone, cortisol, lactate, transferrin, hemoglobin (Hb), triglycerides concentration, and creatinine clearance; strength: peak torque, peak torque/weight, time to peak torque, maximal work, total work, power average at $30^{\circ}\cdot s^{-1}$ and $60^{\circ}\cdot s^{-1}$ angular velocity in leg extension and flexion movements, squat jump (SJ), countermovement jump (CMJ), and abalakov jump (ABK) performance; aerobic performance: probe time, watts at $VO_{2\,max}$, $VO_{2\,max}$ absolute, $VO_{2\,max}$ relative, maximum heart rate, watts at ventilatory threshold, VO_2 relative at ventilatory threshold, VO_2 absolute at ventilatory threshold, and heart rate at ventilatory threshold in a maximal incremental cycling test. The independent variable was the 1700 km ultraendurance event.

2.2. Participants. The six participants in the ultraendurance event were analyzed. The athletes were part of an ONG that promoted this sport challenge in the African continent; they were not professional athletes and for this reason the participants were not experienced athletes and parameters like age were higher than in previous studies. The characteristics of the athletes were (mean ± SD) age 56.2 ± 6.9 years; height 170 ± 0.1 cm; body mass 73.3 ± 10.2 kg, body mass index: 25.1 ± 1.5 $kg\cdot m^2$, 3.1 ± 1.2 years training cyclist, average of 2.5 ± 1.5 days/week, and 8.2 ± 1.3 hours of training per week. Prior to participation, the experimental procedures were explained to all the participants, who gave their voluntary written informed consent. All were given a medical examination prior to participation to assess their health state and detect any medical condition which might result in injury during the study. The study was conducted in accordance with the Declaration of Helsinki.

2.3. The Race. The ultraendurance race consisted of crossing the African continent from the east coast to the west coast by bike. The distance of the race was 1700 km and was performed in 17 days, averaging 100 km per day. The temperature oscillated between 8 and 39°C and the humidity oscillated between 64 and 85%.

2.4. Assessment Protocol. In pre and post samples athletes realized the same assessment protocol to measure the study variables. The protocol was as follows.

Blood Draw in Hospital. All participants performed blood draw fasting between 9:00 and 10:00 am. Samples for biochemical assay were collected into sterile vacutainer tubes.

Measurement of Body Mass. Body mass was analyzed by SECA 222 (Apling, Barcelona, Spain).

Warm-Up. A standardized warm-up consists in 10 min of cycling (140 bpm, 90 rpm) in a cicloergometer (Monark 630 Monark Exercise, AB, Sweden) and 2 series of 10 repetitions of submaximal CMJ.

Flexibility Evaluation. Sit and reach test [13] was performed 3 times; we analyzed the maximal value reached.

Leg Strength Evaluation. Vertical jump test: athletes performed 3 SJ, 3 CMJ, and 3 ABK in an Ergojump System (Bosco System, Ergotest Technology). The rest period between jumps was always 30 seconds. The best jump in terms of height was taken for further analysis. We chose to use vertical jumps as they provide further insight into the force capabilities of leg extensor muscles [14]. Moir et al. [14] suggest that vertical

jump assessment in athletes and recreationally active men can be achievedwith a high degree of reliability.

Isokinetic Leg Strength Evaluation. The knee extensor and flexor muscle peak torque (absolute) of each leg were concentrically measured at $30^{\circ}\cdot s^{-1}$ and $60^{\circ}\cdot s^{-1}$ (5 repetitions each) using a Biodex System 3 isokinetic dynamometer (Biodex Corporation, Shirley, NY) according to standard procedures [15].

The athlete was strapped into the chair, using the lateral femoral condyle as an anatomical reference for the axis of rotation. The length of the lever arm was individually determined, and the resistance pad was placed proximal to the medial malleolus. Gravity correction was applied after direct measurements of the mass of the lower limb lever arm system at 30° knee extension. Range of motion varied from 90° knee flexion to 10° extension (considering 0° as full extension). The values of the peak torques over 5 consecutive contractions for each muscle group tested were used for the data analysis. One min of rest was allowed between assessments at different angular velocities using the protocol described by Bradic et al. [16]. All participants indicated that their right leg was dominant. Participants were instructed to hold their arms across the chest to isolate extension movements in knee joint [17].

Aerobic Capacity Evaluation. Incremental maximum cycling test was performed in a cicloergometer (Monark 630 Monark Exercise, AB, Sweden) using a CPX gas analyzer (Medical Graphics Corporation, St. Paul, MN). Athletes performed the following incremental test protocol: 5 min 50 w warm-up, increments of 50 w per minute until exhaustion. The incremental test was performed with a cadence between 90 and 105 rpm. To evaluate heart rate a polar S810 (Polar Electro Ibérica. Barcelona, Spain) was used. The test finished when the participant reached at least three of the following five criteria [18]: (a) a plateau in the oxygen consumption (VO_2) versus exercise intensity relationship, which has been defined as an increase in VO_2 of less than 2 mL/kg/min with an increase in exercise intensity, (b) elevated respiratory exchange ratio ($r \geq 1.0$), (c) elevated HR (≥90% of [220-age]), (d) a rating of perceived exhaustion (RPE) of 19-20 on the Borg scale, and (e) high levels of blood lactate concentration (≥8 mmol/L).

2.5. Study Variables. The following parameters were evaluated in pre and post samples.

Biochemical parameters: blood (25 mL) was withdrawn from an antecubital vein, using a sterile technique to analyze parameter of urea (mg/dL), creatinine (mg/dL), sodium (mmol/L), potassium (mmol/L), chloride (mmol/L), LDH (UL/L), CK (UI/L), iron (μg/dL), ammonium (μmol/L), testosterone (ng/L), cortisol (μg/dL), and transferrin (mg/dL). The analyses were performed on an Olympus AU 800 Autoanalyzer. Results were corrected for changes in plasma volume as previous research [19]. 32 μL capillary bloods from fingertips were collected to analyze blood lactate concentration (mmol/L), Hb (mg/dL), and triglycerides (mg/dL). Blood lactate was measured using an Accusport Lactate Analyzer (Total Performance Inc., Mansfield, Ohio). This portable lactate analyzer has been found to be valid and reliable [20]. Hb and triglycerides were analyzed by a Reflotron Plus system (Roche Diagnostics S.L., Sant Cugat del Vallès, Barcelona, Spain). Creatinine clearance (mL/min) was estimated by Cockroft and Gault formula that has shown a good correlation with glomerular filtering [21].

(i) Body mass (Kg).

(ii) Vertical jump parameters: jump height in SJ (cm), CMJ (cm), and ABK (cm).

(iii) Isokinetic parameters: peak torque (n·m), peak torque/weight (%), time to peak torque (mseg), maximal work (J), total work (J), and power average (w) in knee extensor and flexor muscle of the legs at velocities of 30°/seg and 60°/seg.

(iv) Flexibility parameter: sit and reach performance (cm).

(v) Aerobic capacity parameters: probe time (seg), watts (w) at maximal oxygen uptake ($VO_{2\,max}$), $VO_{2\,max}$ absolute (mL/min), $VO_{2\,max}$ relative (mL/kg/min), maximum heart rate (bpm), watts at ventilatory threshold (w), VO_2 absolute at ventilatory threshold (mL/min), VO_2 relative at ventilatory threshold (mL/kg/min), and heart rate at ventilatory threshold (bpm).

2.6. Statistical Analysis. The SPSS statistical package (version 17.0; SPSS, Inc., Chicago, Ill.) was used to analyze the data. The Shapiro-Wilk normality test was used to test the normality and homogeneity of each variable. All data presented a nonparametric distribution; therefore a Wilcoxon t-test was performed to compare prerace and postrace data. In order to improve the applicability of the research to exercise professionals the effect size (ES) was tested by Cohen's d test and interpreted according to Rhea classification for recreationally trained athletes [22]. This classification was proposed for determining the magnitude of training interventions that commonly produced a small range of change due to the exquisiteness of training programs required to elicit adaptations [23], especially in small sample sizes or large variance data [22] as in the present study. The level of significance for all the comparisons was $P < 0.05$.

3. Results

Modifications of biochemical parameters analyzed are shown in Table 1. The increase of transferrin and triglycerides presented a large ES as well as the decrease of Hb. However, the increase of iron and ammonium and the decrease of ammonium and creatinine clearance presented a moderate ES. Only the decrease in hemoglobin was significant (P: 0.043).

Body mass before the race was 73.3 ± 10.2 kg and after was 71.6 ± 8.5 kg (−1.78%; Z: −1.753; P: 0.080; ES: −0.016). Flexibility values did not significantly decrease after the race (Pre: 2.8 ± 6.3 cm versus Post: 1.0 ± 2.9 cm; Z: −0.674;

Table 1: Pre and post mean and SD values of the studied biochemical variables.

Parameter	Unit	Samples		% change	Z	P	Cohen' D
		Prerace	Postrace				
Urea	mg/dL	32.3 ± 7.3	29.5 ± 2.0	−8.6	−.674	.500	−0.38
Creatinine	mg/dL	1.1 ± 0.2	1.1 ± 0.2	0	−.674	.500	0.00
Sodium	mmol/L	144.0 ± 0.8	143.3 ± 2.1	−0.5	−.816	.414	−0.87
Potassium	mmol/L	4.5 ± 0.2	4.5 ± 0.1	−1.1	−.447	.655	0.00
Chloride	mmol/L	106.3 ± 1.0	106.5 ± 1.3	0.2	−.272	.785	0.20
LDH	UI/L	424.8 ± 36.3	411.8 ± 47.3	−3.1	−.730	.465	−0.36
CK	UI/L	81.0 ± 23.3	82.3 ± 32.8	1.5	−.674	.500	0.06
Iron	μg/dL	132.0 ± 27.4	172.8 ± 60.6	30.9	−1.461	.144	1.49
Ammonium	μmol/L	62.4 ± 17.1	78.0 ± 13.3	25.0	−1.095	.273	0.91
Testosterone	ng/L	4.2 ± 2.5	3.9 ± 2.6	−6.5	.000	1.000	−0.12
Cortisol	μg/dL	14.3 ± 2.3	14.6 ± 0.6	2.5	−.674	.500	0.13
Transferrin	mg/dL	188.8 ± 7.3	204.5 ± 17.1	8.3	−.674	.500	2.15
Hemoglobin	mg/dL	167.8 ± 9.5	141.6 ± 15.7	−15.6*	−2.023	.043	−2.76
Triglycerides	mg/dL	86.2 ± 9.1	127.0 ± 32.2	70.5	−1.753	.080	4.51
Creatinine clearance	mL/min	143.3 ± 17.3	128.0 ± 28.8	−10.7	−.674	.500	−0.87
Lactate	mmol/L	2.3 ± 0.6	2.2 ± 0.3	−3.5	.000	1.000	−0.17

* $P < 0.05$ versus prerace sample. Ck: creatinine kinase; LDH: lactate dehydrogenase.

Table 2: Mean and SD values of the jump test variables.

Parameter	Unit	Samples		% change	Z	P	Cohen' D
		Prerace	Postrace				
SJ	cm	23.3 ± 4.5	22.7 ± 3.4	−2.5	−.405	.686	−0.13
CMJ	cm	29.4 ± 2.7	25.5 ± 3.7	−13.5*	−2.023	.043	−1.44
ABK	cm	34.6 ± 6.5	29.6 ± 5.0	−14.4	−1.753	.080	−0.77

* $P < 0.05$ versus prerace sample. SJ: squat jump; CMJ: countermovement jump; ABK: abalakov jump.

P: 0.500; ES: −0.28). Regarding jump tests only CMJ values significantly decrease (−13.5%) with a moderate ES (Table 2).

None of the values of isokinetic strength conducted at 30°/seg presented significant differences. Isokinetic strength values are shown in Tables 3 and 4.

By contrast, total work and power average in flexion at 60°/seg significantly decreased (−23.2%; P: 0.043 and −45.0%; P: 0.043, resp.). Time to peak torque in flexion presented a large ES and peak torque/weight, power average and total work, a moderate ES.

The variables of probe time and watts at $VO_{2\,max}$ in the incremental cycling test significantly increased (8.5%; P: 0.043 and 11.4%; P: 0.025, resp.), and VO_2 and HR at VT decreased significantly (−10.3; P: 0.043 and 6.6%; P: 0.043, resp.) (Table 5). Additionally, probe time and watts in $VO_{2\,max}$ presented a large ES and HR at ventilatory threshold, a moderate ES.

4. Discussion

The purpose of the present study was to analyze modifications in selected biochemical, strength, flexibility, and aerobic capacity parameters after a 1700 km ultraendurance cycling race. A significant decrease in Hb, strength, VO_2, and HR at ventilatory threshold was measured. The hypothesis of the study was partially supported because when aerobic capacity improved, strength parameters decreased and flexibility and the majority of the biochemical parameters were not modified.

4.1. Biochemical. The electrolytes concentration (sodium, potassium, and chloride) measured postrace was similar to the basal sample, despite the event being performed in high condition of humidity and temperature. These findings could be related to the fact that electrolyte replacement strategies were correct and could replace the losses suffered during the race, result similar to previous studies in ultramarathon runners [24]. Results obtained in the sodium concentration rule out any possible hyponatremia, and the unmodified serum potassium values obtained after the race were in consonance with previous researches conducted in marathon [25], 56 km run [26], and triathlon [27]. Regarding renal function indicators such as urea, creatinine, and creatinine clearance, they were not significantly modified. The lack of change of creatinine values coincides with the results obtained after running a marathon, a 100 km running race or a 110 km cycling race [28], but is opposite to an increase measured after a 60 km mountain bike race [1], a 460 km cycle race [5], a marathon [29], a 100 km run [30], and a 24 hour event [31]. This increase in creatinine has been related

Table 3: Pre and post isokinetic legs strength at 30°/seg velocity data.

	Parameter	Unit	Prerace	Postrace	% change	Z	P	Cohen' D
Extension	Peak torque	n.m	170.0 ± 27.0	162.4 ± 18.8	−4.5	−.674	.500	−0.28
	Peak torque/weight	%	235.7 ± 37.5	224.4 ± 14.1	−4.8	−.674	.500	−0.30
	Time to peak torque	mseg	674.0 ± 25.1	636.0 ± 61.1	−5.6	−1.355	.176	−1.51
	Maximal work	J	124.6 ± 26.8	124.0 ± 17.6	−0.4	−.135	.893	−0.02
	Total work	J	550.5 ± 123.2	529.3 ± 70.4	−3.9	−.135	.893	−0.17
	Power average	w	43.5 ± 7.2	46.2 ± 5.8	6.3	−.405	.686	0.38
Flexion	Peak torque	n.m	89.6 ± 25.0	87.8 ± 16.5	−2.0	−.405	.686	−0.07
	Peak torque/weight	%	123.4 ± 34.1	120.6 ± 16.0	−2.3*	−.135	.893	−0.08
	Time to peak torque	mseg	886.0 ± 252.8	1090.0 ± 339.7	23.0*	−.674	.500	0.81
	Maximal work	J	81.5 ± 28.3	80.7 ± 20.6	−1.0*	−.135	.893	−0.03
	Total work	J	354.3 ± 129.4	331.9 ± 130.8	−6.3*	−.135	.893	−0.17
	Power average	w	27.1 ± 10.5	22.7 ± 11.5	−17.9*	−.405	.686	−0.42

* $P < 0.05$ versus prerace sample.

Table 4: Pre and post isokinetic legs strength at 60°/seg velocity data.

	Parameter	Unit	Prerace	Postrace	% change	Z	P	Cohen' D
Extension	Peak torque	n.m	153.2 ± 38.3	141.4 ± 34.4	−7.7	−1.214	.225	−0.31
	Peak torque/weight	%	209.5 ± 35.2	194.8 ± 46.2	−7.0	−1.214	.225	−0.42
	Time to peak torque	mseg	434.0 ± 84.4	444.0 ± 27.0	2.3	−.135	.892	0.12
	Maximal work	J	118.8 ± 33.5	115.9 ± 26.3	−2.5	−.135	.893	−0.09
	Total work	J	514.7 ± 165.6	451.0 ± 138.4	−12.4	−.405	.686	−0.38
	Power average	w	73.4 ± 22.5	77.4 ± 23.2	5.4	−.405	.686	0.18
Flexion	Peak torque	n.m	85.2 ± 16.5	96.9 ± 32.2	13.8	−.405	.686	0.71
	Peak torque/weight	%	117.1 ± 14.1	133.0 ± 41.0	13.6	−.405	.686	1.13
	Time to peak torque	mseg	476.0 ± 71.3	938.0 ± 480.3	97.0	−1.753	.080	6.48
	Maximal work	J	78.8 ± 16.8	87.9 ± 34.2	11.5	−.674	.500	0.54
	Total work	J	318.8 ± 88.6	244.9 ± 129.3	−23.2*	−2.023	.043	−0.83
	Power average	w	44.8 ± 13.6	24.6 ± 14.3	−45.0*	−2.023	.043	−1.49

* $P < 0.05$ versus prerace sample.

to the reduced renal blood flow, reduced glomerular filtration rate, and hypovolemia produced in these shorter events [32] and could be related to the higher intensity of these races compared to the 1700 km cycling race. Therefore, the renal function of athletes in the current study was not affected despite the 17 days of race duration.

The variables related to muscle breakdown (CK, LDH, and urea) presented after 5 days values close to the ones obtained in the prerace sample. These data showed that in five days athletes muscles have time to recover despite the high levels of muscle breakdown that usually are measured in these ultraendurance events [4]. It could be due to the fact that the race was performed cycling, which is an activity with no impact and produces a minor damage in the muscle structure. Another parameter traditionally used to control the organic anabolic-catabolic balance, the blood testosterone, presented decreased tendency after the ultraendurance race. This is because the organism still recovering to the catabolic situation that supposed the ultraendurance race, fact also corroborated by the increased tendency in cortisol values [33]. Then, after 5 days the cortisol and testosterone concentrations did not return completely to the basal values; this catabolic status also was reflected in the increased tendency of ammonium values; parameter increased after exercise and related to training workload and effort performed by athletes [34]. In addition, testosterone values measured both before and after the ultraendurance event were lower than in other studies conducted in ultraendurance events, a fact that could be explained because these athletes were younger than in the present research and their testosterone production was higher because of their lower age [35].

The increase in transferrin and iron (large and moderate ES, resp.) after the ultraendurance race could be interpreted as a symptom of haemolytic anaemia that is related to the decrease in Hb [6]. Also the increase in these values could be explained because of the liver overstimulation [27, 28, 30] that increases the production of hepatic enzymes that cause an increase in iron levels. In addition, related to substrates metabolism, triglycerides presented an increase with a large ES after the ultraendurance race that could be due to the discharge of catecholamines induced by the exercise, which stimulated lipolysis in the adipose tissue and led to a release of lipid substrates including triglycerides [4]. Finally, the drop in the Hb concentration was in contrast to the results obtained

Table 5: Mean and SD values of the aerobic performance variables analyzed.

Parameter	Unit	Samples		% change	Z	P	Cohen' D
		Prerace	Postrace				
Probe time	seg	705.4 ± 23.6	765.2 ± 23.2	8.5*	−2.023	.043	2.53
Watts at VO_2 max	w	220.0 ± 10.0	245.0 ± 10.0	11.4*	−2.236	.025	2.50
VO_2 max absolute	mL/min	37.2 ± 2.4	38.7 ± 1.8	3.9	−1.473	.141	0.63
VO_2 max relative	mL/kg/min	2706.4 ± 296.9	2749.0 ± 209.1	1.6	−.674	.500	0.14
Maximum heart rate	bpm	173.2 ± 5.7	176.6 ± 7.1	2.0	−1.841	.066	0.60
Watts at VT	w	140.0 ± 20.0	135.0 ± 12.2	−3.6	−1.00	.317	−0.25
VO_2 absolute at VT	mL/min	26.6 ± 4.3	24.5 ± 1.7	−8.0	−1.214	.225	−0.49
VO_2 relative at VT	mL/kg/min	1957.0 ± 458.4	1755.2 ± 281.5	−10.3*	−2.023	.043	−0.44
Heart rate at VT	Bpm	140.0 ± 9.7	130.8 ± 8.3	−6.6*	−2.023	.043	−0.95

* $P < 0.05$ versus prerace sample. VO_2: oxygen uptake; VT: ventilatory threshold.

after an alpine marathon [1] and also after a 20 hour and 51 min cycling event [5], showing that, in the ultraendurance race analyzed, erythropoiesis was insufficient to compensate the breakdown of red blood cells caused by the extreme effort [6] and possibly related to the extreme ambient condition (temperature and humidity) of the race [36].

4.2. Strength. The decrease of strength parameters after the ultraendurance race might be due to athletes losing muscular mass because of the stress of the continuous effort [36], a fact that could be related to the body mass loss after the race. Another cause of the decrease in strength parameters might be due to the interferences between endurance exercise and strength manifestation [8]. In this line, the decrease in isokinetic leg strength values was similar to the study of Glowacki et al. [9] after performing a low intensity aerobic training. Also, the study of Abernethy [37] found a decrease in isokinetic leg strength and tension after acute endurance activity in athletes. This fact could be explained because oxidative-endurance training causes muscle to respond in an opposite fashion by ultimately degrading and sloughing myofibrillar protein to optimize oxygen uptake kinetics as shown by Kraemer et al. [8] in a group of subjects to develop endurance exercise.

4.3. Flexibility. Participants presented a decrease tendency in flexibility values after the ultraendurance race. This might be due to the constant repetition of a cyclic movement for long time periods. This repetitive movement might cause degeneration in muscle cells that prevented them from showing the initial length. The decrease in flexibility values was in consonance with the results obtained in endurance runner, who presented a decrease in flexibility values because of musculotendinous structures reducing the aerobic demand of submaximal running by facilitating a greater elastic energy return during the shortening phase of the stretch shortening cycle [10].

4.4. Aerobic Capacity. A general improvement in aerobic capacity was measured. Athletes decreased HR and VO_2 at ventilatory threshold, which reflects an improvement on aerobic energy system since participants consumed less oxygen at ventilatory threshold; therefore the energy demand at this intensity was lower [38]. An increase in the HR efficiency was also observed, because athletes performed the same intensity, intensity corresponding to the ventilatory threshold, with a lower HR [39]. These improvements at the intensity of the ventilatory threshold were similar to that obtained after training programs with periods between 12 weeks [40] and 20 weeks [41].

It has been documented that a progressive decline in $VO_{2\,max}$ with age seems to be due to both central and peripheral adaptations, primarily reductions in maximal heart rate and lean body mass [42]. However, athletes in the current research have managed to present an increase tendency in absolute $VO_{2\,max}$ values; therefore even in this age, this parameter can be increased in opposition to previous literature [36].

It is also noteworthy that athletes achieved increases in aerobic capacity despite the low intensity effort performed during the ultraendurance race. Previous literature postulated that high intensity training may reduce the decrease in $VO_{2\,max}$ related to age [43], but the results obtained in the present research demonstrated that long extended aerobic exercise also could improve $VO_{2\,max}$ of athletes with ages in which previous research only measured decreases. The increase in $VO_{2\,max}$ might be explained because of the increase in the maximum heart rate [44] and also could affect the initial performance level of athletes that was not high since they only trained 2.5 ± 1.5 days per week.

4.5. Limitation of the Study. The principal limitation of the study was the low number of participants analyzed, which limits the generalization of the results obtained in the present research. It was because only 6 participants completed the 1700 ultraendurance race, which is a low number of subjects analyzed, but it represents the 100% of finisher athletes of the race. Also the test conducted after the ultraendurance race could be realized immediately after the race to analyze the acute organic response and also repeated 7 and/or 10 days after the race to analyze the evolution of the different variables analyzed. The testing procedure was conducted in

a laboratory in Spain and we had not the option to conduct the tests in the African continent. Then, participants had to fly from Africa to Spain and for this reason we did not conduct the tests immediately after the race, and for lack of funds we cannot repeat the tests in posterior days.

4.6. Practical Application. The results obtained in the present study have demonstrated the effect of an ultraendurance event in different organic parameters. These data can be used to program different training interventions, such as the inclusion of supplementary strength sessions to prevent a decrease in muscle strength when high volume and low intensity aerobic effort are performed; additionally, the recovery times between ultraendurance probes and nutritional and/or ergonomic strategies can be implemented to prevent, for example, participant's weight loss, which could lead to their overtraining states.

It has also been shown that low intensity high volume aerobic efforts produce improvements in aerobic performance markers, factor to consider since currently research in this area has shown the effectiveness of high intensity and low volume training. Possibly the concentration of high volumes may also produce improvements in aerobic fitness as well as high intensity and low volume efforts.

5. Conclusion

Participants analyzed in the present study presented after five days of completing a 1700 ultraendurance race a significant decrease in Hb, strength, VO_2, and HR at ventilatory threshold. Testosterone presented a decrease tendency in opposition to the increase tendency of cortisol and ammonium parameters. Transferrin and iron level presented high values related to overstimulation of the liver, a normal renal function, a tendency to decrease flexibility, and an increase in aerobic capacity, finding a tendency to increase the absolute $VO_{2\,max}$ in contrast to previous studies conducted with subjects with similar age.

Conflict of Interests

The author declares that there is no conflict of interests regarding the publication of this paper.

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Research Article

Prolonged Sleep Deprivation and Continuous Exercise: Effects on Melatonin, Tympanic Temperature, and Cognitive Function

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The purpose of this study was to examine tympanic temperature, melatonin, and cognitive function during a 36-hour endurance event. Nine male and three female participants took part in a 36-hour sustained endurance event without sleep ($N = 12$, mean age $= 31.8 \pm 5.0$ yrs). Participants were stopped for data collection at checkpoints throughout the 36-hour event. Tympanic temperature was assessed, a psychomotor vigilance test (PVT) was administered, and saliva samples were collected. Salivary melatonin was determined via immunoassay. During the 36 hours of competition, melatonin levels were negatively correlated with the day of the race ($rs = -0.277$, $P = 0.039$) and positively associated with nighttime ($rs = 0.316$, $P = 0.021$). Significant main effects of tympanic temperature ($P < 0.001$), day of the competition ($P = 0.018$), and a tympanic temperature $*$ day of competition interaction ($P < 0.001$) were used to predict minor lapses in attention. No associations between melatonin levels and cognitive function were observed ($P > 0.05$). During the event tympanic temperature declined and was associated with an increase in lapses in attention. With sustained endurance events becoming more popular future research is warranted to evaluate the physiological impact of participation.

1. Introduction

Ultra-endurance events, similar to extended military operations, are now making their way into the general population in the form of competitive sporting events. These events generally consist of prolonged exercise bouts, sleep deprivation, and continuously changing environmental conditions. Given the rapidly growing popularity of these ultra-endurance events, it is important to examine the physiological changes that occur under these conditions in order to provide effective training recommendations for both athletes and military personnel.

Due to the severe physical and mental demands of military operations, many studies examining sleep deprivation and prolonged exercise have come from military settings. While these studies do provide useful information to the general population, military personnel often carry excess weight (e.g., backpacks), unlike ultra-endurance athletes. Studies have shown that extended exercise while carrying excess weight could lead to significantly impaired cognitive processing [1, 2]. In addition, while sleep deprivation alone does not seem to significantly impair physical work capability, exercise compounded with sleep deprivation appears to increase vulnerability for negative mood disturbances and impaired reaction times [3–5]. Thus, when sleep deprivation is combined with excess physical exertion, cognitive function is negatively affected.

In addition to sleep deprivation, military personnel and ultra-endurance athletes can experience drastically different environments depending upon the location and time of year they choose to compete. While acute cold exposure had no major effects on the metabolism of sleep-deprived individuals, repeated cold exposure was shown to negatively affect cognitive function [6–9]. Not only does cold exposure impact physical and mental capabilities during sustained, sleep-deprived exercise, sleep deprivation itself disrupts coordination of fluctuations in individual thermoregulation, a process crucial to normal cognitive function [10–14]. Should environmental temperature significantly drop during exercise, individuals could also experience a reduction in core

body temperature, potentially leading to a change in cognitive function.

Previous studies have found that restricted sleep, in the absence of physical exertion, compromises many components of cognitive processing, alertness, and performance [15–22]. It is associated with a decline in core temperature and a concomitant decline in cognitive function [10–12] and a rise in the hormone melatonin, which governs the body's sleep-wake cycles and protects against sleep deprivation-induced behavioral and biochemical alterations [23]. Elevated levels of melatonin can also lead to the aforementioned impairment in cognitive function, as well as psychomotor vigilance [24, 25]. Furthermore, melatonin is known to increase long duration exercise, in which the body continuously works through the normal period of nighttime sleep, leading to altered circadian rhythms [26]. Taken together, these findings suggest that cognitive function, core temperature, and melatonin production all become altered during sleep deprivation in combination with prolonged exercise. Thus, the purpose of the present investigation was to examine the impact of a 36-hour ultra-endurance event on cognitive function, melatonin, and tympanic temperature among a group of competitors. We hypothesized that the combined effects of sleep deprivation and prolonged exercise would cause a drop in core temperature and cognitive function, while melatonin levels would rise throughout the event.

2. Methods

2.1. Subjects. The subjects consisted of 9 male and 3 female participants who took part in a 36-hour sustained endurance without sleep ($N = 12$, mean age = 31.8 ± 5.0 yrs). All subjects gave written informed consent prior to participating in the data collection. The participants completed a Leisure Physical Activity Survey [1] prior to the event (Total Aerobic Exercise Score = 7.1 ± 1.8, Mean Total Weightlifting Score = 6.7 ± 1.9) with results suggesting high levels of physical activity.

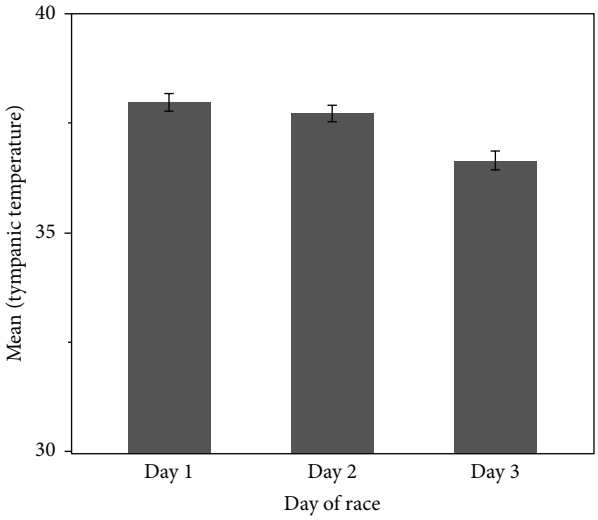
2.2. Ultra-Endurance Event. The event was held in rural Illinois (Cuba, IL, USA) during the late summer. The event was 36 hours in duration and consisted of a variety of different tasks. Tasks included extended bucket carries (buckets approximately 22.5 kg each for men and 15 kg each for women), extended marches with heavy packs, weightlifting, swimming, body weight exercises, and running in a predetermined order that remained unknown to the participants. Each task was completed as quickly as possible by each participant and, following the completion of each task, participants returned to the starting tent to receive the next task assignment from race officials. All participants completed the same tasks in the same order, which minimized the variation in environmental conditions and required physical exertion. Each task required several hours to complete and some participants completed tasks several minutes faster than others. The participants were allowed rest breaks at the conclusion of each task; however, they were disqualified if they slept. Specific rest times varied between individuals, tasks, and total elapsed time. Participants had shorter rest times (approximately 10 minutes) early in the event and following lower-intensity tasks, whereas participants had longer rest times later in the event and following higher-intensity tasks (approximately 30 minutes). The order of finish was determined by the amount of work performed during the 36 hours. The average temperature, barometric pressure, and humidity during the competition were 28.1°C, 739.6 mmHg, and 75.5% RH, respectively. The average daytime temperature was 31.8°C. Research personnel remained at the starting tent for the duration of the event and collected data from participants between tasks, thus data were collected at five time-points throughout the 36-hour event.

2.3. Tympanic Temperature. After resting for at least ten minutes, body temperature was assessed via a tympanic thermometer (Genius 2 Tympanic thermometer, Covidien LLC, Mansfield, MA). The participant was seated in a chair and the researcher guided the tip of the thermometer into the ear canal in such a manner as to follow the natural anatomy.

2.4. Saliva Collection and Analysis. One-milliliter samples of whole unstimulated saliva were collected using a saliva collection aid and frozen on dry ice at the event location. Upon arrival at the lab, samples were transferred to a −30°C lab freezer until analysis. Melatonin levels were analyzed using a commercial ELISA assay kit (Salimetrics LLC, State College, PA). The intra-assay coefficient of variation was 2.75%.

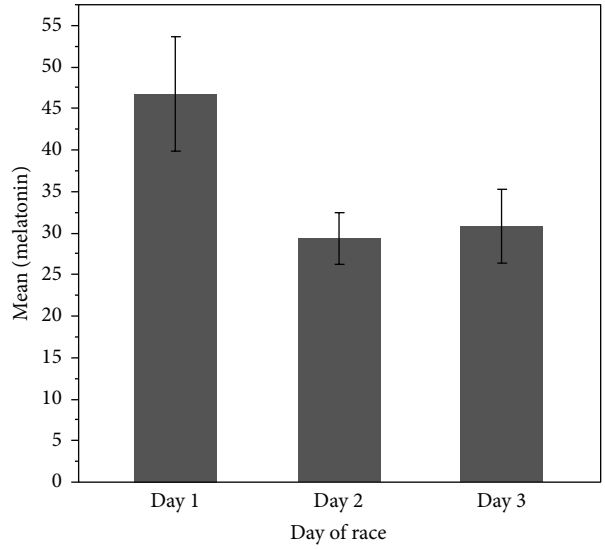
2.5. Psychomotor Vigilance Testing. In order to quantify the effects the ultra-endurance event on cognitive function, a psychomotor vigilance test (PVT) was administered to the participants after resting for at least ten minutes. In an effort to avoid the potential of practice effects occurring during subsequent testing, participants were familiarized and practiced the test after giving informed consent. The PVT is a test of simple visual reaction time and was developed at the Walter Reed Army Institute of Research [16, 17]. The PVT was used to assess mean reaction time over a 5-minute time course. The test used random periods of time in which a target stimulus was displayed on the screen of a Palm handheld device. The program was set to display approximately 100 stimuli in the 300-second (5 minutes) period at randomly spaced intervals [17]. This program computed a mean reaction time to each stimulus. Both right-handed and left-handed individuals were accommodated.

2.6. Statistical Analysis. Data were analyzed for relationships with the day of the race (days 1, 2, and 3) and light/dark via nonparametric correlations. This analysis was conducted both for tympanic temperature measurements and for melatonin concentrations. Generalized linear modeling analysis was used to examine the relationship between minor lapses in attention (>500 ms in duration, but less than 1000 ms) and tympanic temperature and melatonin with day of the competition included in the model. All statistical analyses were conducted with a modern computerized statistical



Each error bar is constructed using 1 standard error from the mean.

Figure 1: Mean tympanic temperature by day of competition. Error bars represent ± 1 SEM.



Each error bar is constructed using 1 standard error from the mean.

Figure 2: Mean melatonin by day of competition. Error bars represent ± SEM.

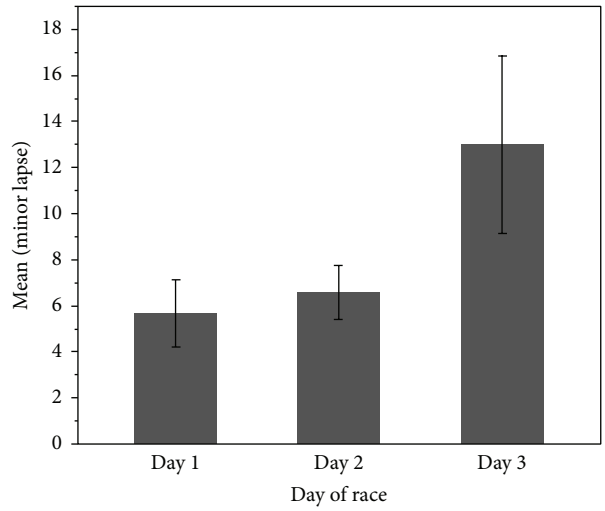
software package (JMP 11.0). Statistical significance was set a priori at alpha <0.05.

3. Results

3.1. Tympanic Temperature. Over the course of the 36-hour event, there was a significant correlation between tympanic temperature and day of race ($r = -0.444$, $P = 0.001$; see Figure 1) with steady tympanic temperature decline (day 1: 38.0±0.7°C, day 2: 37.7±1.0°C, and day 3: 36.6±0.7°C) during the event. Analysis also revealed a significant correlation between tympanic temperature and the light/dark cycle ($rs = -0.612$, $P = 0.000$) (Mean Light Condition = 38.1 ± 0.86, Mean Dark Condition = 37.1 ± 0.86).

3.2. Melatonin. During the 36 hours of competition melatonin levels were negatively correlated with the day of the race ($rs = -0.277$, $P = 0.039$; day 1: 46.8 ± 21.9 pg/mL, day 2: 29.3 ± 14.5 pg/mL, and day 3: 30.8 ± 12.6 pg/mL; see Figure 2) and positively associated with nighttime ($rs = 0.316$, $P = 0.021$; night: 40.5 ± 16.8 pg/mL, day: 24.2 ± 14.1 pg/mL).

3.3. Cognitive Function. General Linear Model analysis was conducted to predict minor lapses in attention (Omnibus Test $P < 0.001$) and resulted in significant main effects for tympanic temperature ($P < 0.001$), day of the competition ($P = 0.018$), and a tympanic temperature ∗ day of competition interaction ($P < 0.001$). During the 36 hours of the endurance event the participants' tympanic temperature declined (day 1: 38.0 ± 0.7°C, day 2: 37.7 ± 1.0°C, and day 3: 36.6 ± 0.7°C) and was associated with an increase in lapses in attention (day 1: 5.7±4.3 lapses, day 2: 6.6±5.7 lapses, and day 3: 13.0 ± 7.5 lapses). Major lapses by day of the competition can be seen in Figure 3.



Each error bar is constructed using 1 standard error from the mean.

Figure 3: Mean major lapses by day of race. Error bars represent ± SEM.

4. Discussion

The decline in cognitive function associated with sleep deprivation has been well established. The current study is in agreement with previous research findings and further indicates that cognitive function may be dependent on tympanic temperature. The primary outcomes of the current study suggest that consecutive days of sleep deprivation result in an overall significant decline in cognitive function. In addition, the significant decrease in tympanic temperature throughout the 36-hour race despite the absence of a significant increase in salivary melatonin suggests that cognitive

function may be directly affected by tympanic temperature, independent of melatonin concentration. These findings are supported by a previous research study which demonstrated that lower temperatures in the brain are associated with a greater number of lapses on the PVT during dark hours and that body temperature directly regulates cognitive function, independent of circadian rhythm [12]. Additional research, however, has indicated that decreased time in rapid eye movement sleep cycles (deep sleep) and increased time in wake cycles are associated with changes in core temperature and cognitive function [27]. Thus, altered sleep cycles, which are known to affect circadian patterns, likely affect cognitive function via direct changes in core temperature, rather than direct changes in melatonin production. The results of the current study further reinforce this notion.

Salivary melatonin concentrations followed typical light/dark oscillations throughout the race, yet melatonin concentrations were not higher during the second dark cycle versus the first dark cycle despite the prolonged sleep deprivation and drop in tympanic temperature. Previous sleep deprivation studies have shown that melatonin release increases with sleep deprivation [28, 29] and that the amplitude of melatonin release is dependent upon the duration of sleep deprivation [30], with longer sleep deprivation resulting in greater melatonin release and a greater drop in body temperature. This response to sleep deprivation is likely a physiological safeguard that encourages humans to sleep in order to regain normal physiological function. The current literature offers some potential insight as to why the current study did not demonstrate a significant change in melatonin amplitude despite a drop in tympanic temperature and prolonged sleep deprivation.

The most likely cause for the lack of change in melatonin observed in the current study was due to the physical exercise stimulus. Exercise at night has been shown to significantly blunt normal melatonin responses to dark cycles [31]. Thus, although melatonin concentrations were significantly higher during the dark cycles versus the light cycles, it is likely that the continuous stimulus of exercise was enough to attenuate an expected rise in melatonin during the second dark cycle of the 36-hour race. Physical exercise, however, did not alter the sleep-deprivation-associated drop in core temperature and cognitive function, further suggesting that sleep deprivation directly alters cognitive function via decreases in core temperature.

In addition to physical stress, many circadian rhythms are known to be affected by light and temperature and, therefore, it is also possible that the elevated environmental temperature in combination with the stressful conditions may have compromised the expected increase in melatonin release during the second dark cycle. It is well documented that core temperature is dependent upon environmental temperature, relative humidity, level of physical activity, and melatonin release [32, 33]. It is important to note that the environmental conditions for the current study were not controlled. At the peak environmental temperature of 36°C, the average tympanic temperature was 38.2°C; at the lowest environmental temperature of 21°C, the average tympanic temperature was 36.6°C. The coolest environmental temperature occurred at the end of the 36-hour race during the second dark cycle, so the possibility that sleep deprivation was responsible for the drop in tympanic temperature cannot be ruled out. However, melatonin levels were not significantly elevated during the second dark cycle when compared to the first dark cycle. Furthermore, tympanic temperature during the first dark cycle of the race, when participants were well-rested, averaged 38°C, versus 36.6°C during the second dark cycle, while the environmental temperature during the first dark cycle averaged 26.6°C, versus 21°C during the second dark cycle. These results indicate that although the participants were exerting themselves physically and mentally throughout the 36-hour period, tympanic temperature appeared to be more dependent upon the environment, rather than physical activity or melatonin levels. Tympanic temperature did change with the light/dark cycles and subsequent changes in melatonin concentration, with higher tympanic temperatures associated with lower salivary melatonin levels during light hours. However, environmental temperature was higher during light hours as well and melatonin concentration was not significantly altered during the second dark cycle yet tympanic temperature was significantly lower. This further reinforces the notion that tympanic temperature was more dependent on the environment than light/dark cycles or melatonin concentration. However, it cannot be ruled out that prolonged sleep deprivation played a major role in the decline in tympanic core temperature as well. It is worth noting that while rectal temperature provides the most accurate reflection of core temperature, such measurement techniques were not feasible given the nature of the current study. In addition, tympanic temperature measurements have been shown to be highly reliable [34], and since changes in core temperature were a primary outcome variable, rather than absolute core temperature values, tympanic measurements were appropriate.

While several sleep deprivation studies have been completed, this is the first study, to our knowledge, to examine the effects of 36 hours of continuous exercise. This type of activity is not common among the general public but may be more frequently observed in military training or missions as well as long-duration ultra-endurance races. Therefore, the findings presented here may prove particularly useful for groups of people that may undergo several hours or days of strenuous activity with minimal or no sleep time.

5. Conclusions

Clearly, the addition of exercise to prolonged sleep deprivation is effective at blunting melatonin responses to dark cycles, which may minimize the urge to sleep. However, decreased core temperature during prolonged activity and sleep deprivation was associated with compromised cognitive function demonstrating a need for the sleep to maintain cognitive function. Whether or not short sleep cycles (i.e., 1-2 hours) are effective enough to offset the drop in tympanic temperature and cognitive function associated with sleep deprivation is yet to be established but could prove useful, especially during prolonged activity.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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Research Article

Anthropometric Characteristics and Sex Influence Magnitude of Skin Cooling following Exposure to Whole Body Cryotherapy

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This study explored whether anthropometric measures influence magnitude of skin cooling following exposure to whole body cryotherapy (WBC). Height, weight, body fat percentage, and lean mass were measured in 18 male and 14 female participants. Body surface area, body surface area to mass ratio, body mass index, fat-free mass index, and fat mass index were calculated. Thermal images were captured before and after WBC (−60°C for 30 seconds, −110°C for 2 minutes). Skin temperature was measured at the chest, arm, thigh, and calf. Mean skin temperature before and after WBC and change in mean skin temperature (ΔT_{sk}) were calculated. ΔT_{sk} was significantly greater in females (12.07 ± 1.55°C) than males (10.12 ± 1.86°C; $t(30) = -3.09$, $P = .004$). A significant relationship was observed between body fat percentage and ΔT_{sk} in the combined dataset ($P = .002$, $r = .516$) and between fat-free mass index and ΔT_{sk} in males ($P = .005$, $r = .622$). No other significant associations were found. Skin response of individuals to WBC appears to depend upon anthropometric variables and sex, with individuals with a higher adiposity cooling more than thinner individuals. Effects of sex and anthompometrics should be considered when designing WBC research or treatment protocols.

1. Introduction

Whole body cryotherapy (WBC) involves a short exposure to very cold air whilst wearing minimal clothing. It has been used in both clinical and sporting populations for treatment of depression [1], rheumatic conditions [2], ankylosing spondylitis [3], and exercise-induced muscle damage [4]. The thermodynamics of WBC centre on the principle of unidirectional heat transference from high to low temperatures [5], where body tissues lose their heat to the cryotherapy modality. The interaction between body and cryochamber occurs principally at the skin, and skin temperature directly reflects the balance of heat loss to the environment and heat deposited by metabolically active tissue [6]. Skin temperature has been shown to reduce significantly immediately after WBC exposure and then increase up to 1 hour after exposure, with concomitant decreases in muscle and core temperature [7, 8]. Heat transfer mechanisms and processes have not been previously examined with WBC, but, with application of ice packs, the transfer of heat from the body depends on several factors including size of contact area, the difference in starting temperatures, and the heat capacity of each material [9]. Thickness of adipose tissue also affects cooling time, with thicker skinfolds requiring longer ice exposure than thinner skin folds to produce a standard temperature in deeper tissues [10]. With WBC, differences in magnitude of cooling have been observed in individuals with a high body mass index (BMI) compared to low body mass index [6]. Collectively, the evidence from ice pack and WBC research suggests that standardised WBC protocols may result in differences in cooling in individuals with different anthropometric characteristics. To the best of our knowledge, no study has explored whether a range of anthropometric measures influence the magnitude of skin cooling when using WBC. Potentially important anthropometric factors include the size of contact area between cold air and skin (body surface area (BSA)), level of subcutaneous fat, or calculated ratios of anthropometry, for example, BMI or BSA

to mass ratio (BSA : mass). Establishing this knowledge has implications for determining whether individualised doses based on anthropometric measures are warranted for WBC, as is found in other areas of medicine including the use of BSA for dosing chemotherapy [11].

Therefore the aims of this exploratory study were to

(i) examine the relationship between anthropometric measures and magnitude of skin cooling following exposure to WBC;

(ii) explore whether anthropometric measures can be used to predict magnitude of skin cooling following exposure to WBC;

(iii) compare skin cooling responses to WBC in males and females.

2. Materials and Methods

2.1. Participants. Thirty-two recreationally active participants (males: $n = 18$, mean age: 29.5 ± 4.4 years; females $n = 14$, mean age: 28.3 ± 6.4 years) gave written informed consent to participate in the study. Each participant completed a medical form and declared that they were free from medical conditions including Raynaud's phenomenon and other cold sensitivities, heart conditions, claustrophobia, and allergy to adhesive tape. The study was approved by the Moulton College Research Ethics Committee in accordance with the Declaration of Helsinki.

2.2. Instrumentation and Measures. Height and weight were measured using laboratory scales and stadiometer. Body composition was measured using bioelectrical impedance (BIA) at a frequency of 50 kHz (Biostat 1500, Isle of Man) yielding information on body fat percentage and lean mass. From the anthropometric measures taken, height and weight, BMI, body surface area using the Du Bois equation [12], and BSA : mass were also calculated. As the BMI concept has been criticised for not accounting effectively for body composition, fat-free mass index (FFMI; fat-free mass/height^2) and fat mass index (FMI; fat mass/height^2) were also calculated [13]. A summary of the anthropometric characteristics of the participants can be seen in Table 1.

Skin temperature was measured by taking thermal images using a factory calibrated FLIR Thermal Imaging Camera (E40BX FLIR systems, Danderyd, Sweden). The thermographs were taken according to the standard protocol for infrared imaging in medicine [14]. Thermal imaging has been shown to be reproducible between users, although being variable within individuals over time [15], and was suggested to be an accurate and reliable method for assessing skin temperature following cryotherapy in a recently published review [16]. The camera was positioned on a tripod, 3 metres from the participants, with an emissivity factor of 0.98 which is appropriate for skin [16, 17]. The thermographs were later analysed using FLIR QuickReport to establish mean skin temperature within regions of interest (ROI) on the chest, anterior thigh, posterior upper arm, and calf, for both pre-WBC (preT_{sk}) and post-WBC (postT_{sk}) images. Mean skin temperature (T_{sk}) was calculated from four body sites using the equation of Ramanathan [18]. The change in skin temperature (ΔT_{sk}) from before WBC to after WBC (outcome variable) was calculated by subtracting postT_{sk} from preT_{sk}.

Table 1: Anthropometric characteristics of study participants.

	Males		Females	
	Mean	SD	Mean	SD
Height (cm)	179.2	6.2	164.6	6.5
Mass (kg)	80.6	9.4	64.1	12.4
BMI (kg/m^2)	25.0	2.3	23.7	4.6
FFMI (kg/m^2)	21.1	1.4	16.8	1.2
FMI (kg/m^2)	4.0	1.3	6.9	3.8
BSA (m^2)	2.0	0.1	1.7	0.2
Lean mass (kg)	67.7	6.2	45.7	4.9
Body fat percentage (%)	15.6	4.0	27.4	8.8
BSA : Mass (cm^2/kg)	2.0	0.1	3.0	0.3

Cryotherapy exposures took place in a liquid nitrogen cooled cryogenic chamber at The Chris Moody Sports Injury and Rehabilitation Centre in Northamptonshire, United Kingdom. The unit was purpose-built and temperature controlled (Juka, Poland), comprising two chambers (−60°C and −110°C) connected by an internal door.

2.3. Procedure. Participants were instructed to abstain from consuming caffeine, smoking, or taking part in exercise on the day of testing and had not consumed food within 2 hours of WBC exposure. They were asked to remain hydrated. Height and weight were first measured; then participants acclimated for 20 minutes to ambient room temperature. Four-electrode BIA was undertaken and connected at two sites (wrist/hand and ankle/foot).

In preparation to enter the cryotherapy chambers, participants undressed to shorts (males) and shorts and vest (females). Protective garments worn by the participants included gloves, socks, clogs, tubular bandages to cover elbows and knees, headband to cover the ears, and surgical mask over the mouth. Glasses, jewellery, and piercings were removed before entering the chamber. Thermally inert markers were attached using adhesive tape to eight sites on the participants' bodies to create ROI for temperature analysis, in a similar fashion to that described by Costello et al. [16]. The sites used were the acromion process, olecranon process, coracoid process, 5 cm below the sternoclavicular joint, anterior superior iliac spine, 5 cm above the patella, popliteal crease, and the distal musculotendinous junction of gastrocnemius. Participants were instructed to stand in anatomical position in a thermally controlled room (21.6 ± 1.6°C), and two pre-WBC thermographic images of the whole body (anterior and posterior) were taken.

Following a safety briefing from the cryochamber operators, participants (in pairs) entered the antechamber for 30 seconds at −60 ± 4.7°C and transferred through an internal door to the main chamber for 2 minutes at −110 ± 2.3°C. This replicates commonly reported time and temperature protocols [2, 19–23]. At the completion of the WBC exposure,

participants transferred immediately to the adjacent thermally controlled area to capture post-WBC thermal images.

2.4. Statistical Analysis. Descriptive analysis of pre- and post-WBC temperatures for each of the ROI and T_{sk} measures was undertaken. Differences in mean decrease in T_{sk} between sexes (ΔT_{sk}) were examined using an independent t-test, with significance accepted at $P \leq .05$. Bivariate correlation between six anthropometric measures (BMI, FFMI, FMI, BSA, body fat percentage, and BSA : mass) and ΔT_{sk} were calculated using a Pearson's correlation coefficient (r). A Bonferroni adjusted alpha value of 0.008 was used as multiple correlations were performed [24]. For significantly associated variables, simple linear regression was calculated.

3. Results

3.1. Analysis of Change in ΔT_{sk} after WBC. The mean temperatures of all body regions (T_{Chest}, T_{Arm}, T_{Thigh}, and T_{Calf}) and of T_{sk} decreased after exposure to whole body cryotherapy in both males and females (Table 2). The lowest temperatures reached were in the calf (19.54 ± 3.28 and $15.05 \pm 2.04^{\circ}$C for males and females, resp.). ΔT_{sk} was significantly greater in females ($12.07 \pm 1.55^{\circ}$C) than males ($10.12 \pm 1.86^{\circ}$C), $t(30) = -3.09$, $P = .004$. When considering the data set as a whole, ΔT_{sk} was $11.0 \pm 1.96^{\circ}$C, equivalent to a 34.2% decrease in skin temperature from pre- to post-WBC exposure.

3.2. Exploring Relationships between Anthropometric Measures and ΔT_{sk}. For combined male and female data, a highly significant moderate relationship was observed between body fat percentage and ΔT_{sk} ($P = .002$, $r = .516$). Figure 1 shows the effect of adiposity on ΔT_{sk}, with females demonstrating higher levels of both adiposity and cooling than males. A very similar relationship is seen between FMI and ΔT_{sk} (Figure 2), although this is not significant. No other anthropometric variables were significantly associated in the combined group (Table 3). A significant moderate association was observed for FFMI with ΔT_{sk} in males ($P = .005$, $r = .622$) and an effect of sex and FFMI appears to be present (Figure 3), where the two sexes are distinctly clustered along similarly sloped lines with different intercepts. ΔT_{sk} increased with FFMI in both male and female participants, but the female cluster of data points are situated to the left on the graph, indicating that, for a given FFMI, females cooled more than males. There were no significant associations between ΔT_{sk} and anthropometric measures in females.

3.3. Examining Predictors for ΔT_{sk}. Simple linear regression of the significantly associated variable of body fat percentage with ΔT_{sk} gives the following regression equation to predict magnitude of cooling in males and females:

$$\Delta T_{sk} = 0.12\ (\text{body fat percentage}) + 8.6\ (\pm 1.7). \quad (1)$$

Similarly, FFMI can be used as a predictor of ΔT_{sk} in males using the following equation:

$$\Delta T_{sk} = 0.81\ (\text{FFMI}) - 6.9. \quad (2)$$

4. Discussion

The response of individuals to WBC appears to depend upon sex and anthropometric variables, with body fat percentage demonstrating a significant positive correlation with ΔT_{sk} in a combined dataset of males and females. Females had higher levels of adiposity than males and experienced a greater degree of skin cooling. A significant difference was observed for ΔT_{sk} in males and females, with females' skin cooling more than that of males, suggesting that optimal WBC dosage may differ between sexes. Differences in local skin cooling were also observed, with the greatest difference found in the calf, where ΔT was almost 4°C more in females. The differences observed between males and females might be explained by anthropometric and thermoregulatory differences; females have 20% smaller body mass, 14% more fat, 33% less lean body mass and 18% less surface area than males [25], a higher subcutaneous to visceral fat ratio [26], and a smaller ratio of FMI to FFMI than males [13]. Furthermore, females' BSA : mass is higher than males, and the greater the ratio of BSA to mass is the greater the heat lost is [27]. Under cold stress, females have a more extensively vasoconstricted periphery, with greater surface heat losses [25] and show a significantly reduced sensitivity of the shivering response [28].

FFMI was significantly associated with ΔT_{sk} for male participants and appeared to also demonstrate a trend in females although this was not significant. Similarly, BMI (in males) and FMI (in males and females combined) appeared to demonstrate trends that were not significant; however the lack of statistical significance may be explained by the very conservative Bonferroni adjusted alpha value used in this study, potentially increasing the risk of Type II error. These factors suggest that adiposity and sex alone do not fully explain the differences in cooling observed; rather there appears to be an effect where the bigger the individual is (be that through body fat percentage, BMI, or either of its two constituents, FMI or FFMI), the greater the amount of heat they lose at the skin following WBC. Research examining the effects of extreme cold on the skin in individuals of different levels of fatness is scant, as researchers have mainly focused on core temperature and tolerance to cold environments. However, some explanations have been offered that could explain the findings reported here. Matsumoto et al. [29] reported that obese women displayed significantly lower sympathetic responsiveness to cold air (10°C) than nonobese women, which would result in a greater degree of cutaneous heat loss following the reduced degree of vasoconstriction. Alternatively, individuals with higher subcutaneous fat stores tend to have a lower T_{sk} than their thinner counterparts as fat insulates the skin from the warming effect of deeper tissues [27]. A similar relationship between BMI and ΔT_{sk} following WBC has previously been reported in a sample of males and females [6], where individuals with a higher BMI cooled

Table 2: Mean temperature of body regions (°C) before and after exposure to WBC.

	Males			Females			Combined		
	Before WBC (°C)	After WBC (°C)	ΔT (°C)	Before WBC (°C)	After WBC (°C)	ΔT (°C)	Before WBC (°C)	After WBC (°C)	ΔT (°C)
T_{Chest}	33.58 ± 0.76	25.45 ± 1.97	8.13 ± 1.57	34.15 ± 0.73	25.25 ± 2.98	8.90 ± 2.48	33.83 ± 0.79	25.3 ± 2.42	8.47 ± 2.02
T_{Arm}	31.17 ± 1.14	21.20 ± 2.00	9.98 ± 1.81	31.52 ± 1.07	20.40 ± 2.40	11.13 ± 1.84	31.33 ± 1.10	20.85 ± 2.22	10.48 ± 1.88
T_{Thigh}	31.47 ± 1.14	20.50 ± 2.56	10.97 ± 2.36	31.23 ± 0.86	17.38 ± 1.76	13.85 ± 1.21	31.36 ± 1.02	19.14 ± 2.72	12.23 ± 2.41
T_{Calf}	32.20 ± 0.57	19.54 ± 3.28	12.66 ± 3.06	31.50 ± 0.76	15.05 ± 2.04	16.46 ± 1.96	31.9 ± 0.74	17.57 ± 3.57	14.33 ± 3.2
T_{sk}	***32.16 ± 0.77***	***22.0 ± 2.10***	***10.12 ± 1.86***	***32.25 ± 0.73***	***20.18 ± 2.11***	***12.07 ± 1.55***	***32.2 ± 0.74***	***21.20 ± 2.26***	***11.0 ± 1.96***

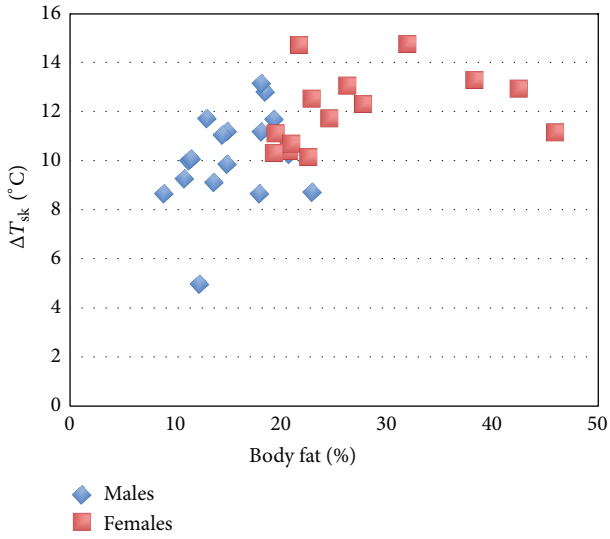

Figure 1: Significant association between body fat percentage and ΔT_{sk} in combined males and females.

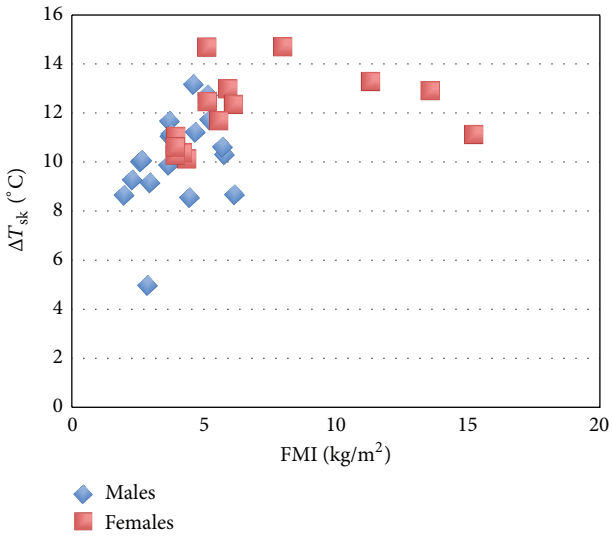

Figure 2: Nonsignificant association between FMI and ΔT_{sk} which demonstrates a highly similar pattern to that observed with body fat percentage and ΔT_{sk}.

more than those with a lower BMI. Despite some differences in study protocols and subject characteristics, those findings corroborate the findings presented here.

Skin temperatures recorded at the different anatomical sites sampled for combined sexes showed a trend that is also in agreement with previous work in this area. ΔT was greater in the lower extremities (calf 14.3°C; thigh 12.2°C) than the chest or forearm (8.5°C and 10.5°C, resp.), which has also been shown in previous cryotherapy studies [6, 30].

4.1. Methodological Considerations. Like many studies of WBC, skin temperature was measured by infrared thermography in this study. Infrared cameras have the advantage

Table 3: r values and significance of correlations of anthropometric measures with ΔT_{sk}.

	Males		Females		Combined	
	r	P	r	P	r	P
BMI	.590	.010	.402	.154	.286	.113
FFMI	.622	.005*	.653	.009	−.013	.478
FMI	.380	.118	.288	.315	.444	.010
BSA	.115	.649	.417	.138	−.216	.234
Body fat %	.326	.187	.313	.276	.516	.002*
BSA : Mass	−.512	.030	−.501	.068	−.137	.455

*Statistically significant (≤.008).

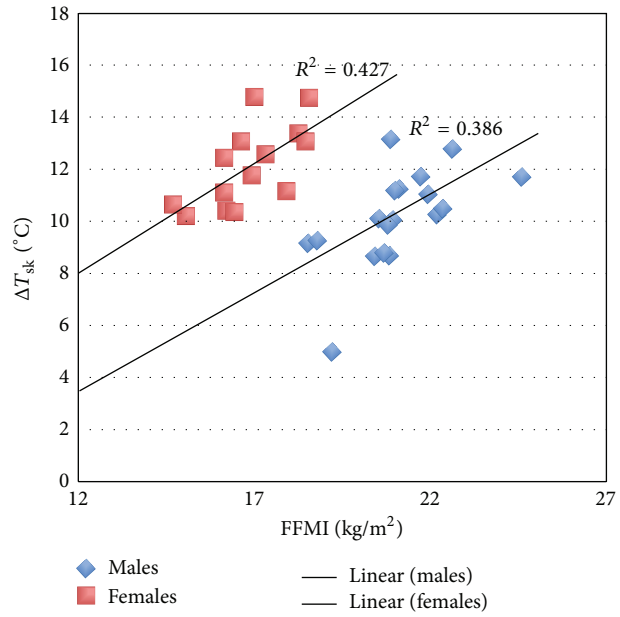

Figure 3: Association between FFMI and ΔT_{sk} which was significant for males but not females.

over other methods of assessing skin temperature (such as thermocouples, thermistors, or wireless sensors) that they do not require contact with the skin and therefore do not create any local area of insulation [16]. However, no verification of the temperatures recorded was made during this study, and due to the lack of agreed guidelines on the creation of ROI the regions used for this study may not be directly comparable with those used in other studies.

BIA is a popular commercial method for measuring body composition and has been shown to be reliable and valid when compared to skinfold analysis and hydrostatic weighing [31]. However it can be affected by several factors including hydration, food consumption, and exercise. Participants in the study were required to remain hydrated, to have abstained from eating within the two hours prior to WBC exposure, and to have abstained from any exercise that day in order to minimise these effects. Despite this, it is possible that the limitation of using BIA compared to a gold standard or reference measure of body composition may have adversely affected these data. Hydration status could also theoretically

influence heat dissipation after WBC, and future work could address this additional factor.

Skin temperature is clearly an important parameter in influencing internal cooling following WBC [7]; however it is a limitation of this study that it is unknown whether there were differences present in core temperature as a result of the differences in T_{sk} observed between individuals. Core temperature has previously been shown to display a predictable relationship with T_{sk} following WBC [8] and this would be an interesting area for future study in order to further explain the findings reported here. Further research using a measure of core temperature such as a rectal probe or temperature-sensitive ingestible capsule would be needed to make those comparisons, which were not available for this study. Furthermore, time-course data would be required to evaluate if rewarming of the skin and core varied between individuals of different anthropometry.

One issue that was not accounted for in female subjects was potential variation in basal body temperature according to the phase of the menstrual cycle that individuals were in at the time of testing. Some attempt was made by the research team to quantify the cycle phase to explore whether it was confounding; however three-quarters of the female participants were using hormonal contraception, and consequently some participants had not experienced menses for some time. Therefore it was not possible to examine any potential effects of this variable. The effects of this on the findings are likely to be minimal and, however, should be taken into consideration when interpreting the female portion of the data presented here.

The sample size used for this study is greater than many studies that have explored the effects of WBC; however at 32 it was still relatively small when considering a heterogeneous group of people. The female dataset was more heterogeneous than the male dataset, with large standard deviations observed for mass, BMI, and body fat percentage. A larger number of participants would result in a more representative study population, and further research with much larger numbers of participants is needed to validate the findings presented here. A larger sample size would also facilitate performing multiple regressions on the data to explore whether multiple anthropometric predictors exist for skin cooling following WBC; this was not possible in this data set as the test would be underpowered for 6 predictors in 32 participants. Further research should also examine whether the same effects occur in athletic populations, where anthropometric characteristics are diverse and where WBC has become a popular tool for recovery [3].

4.2. Practical Implications and Conclusions. The findings of this study appear to suggest that individuals with different anthropometric characteristics receive different therapeutic effects in terms of changes in skin temperature from identical doses of WBC, with individuals with higher adiposity cooling more at the skin than thinner individuals. Similarly, males and females appear to respond differently to WBC exposure, and this should be considered when designing and interpreting research studies and determining commercial treatment protocols for WBC. These findings could have implications for the individualisation of dosage for WBC. Personalised medicine is becoming an increasingly popular model of healthcare and these findings lend support to the idea of individualising dosage, although more research would be needed to determine a multitude of parameters in order to achieve this. As well as application for medical usage, this may have important implications in determining WBC dosage in elite athletic populations, for those that have access to cryotherapy chambers. Performance margins are very small in elite sport, so individualising a dose of WBC may assist in creating optimal effects in individual elite athletes. Manipulating the parameters of time and temperature of exposure adjusts the therapeutic dose given to users, but the question of "what are the optimal modality, temperature, and duration required to elicit the required physiological response?" posed by Costello et al. [7] highlights the current "holy grail" in WBC research. As presently the optimal WBC dose (in terms of duration and temperature) has yet to be elucidated, manipulation of dose based on individual characteristics to achieve optimal effects might be a goal for the future. However, further work should be conducted towards this to help better understand the use and application of WBC in different populations.

Conflict of Interests

Whole body cryotherapy took place at The Chris Moody Sports Injury and Rehabilitation Centre which is a commercial arm of Moulton College, an educational institution. Authors S. Cuttell and J. Meyler are employed on academic contracts at Moulton College and are not involved in commercial activity.

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Clinical Study

Effects of a Meal on the Hemorheologic Responses to Exercise in Young Males

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Aim. This study investigates the changes in hemorheologic parameters resulting from exercise followed by a standard meal. *Methods*. In twelve moderately active men a period of exercise on a bicycle ergometer for 30 min at 60% VO_{2max} was followed by a test meal or by 30 min rest. Venous blood was sampled for further analysis at baseline, after exercise, and after the meal/rest period. *Results*. The elongation index (EI) was reduced and a marked rise in plasma viscosity was observed after exercise. A significant decrease in half time of total aggregation ($T_{1/2}$) and a rise in aggregation index (AI) after exercise were observed; however, after the postexercise period these changes were reversed. *Conclusion*. The present study demonstrates that physical exercise causes several changes in blood rheology parameters, such as an increase of blood viscosity, a decrease in EI and an increase in AI, and a fall in the $T_{1/2}$ values. The meal eaten in the postexercise period caused a further reduction in EI values indicating higher red cell rigidity, but not in plasma viscosity or aggregations indices. Such alterations in hemorheologic parameters should not impair the function of the cardiovascular system in fit and healthy people but it could constitute a serious risk under various pathophysiological conditions.

1. Introduction

The importance of physical activity in preventing most chronic cardiovascular and metabolic diseases and the role of exercise as an adjunctive therapy regime is widely accepted. Generally, the beneficial effects of exercise are associated with metabolic improvement and neutralization of some of the risk factors connected with a sedentary lifestyle [1]. The protective effect of exercise may also be attributed to its anti-inflammatory effect which is at least partly caused by muscle-derived peptides, the so-called "myokines" [2]. The contracting skeletal muscles release myokines with endocrine effects mediating direct anti-inflammatory effects and/or specific effects on visceral fat [2]. Systematic exercise could lead to some adjustments of the rheological properties of blood that could potentially be involved in its beneficial effects [3, 4].

However, it should be considered that intensive, acute exercise may sometimes constitute a risk of acute cardiovascular accidents. Acute exercise leads to several changes in blood rheological parameters, including increased blood viscosity. A sudden rise in blood viscosity may impair the microcirculatory blood flow and oxygen supply to the tissues. Increased blood viscosity is treated as an important risk factor for cardiovascular and cerebrovascular disorders; decreased deformability and increased aggregation of red blood cells (RBC) were reported in such diseases as hypertension, diabetes mellitus (DM), and coronary artery disease [3, 5–9].

Rheological properties of blood are influenced by several factors, including metabolic and humoral factors [10]. It is possible that nutritional factors could also influence the rheological changes associated with exercise but these effects are relatively understudied and only observed in a specific model when a meal preceded exercise [11, 12]. It was suggested,

however, that the meal which was consumed (particularly the carbohydrate component) not only influences the rheological blood properties but also accelerates myocardial ischemia, reduces exercise capacity, and is associated with a more rapid increase in the determinants of myocardial oxygen consumption [12]. An increased food intake in response to acute exercise was reported and is considered to be the result of cognitive factors including attitudes and beliefs associated with exercise, such as the common behavior of using food as a reward for exercising [13]. To our knowledge the effects of a meal following exercise on hemorheologic parameters, such as viscosity, deformability, and increased aggregation of red blood cells, were not tested.

The aim of this study was to analyze the changes in hemorheologic parameters resulting from exercise followed by a standard meal.

2. Material and Methods

2.1. Participants. Twelve moderately active, nonsmoking male volunteers (mean ± SEM, age = 28.7 ± 3.7; height = 1.86 ± 0.1 m; weight = 78.2 ± 9.7, body mass index (BMI) 22.6 ± 0.3 kg/m^2) were recruited to participate in this study. None of the subjects had signs or symptoms of acute or chronic disease or took any medication. Volunteers received monetary compensation for their participation. They were fully informed of the study details and expressed their written consent prior to taking part in the study. The study protocol was approved by the Jagiellonian University Bioethics Committee, and all the procedures complied with the Declaration of Helsinki.

2.2. Study Protocol. All subjects were instructed to consume a staple diet containing 40–50% carbohydrates, 15–25% proteins, and 30–40% fat (energy percent) at least two weeks prior to and throughout the study period. They were asked to refrain from taking vigorous exercise and ingesting caffeine or alcohol 24 h prior to the main trials. All experiments started at 8.00 a.m., after a 12-hour overnight fast.

Subjects attended the laboratory for an initial session during which anthropometric data was collected and they were familiarized with the equipment. A submaximal fitness test was used to estimate $VO_{2\,max}$, bearing in mind the considerable limitations of that approach [14]. The test was performed on a Monark cycle ergometer with the seat height adjusted so that the subject's knee was slightly flexed when the ball of the foot rested on the pedal at the lowest point in a revolution. Heart rates were monitored and recorded using a HR monitor (Polar F1—Polar Electro Oy, Kempele, Finland). The ambient temperature range was about 23.8°C. Initial work loads of 120 W at 60 pedal revs. per min were used. If the heart rate after 2 minutes was less than 120 bpm, the work load was increased to 180 W and the test was continued. If in the 4th minute the heart rate was less than 120 bpm, the work load was increased to 210 W and the test was continued until that criterion was met. The predicted $VO_{2\,max}$ was read from a nomogram or accompanying tables and multiplied by the von Dobeln age correction factors [15, 16].

During actual intervention, the subjects underwent two test sessions: a high intensity exercise on a bicycle ergometer for a 30 min period at 60% predicted $VO_{2\,max}$ followed by a test meal (test meal series) or by a period of 30 min of rest (control series) in random order. During the test meal the subjects received sandwiches consisting of bread, butter, and ham (2.73 kcal/g, energy percent: 44.4% carbohydrates, 16.2% proteins, and 39.4% fat) which they were supposed to consume until reaching satiety. The two test sessions were performed on two separate days, with a minimal period of 14 days between the sessions. The participants had free access to water in the postexercise period.

Blood samples were collected into EDTA tubes from the antecubital vein at baseline, after exercise, and after eating. The following basic hematological parameters were determined: (1) red blood cell count (RBC, 10^6/mm^3), (2) hemoglobin (Hb, g/dL), and (3) hematocrit (HCT, %).

2.3. Rheological Analysis. The rheological analysis was conducted as described above [17, 18]. The deformability and aggregation of red blood cells were determined using a laser-assisted optical rotational cell analyzer (LORCA, RR Mechatronics, Holland) according to Hardeman's method [7, 8]. The deformability was expressed using the elongation index (EI). Additionally, the maximum EI (EI_{max}) and the shear stress required to cause one half of this maximum ($SS_{1/2}$) were determined using the Lineweaver-Burk approach and nonlinear curve fitting using a nonlinear curve-fitting algorithm available in a commercial statistical package (Prism 6.2, GraphPad Software Inc., La Jolla, CA). The $SS_{1/2}/EI_{max}$ ratio was calculated as a normalized measure of $SS_{1/2}$. $SS_{1/2}/\ EI_{max}$ is inversely related to RBC deformability such that a lower value indicates better deformability. Details and justification of this method have been described elsewhere [18, 19].

The following aggregation parameters were estimated: (1) aggregation index (AI, %), (2) the amplitude and total extent of aggregation (AMP, arbitrary units), and (3) the half time ($T_{1/2}$, s) which describes the kinetics of the aggregation process and which is proportional to the time of reaggregation of the disintegrated red cell complexes. Measurements of aggregation parameters were carried out at native hematocrit. The temperature in the LORCA was adjusted to 37°C. All other preparations and measurements were carried out at room temperature (22 ± 1°C).

For each measurement of deformability, a 25 μL blood sample was prepared in 5 mL of a 0.14 mM polyvinylpyrrolidone (PVP, M = 360,000, η = 31.8 mPa·s at 37°C in PBS) solution. The samples were injected into the LORCA measuring system, where they were subjected to varying levels of shear stress. That process was fully automated (a shear stress between 0.30 Pa and 59.97 Pa was applied). The EI for erythrocytes was calculated according to the formula:

$$\mathrm{EI} = \frac{(A - B)}{(A + B)}, \tag{1}$$

where A and B represent the vertical and horizontal axes of the ellipsoid, respectively. EI allowed for the estimation of erythrocyte elasticity and was calculated based on the change

Table 1: Mean values (±SD) or median values (interquartile ranges) of plasma viscosity (PV), hematocrit (HCT), plasma lactate, and fibrinogen in meal series ($n = 12$).

Parameter	Basal	Exercise	Meal	P Basal versus exercise	P Exercise versus meal
PV (mPa·s)	1.04 ± 0.1	1.3 ± 0.09	1.28 ± 0.18	0.000***	0.808
HCT (%)	45.91 ± 1.1	49.26 ± 1.13	44.99 ± 1.31	0.000***	0.000***
Lactate (mmol/L)	1.32 ± 0.1	7.32 ± 0.81	5.43 ± 0.971	0.023*	0.067*
Fibrinogen (g/L)	3.41 (3.31–3.57)	3.79 (3.56–4.24)	3.14 (3.05–3.59)	0.004**	0.003**

*Significantly different ($P < 0.05$).
**Significantly different ($P < 0.001$).
***Significantly different ($P < 0.001$).

Table 2: Mean values (±SD) or median values (interquartile ranges) of plasma viscosity (PV), hematocrit (HCT), plasma lactate, and fibrinogen in control (without meal) series ($n = 12$).

Parameter	Basal	Exercise	Rest	P Basal versus exercise	P Exercise versus rest
PV (mPa·s)	1.06 (1.01–1.08)	1.26 (1.22–1.32)	1.46 (1.33–1.54)	0.002**	0.023*
HCT (%)	47.55 ± 2.54	50.18 ± 0.96	48.24 ± 2.4	0.002**	0.005**
Lactate (mmol/L)	1.41 ± 0.1	7.45 ± 0.81	4.78 ± 1.2	0.037*	0.069*
Fibrinogen (g/L)	3.79 (3.54–4.81)	3.72 (3.62–3.92)	3.58 (3.35–3.88)	0.959	0.041*

*Significantly different ($P < 0.05$).
**Significantly different ($P < 0.001$).

in erythrocyte shape (from round to ellipsoid) under the influence of shear stress.

Aggregation measurements obtained from the LORCA aggregometer were based on the detection of laser backscattering from the sheared (disaggregated) and unsheared (aggregating) blood using a computer-assisted system. Each 2 mL sample of blood was transferred into a glass vessel and oxygenated for 10 to 15 min prior to obtaining measurements. A 1 mL sample of blood was injected into the gap between the outer cylinder "cup" and inner cylinder "bob" of the LORCA. During the measurement, the cup was driven by a computer-controlled stepper motor. The blood sample was sheared at 400 s^{-1}, with the shear rate decreasing rapidly to zero. The backscattering data was evaluated by the computer and the AI was calculated from the syllectrogram (light scatter versus time curve during a 120 s period). This method relies on the fact that there is less light backscattered from aggregating red cells.

2.4. Other Measurements. The viscosity of plasma was determined in a viscosimeter (type D-52159 Roetgen, Myrenne, Germany) with results displayed in mPa · s. Concentrations of fibrinogen were determined with a Chrom 7 coagulometer (Slamed Ing GmbH, Germany), with results displayed as g/L plasma lactate concentration ([La]pl) and measured using an automatic analyzer (Ektachem XR 700, Kodak, USA).

2.5. Statistical Analysis. Results are presented as mean and SD or as median and interquartile range according to the distribution of the data. The Kolmogorov-Smirnov test was used to evaluate the normal distribution of the continuous data. The paired Student's t-test or the Wilcoxon matched-paired ranked-signs test was used to compare the changes between measurements. Student's t-test was used to compare means between the meal and the control series of normally distributed variables. For comparison of the not normally distributed variables the nonparametric Mann-Whitney U test was used. $P < 0.05$ was considered statistically significant. All of the above statistical analyses were performed using SPSS version 20 for Windows.

3. Results

3.1. Hematological Parameters. There was no significant change detected in the values of RBC and Hb between measurements and these results were omitted for the sake of clarity. HCT values increased significantly after exercise but returned to normal within the next 30 min in both series as shown on Tables 1 and 2.

When comparing HCT values between the experimental series we observed that after the test meal they were significantly higher than after the rest period in the control series ($P = 0.001$).

3.2. Deformability Measurements. RBC deformability (assessed as the elongation index, EI) was found to be significantly reduced immediately after exercise at shear stress levels of 0.58 Pa–31.04 Pa (Tables 3 and 4).

This effect was sustained after exercise during the 30 min rest period in the control series, but after the test meal we observed a further statistically significant reduction in

Table 3: Mean values (±SD) or median values (interquartile ranges) of the total extent of aggregation (AMP), half time of total aggregation ($T_{1/2}$), aggregation index (AI), and the elongation index (EI) at various levels of shear stress in meal series ($n = 12$).

Parameter	Basal	Exercise	Meal	P Basal versus exercise	P Exercise versus meal
AMP (au)	18.31 (17.51–18.85)	18.65 (17.83–19.11)	17.62 (16.58–18.46)	0.347	0.05
$T_{1/2}$ (s)	3.29 (2.98–3.48)	2.82 (2.7–2.97)	3.41 (3.24–3.91)	0.025*	0.028
AI (%)	50.43 (49.53–52)	59.04 (58.21–59.78)	53.8 (49.14–56.08)	0.002**	0.005**
EI (0.3 Pa)	0.04 (0.03–0.05)	0.04 (0.03–0.07)	0.06 (0.06–0.07)	0.875	0.116
EI (0.58 Pa)	0.06 (0.05–0.08)	0.06 (0.05–0.1)	0.1 (0.06–0.11)	0.666	0.077
EI (1.13 Pa)	0.11 ± 0.03	0.1 ± 0.03	0.1 ± 0.03	0.034*	1.000
EI (2.18 Pa)	0.23 (0.14–0.24)	0.21 (0.11–0.24)	0.2 (0.12–0.23)	0.003*	0.505
EI (4.24 Pa)	0.34 (0.24–0.35)	0.32 (0.21–0.35)	0.3 (0.23–0.33)	0.015*	0.125
EI (8.23 Pa)	0.45 (0.43–0.46)	0.42 (0.33–0.44)	0.41 (0.32–0.41)	0.002*	0.014*
EI (15.98 Pa)	0.52 (0.49–0.53)	0.51 (0.42–0.52)	0.46 (0.43–0.5)	0.025*	0.026*
EI (31.04 Pa)	0.58 (0.55–0.58)	0.57 (0.49–0.58)	0.53 (0.49–0.56)	0.135	0.004**
EI (59.97 Pa)	0.61 (0.59–0.61)	0.6 (0.54–0.61)	0.59 (0.53–0.6)	0.13	0.008**

*Significantly different ($P < 0.05$).
**Significantly different ($P < 0.001$).

Table 4: Mean values (±SD) or median values (interquartile ranges) of the total extent of aggregation (AMP), half time of total aggregation ($T_{1/2}$), aggregation index (AI), and the elongation index (EI) at various levels of shear stress in control (without meal) series ($n = 12$).

Parameter	Basal	Exercise	Rest	P Basal versus exercise	P Exercise versus rest
AMP	18.74 ± 0.82	18.78 (18.32–19.52)	19.48 (17.98–19.99)	0.816	0.06
$T_{1/2}$ (s)	3.86 ± 0.91	2.76 ± 0.41	3.68 ± 0.62	0.000***	0.000***
AI (%)	53.22 ± 5.82	59.45 ± 4.93	50.9 ± 5.15	0.001**	0.000***
EI (0.3 Pa)	0.05 (0.04-0.05)	0.06 (0.05-0.06)	0.05 (0.04-0.05)	0.068	0.059
EI (0.58 Pa)	0.07 ± 0.01	0.07 ± 0.01	0.08 ± 0.02	0.435	0.057
EI (1.13 Pa)	0.11 ± 0.02	0.1 ± 0.03	0.1 ± 0.02	0.058	0.966
EI (2.18 Pa)	0.22 (0.18–0.24)	0.2 (0.18–0.21)	0.19 (0.18–0.21)	0.019*	0.41
EI (4.24 Pa)	0.33 (0.28–0.34)	0.31 (0.23–0.33)	0.3 (0.26–0.34)	0.045*	0.695
EI (8.23 Pa)	0.44 (0.42–0.45)	0.43 (0.33–0.43)	0.41 (0.39–0.44)	0.002**	0.53
EI (15.98 Pa)	0.52 (0.49–0.53)	0.5 (0.44–0.51)	0.48 (0.46–0.52)	0.023*	0.695
EI (31.04 Pa)	0.57 (0.56–0.58)	0.56 (0.51–0.58)	0.55 (0.52–0.57)	0.016*	0.929
EI (59.97 Pa)	0.6 (0.59–0.6)	0.59 (0.56–0.6)	0.59 (0.58–0.61)	0.084	0.594

*Significantly different ($P < 0.05$).
**Significantly different ($P < 0.001$).
***Significantly different ($P < 0.001$).

median EI at shear stress levels 8.23 (Pa)–59.97 (Pa) (Tables 3 and 4).

Effects of exercise on EI_{max}, $SS_{1/2,}$ and $SS_{1/2}/EI_{max}$ are presented in Tables 5 and 6. We observed a significant increase in $SS_{1/2}$value and decrease in $SS_{1/2}/EI_{max}$ ratio after exercise but not after meal or rest period.

3.3. Aggregation Indices and Plasma Viscosity. We observed similar, marked, and significant rises in plasma viscosity immediately after exercise in both series (Tables 1 and 2) and in the control series we observed further significant increases in PV (Table 2) during the 30 min rest period. This effect, however, was not observed after the test meal as shown in Table 1. We also observed significant decreases in half time of total aggregation ($T_{1/2}$) and significant rises in aggregation index (AI) immediately after exercise similarly in both series as shown on Tables 3 and 4, but after the 30-minute rest period in the control series, these changes were reversed (Table 4). Similarly, a statistically significant but less pronounced effect was seen after the test meal as shown in Table 3.

3.4. Other Measurements. The exercise caused a pronounced and significant increase in plasma lactate in both series as shown on Tables 1 and 2. Within the next 30 min we observed a slight decrease, similar to the postprandial and rest periods, but this effect was not statistically significant (Tables 1 and 2). There was a slight but statistically significant increase in fibrinogen plasma levels after exercise in the test meal series (Table 1). On the other hand we observed a significant

Table 5: Median values (interquartile ranges) of half-maximal shear stress ($SS_{1/2}$), maximum elongation index (EI_{max}), and $SS_{1/2}/EI_{max}$ ratio in meal series ($n = 12$).

Parameter	Basal	Exercise	Meal	P Basal versus exercise	P Exercise versus meal
$SS_{1/2}$ (Pa)	4.26 (3.873–5.267)	4.71 (4.174–6.706)	4.54 (4.146–6.444)	0.0342*	0.9375
EI_{max}	0.66 (0.641–0.661)	0.65 (0.616–0.659)	0.62 (0.597–0.632)	0.2244	0.0907
$SS_{1/2}/EI_{max}$	6.45 (5.918–8.532)	7.24 (6.320–11.592)	7.28 (6.713–11.195)	0.0022**	0.8139

*Significantly different ($P < 0.05$).
**Significantly different ($P < 0.001$).

Table 6: Median values (interquartile ranges) of half-maximal shear stress ($SS_{1/2}$), maximum elongation index (EI_{max}), and $SS_{1/2}/EI_{max}$ ratio in control (without meal) series ($n = 12$).

Parameter	Basal	Exercise	Rest	P Basal versus exercise	P Exercise versus meal
$SS_{1/2}$ (Pa)	4.18 (4.028–5.193)	4.94 (4.765–5.872)	4.68 (4.418–5.265)	0.0022**	0.1579
EI_{max}	0.65 (0.645–0.658)	0.65 (0.622–0.659)	0.64 (0.611–0.659)	0.4240	0.6234
$SS_{1/2}/EI_{max}$	6.38 (6.150–8.026)	7.54 (7.133–9.878)	7.53 (6.731–8.550)	0.0022**	0.1823

**Significantly different ($P < 0.001$).

decrease in fibrinogen plasma levels after the next 30 min both in the test meal and control series (Tables 1 and 2).

4. Discussion

Until now, no study had investigated the effects of a meal consumed after intensive exercise on hemorheologic factors (i.e., blood viscosity, RBC deformability, and aggregation properties).

We have demonstrated a sharp increase of plasma viscosity following high intensity exercise accompanied by a hematocrit elevation. A postexercise rise in blood viscosity is a well-reported phenomenon. Hematocrit and plasma viscosity are two of the major determinants of blood viscosity. There are several mechanisms responsible for a postexercise rise in blood viscosity: a fluid shift, a rise in the number of circulating erythrocytes due to splenic contraction and their redistribution, an increase of plasma protein concentration, and loss of water caused by thermoregulatory mechanisms and its entrapment in muscle cells [3]. Considering oxygen supply to the tissue, changes in plasma viscosity are more important because the increase in hematocrit, although reducing blood flow, increases oxygen carrying capacity [20]. Rise in plasma viscosity is associated with a rise in plasma proteins and particularly fibrinogen [4].

Changes to rheological properties of blood could depend on the type of exercise. Different effects of cycling and running on blood rheology were observed. Cycling exercise similar to that used in our study leads to a rise in blood viscosity, due to alterations in plasma viscosity and hematocrit. Outdoor running, however, does not increase blood viscosity or hematocrit [21–23].

It is of interest that we observed further increases in plasma viscosity after the 30 min rest period in the control series, but not after the test meal. Although participants in both groups had free access to water in the postexercise period, maybe those consuming a meal were better hydrated. It has been recommended that the sampling should be done after overnight fasting for the determination of blood and plasma viscosity [24] but several postprandial studies reported a decrease in viscosity suggesting relative overnight dehydration [25, 26]. However in active healthy people moderate dehydration occurring during exercise has only a slight effect on blood viscosity [27, 28].

We also observed significant changes in erythrocytes deformability after exercise, as shown by a statistically significant decrease in EI and rise in $SS_{1/2}/EI_{max}$ ratio. This is in agreement with several studies demonstrating that physical exercise could also affect rheological properties of erythrocytes. However, it was shown that these alterations were not observed when red cell rheology was investigated after resuspension of cells on a buffer, which indicates that those changes were mostly due to plasma factors rather than to intrinsic red cell properties [3, 29].

In the present study we also demonstrated a marked increase in plasma lactate levels after exercise. Increased lactate could be, at least in part, responsible for observed changes in deformability. It was shown that in experimental conditions lactate shrinks the red cells and decreases their flexibility. The correlation between lactate concentrations and red cell rigidity after exercise intervention has been shown in some studies [3, 29]. It is, however, of interest that in highly trained athletes after intensive exercise an increase in red cell flexibility in spite of high blood lactate levels does not rigidify the red cell, unlike in sedentary or in the moderately active subjects [30, 31]. It is noteworthy that when exercise in the present study was followed by a meal, a further reduction in EI values was observed, indicating higher red cell rigidity even though at the same time lactate concentration has a tendency to decrease.

The present study also demonstrated an alteration of the aggregation parameters illustrated by a significant increase in AI and a fall in $T_{1/2}$ values. This is in agreement with some previous studies which have shown a similar tendency [4].

The mechanisms of these rheological changes are not clear but fibrinogen is the major plasma component responsible for red cell aggregation in blood [32]. In our study we observed increases in fibrinogen concentrations after exercise but the effect was transient and present only in one series. The changes in aggregation parameters were reversed in the postexercise period irrespective of the consumed meal and this was accompanied by significant decreases in fibrinogen concentrations in both groups.

In theory most alterations in blood rheology observed after acute exercise could exert negative effects particularly on the cardiovascular system and oxygen supply to the tissues. According to Poiseuille's law, a rise in blood viscosity could lead to increased vascular resistance and cardiac afterload [4]. However, some studies have shown that the effects of increased blood viscosity may be in healthy persons quite the opposite. Increased shear stress stimulates nitric oxide (NO) release from endothelium and leads to a decrease in vascular resistance [33, 34]. Connes et al. [32] have recently reported a positive correlation between a rise in blood viscosity and an increase in NO production during exercise and a negative correlation between an increase in blood viscosity and a decrease in vascular resistance. The increase in blood viscosity could be a physiological mechanism necessary for increased NO biosynthesis and adequate vasodilation. However, this physiological compensatory response could be effective only in normally functioning endothelium and in cases of endothelial dysfunction the increase in blood viscosity might be more detrimental to the cardiovascular system [35].

Nutritional factors could affect hemorheologic alterations associated with exercise. The most important factor is dehydration, which not only increases hematocrit, plasma osmolality, and blood and plasma viscosity but also red blood cell aggregation proportional to a rise in plasma globulin. Adequate hydration could almost completely prevent the increase in red cell rigidity induced by 1 hour of submaximal exercise [36].

There are a few reports about the effects of a preexercise meal on blood rheological responses to exercise. In a study by van der Brug et al. [37] no effects of different kinds of feedings on the hemorheological response to prolonged exercise have been found. Guezennec et al. [38] have shown that polyunsaturated fatty acids of the omega-3 family improved red cell flexibility. Brun et al. [11] tested the effects of breakfast eaten before a cycling session and found that a meal prevented a reduction in red cell deformability and increased plasma viscosity after exercise.

In conclusion, the present study demonstrated that high-intensity exercise causes several changes in blood rheology parameters, such as an increase in blood viscosity accompanied by hematocrit elevation, a decrease in EI and increase in AI, and a fall in $T_{1/2}$ values. The meal eaten in the postexercise period caused a further reduction in EI values, indicating higher red cell rigidity, but not in plasma viscosity or aggregations indices. Such alterations in hemorheologic parameters should not impair cardiovascular system functions in healthy and fit people but could constitute a serious risk under various pathophysiological conditions.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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