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Running Title: Volume effect of pre-cooling

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Key words: thermoregulation, team-sports, heat-stress, fatigue, performance

Purpose: To assess the effects of pre-cooling volume on neuromuscular function and performance in free-paced intermittent-sprint exercise in the heat. **Methods:** Ten male, team-sport athletes completed four randomized trials involving an 85-min free-paced intermittent-sprint exercise protocol in $33^{\circ}\text{C} \pm 33\%$ relative humidity. Pre-cooling sessions included whole body (WB), head+hand (HH), head (H) and no cooling (CONT), applied for 20-min pre-exercise and 5-min mid-exercise. Maximal voluntary contractions (MVC) were assessed pre- and post-intervention and mid- and post-exercise. Exercise performance was assessed with sprint times, % decline and distances covered during free-paced bouts. Measures of core (T_c) and skin (T_{sk}) temperatures, heart rate, perceptual exertion and thermal stress were monitored throughout. Venous and capillary blood was analyzed for metabolite, muscle damage and inflammatory markers. **Results:** WB pre-cooling facilitated the maintenance of sprint times during the exercise protocol with reduced % decline ($P=0.04$). Mean and total hard running distances increased with pre-cooling 12% compared to CONT ($P<0.05$), specifically, WB was 6-7% greater than HH ($P=0.02$) and H ($P=0.001$) respectively. No change was evident in mean voluntary or evoked force pre- to post-exercise with WB and HH cooling ($P>0.05$). WB and HH cooling reduced T_c by $0.1\text{-}0.3^{\circ}\text{C}$ compared to other conditions ($P<0.05$). WB T_{sk} was suppressed for the entire session ($P=0.001$). HR responses following WB cooling were reduced ($P=0.05$; $d=1.07$) compared to CONT conditions during exercise. **Conclusion:** A relationship between pre-cooling volume and exercise performance seems apparent, as larger surface area coverage augmented subsequent free-paced exercise capacity, in conjunction with greater suppression of physiological load. Maintenance of MVC with pre-cooling, despite increased work output suggests the role of centrally-mediated mechanisms in exercise pacing regulation and subsequent performance.

INTRODUCTION

Paragraph Number 1 Hot and humid conditions compound the physiological strain of increased metabolic heat production associated with exercise, whilst reducing avenues for heat dissipation to the surrounding environment (31). Accordingly, both physiological and behavioural responses control the rise in thermoregulatory load, often to the detriment of exercise performance (40). These noted responses may be particularly pertinent for team-sport athletes competing in warm environments, owing to increased thermal loads associated with intermittent-sprint activity compared to continuous modes at matched intensities (5,16). Given the observed exacerbated loads and reduced performances in the heat, the popularity of pre-cooling has increased as team-sport athletes seek to counter the reduction of exercise performance in the heat.

Paragraph Number 2 A large quantity of laboratory-based research supports the efficacy of pre-cooling for endurance exercise performance in the heat (13,27,33). Regardless of traditionally equivocal findings (13,27,33), more recent studies of prolonged intermittent-sprint exercise following pre-cooling demonstrate ergogenic benefits (8), particularly in the maintenance of self-paced sub-maximal work (11,12). Typically, whole body methods utilizing cold micro-climates, specifically water immersion or cold air, can result in improved exercise performance and/or reductions in thermoregulatory strain (13,27,33). Despite wide support (13), whole body cold-water immersion may provide environmental and logistical concerns surrounding their field-based application, resulting in problematic (water access) and/or impractical (cooling a full team) implementation (27). Recently, the dosage effect demonstrated when comparing whole- versus part-body cooling methods (8,9,11) has provoked interest in combining multiple part-body techniques, thus maintaining surface area coverage and enhancing the practicality of pre-cooling

(12,34). However, the explicit effect of the volume of the imposed cooling stimulus necessary for optimal physiological, metabolic and performance outcomes remains equivocal.

Paragraph Number 3 Augmented heat storage capacity following pre-cooling aids in the suppression of increased thermoregulatory load associated with exercise in the heat (27). Whilst related reductions in heart rate along with skin and/or core temperature may indicate the maintenance of central blood volume (19), the mechanisms of pre-cooling may relate to the role of higher central regulation (30). Elevated core temperature impairs CNS motor drive, consequently reducing neuromuscular recruitment, force output and voluntary activation (21,28,30,37). Hence, blunting the rise in core temperature by pre-cooling may lead to better pacing during self-paced exercise (14,21,22). Further, the reduction in thermoregulatory and/or physiological load may also ease generic stress responses relating to alterations in metabolic processes (17) and elevated damage and inflammatory markers following exercise in the heat (2). As such, the cause-effect relationship and underlying mechanisms of the imposed pre-cooling stimulus requires further attention to provide insight as to the mechanisms on neuromuscular function which might improve exercise in the heat and also allow for the development of ecologically valid, evidence-based cooling techniques.

Paragraph Number 4 Whilst the rationale for pre-cooling athletes for the protection of acute exercise performance in the heat is accepted (13,27,33), practical limitations may restrict the application of whole body immersion techniques in the field. Mixed-method approaches to pre-cooling demonstrate advantageous effects on exercise performance, physiological loads and perceptual state (12,34). However, a lack of data exists to demonstrate the optimal volume of

cooling stimulus necessary for ergogenic benefit. Moreover, apparent influences of pre-cooling on CNS activity and subsequent self-selected work output require further investigation. Therefore, the purpose of this study was to determine the effects of pre-cooling volume on free-paced intermittent-sprint performance and physiological responses in heat stress. A complementary aim was to determine the effects of pre-cooling on voluntary force and evoked twitch properties and their relationship to exercise performance in the heat.

METHODS

Participants

Paragraph Number 5 Ten, well trained, male team-sport athletes (Mean \pm SD: age 20.9 ± 2.6 yr; height 182.1 ± 8.8 cm; body mass 77.8 ± 6.7 kg; body surface area 1.98 ± 0.12 m²) volunteered to participate in this study. Participants regularly competed in regional level team-sport competitions (cricket, rugby union) and reported ≥ 3 training days per week including sports specific skill-based and strength and conditioning sessions. Following disclosure of all risks and benefits, all participants provided verbal and written consent prior to the commencement of all testing procedures. Experimentation was approved by the Ethics in Human Research Committee of the University.

Overview

Paragraph Number 6 A randomized, repeated measures cross-over design was used to determine the effects of pre-cooling volume on performance, physiological, biochemical and perceptual responses to an intermittent-sprint exercise protocol. The study was conducted as part of a series of cricket related studies and hence the exercise protocol was based on fast bowling

specific intermittent-sprint exercise (32); but, was adapted to increase the volume of work required to allow reporting of a more generic protocol for team-sport activity as previously suggested (11). An initial equipment and procedural familiarisation session was performed before commencing data collection. This included completion of the entire exercise protocol in hot conditions, application of neuromuscular assessments and cooling techniques. Testing sessions were standardised for each participant and separated by 5-7 days to allow full recovery. Physical activity, diet and fluid intake were all documented in food and physical activity diaries in the 24 h prior to the first testing session and replicated for the following sessions. Participants were educated on keeping dietary and workload records, with standardization compliance qualitatively assessed prior to commencement of each session. Sessions were conducted on an enclosed 20 m synthetic running track in hot conditions ($33.0 \pm 0.7^{\circ}\text{C}$ and $33.3 \pm 3.9\%$ relative humidity). Environmental temperatures were controlled by a customized gas heating system and four electronic 2,000 W room heaters (Kambrook, Australia) positioned at 5 m increments alongside the running track. All testing sessions were identical so that pre-cooling volume was the only manipulated variable throughout. Participants performed five sessions including a familiarization session, control session (no pre-cooling), head cooling session (pre-cooling with an iced towel), head and hand cooling session (pre-cooling with an iced towel and container of cold water) and a mixed-method whole body session (pre-cooling with iced towel, container of cold water, ice vest and ice packs applied to the quadriceps). Participants were required to abstain from strenuous exercise and alcohol 24 h before and all caffeine and food substances 3 h before each testing session. A standardized volume of water (500 mL) was consumed 1 h pre-exercise to regulate hydration status.

Exercise protocol

Paragraph Number 7 Participants performed an intermittent-sprint running protocol comprising of 2 x 35 min spells of exercise (Spell 1 and 2), separated by a 15 min recovery period on the enclosed 20 m running track. Prior to commencement, a 5 min warm-up period was completed in hot conditions involving 20 m shuttle running with increments in running speed each minute and 6 repeated 15-m maximal sprints. The 2 x 35 min exercise protocol consisted of 10 (2 x 5) bouts of 6 x 15 m maximal sprint efforts separated by 5 min bouts of self-paced activity at different intensities; designed to incorporate the movement requirements for cricket fast bowlers (32). Participants performed a set of 6 x 15 m sprints commencing at 30 s intervals, emulating a 6-ball cricket over. During the 5 min interval between sets of sprints, participants performed 1 min periods of self-paced, sub-maximal exercise, including walking, jogging and hard running (11). Participants were informed of the required intensity on a minute by minute basis. Self-paced, sub-maximal activity was performed on the 15 m running track in a shuttle run fashion and participants were requested to return to the starting position at 50 s of each self-paced min to then commence the subsequent intensity. Each data collection session involved 10 sets of (6) sprints and 8 periods of 5 min self-paced sub-maximal running bouts, thus incorporating the demands of 2 x 5 over spells of fast bowling. Participants were offered verbal support and encouragement throughout to cover the greatest distance during the hard running bouts, and jog or walk at a self-selected pace during the respective jogging and walking bouts. All fluid consumption was restricted throughout the exercise protocol. Previously collected but unpublished reliability data demonstrate the Intra-Class Correlation of mean sprint times, self-paced distances and hard running distances covered was $r = 0.94 - 0.98$, while the Technical Error of Measurement was 0.5 – 2% and Co-efficient of Variation was 0.6 – 2%.

Pre-cooling intervention

Paragraph Number 8 Following all resting measures, a pre-cooling intervention was performed for 20 min pre-exercise and for 5 min during the 15 min mid-session recovery period. In order to induce a volume effect, a step-wise approach to part-body cooling was used, with pre-cooling sessions involving no cooling (control), head cooling (H), head+hand cooling (HH), or mixed-method whole body cooling (WB). During the H cooling session, participants were cooled using an iced towel soaked in water ($5.0 \pm 0.5^{\circ}\text{C}$) prior to being placed over the head and neck. HH cooling was achieved using an iced towel ($5.0 \pm 0.5^{\circ}\text{C}$) covering the head and neck whilst each hand was immersed up to the wrist in separate containers of cold water actively maintained at $9.0 \pm 0.5^{\circ}\text{C}$. During the WB cooling session, participants were cooled with an iced towel over the head and neck, hands immersed to the wrist in cold water, ice-vest covering the torso (Arctic Heat, Brisbane, Australia) and frozen ice-packs applied to the quadriceps (Techni Ice, Frankston, Australia). Ice-vests and ice-packs were stored at -20°C prior to and following use. The mixed-method approach was selected based on the practical ease and portability of equipment compared to cold water immersion (14). No cooling stimulus was applied during the control condition (CONT). Standardized warm-up procedures commenced immediately following neuromuscular assessment, approximately 5 min post-intervention completion. Reapplication of cooling methods during the mid-exercise recovery period occurred within approximately 10 min to accommodate the collection of neuromuscular and biochemical measures. All treatments were performed in a seated position within controlled laboratory conditions of 33°C and 33% relative humidity. Percentage surface area volumes covered during pre-cooling were estimated at 10%, 15% and 35% for H, HH and WB trials respectively (23).

Measures

Performance

Paragraph Number 9 Exercise performance was determined by 15 m sprint time and distance covered throughout sub-maximal exercise bouts. An infra-red timing system (Speed-Light, Swift, Australia) was used to record sprint times, whilst % decrement was calculated according to Dawson et al. (10). Incremental 1 m markings along the 15 m synthetic running track allowed for calculation of distances covered during each individual sub-maximal exercise bout. Participants wore personal athletic training attire (T-shirt, shorts, socks and running shoes) that were standardized throughout. Data were reported as mean or total for individual exercise modes (walk, jog, hard run).

Neuromuscular

Paragraph Number 10 Measures of voluntary force and evoked twitch properties of the right knee extensors were assessed pre-intervention, post-intervention, mid-exercise and post-exercise using an isokinetic dynamometer (Kin-Com, Model 125, Chattanooga Group Inc., Hixson, TN, USA) connected to a host computer and customized software (v8.0, LabVIEW; National Instruments, North Ryde, NSW, Australia). Participants were seated in an upright position, with the axis of rotation of the dynamometer visually aligned with the lateral femoral epicondyle and the lower leg attached to the lever arm 1 cm above lateral malleolus on the right leg. Seating posture was standardised with the knee and hip positioned at 90° flexion (0° represents full extension) and securely fastened to the dynamometer using conventional waist and shoulder straps. Supra-maximal transcutaneous electrical stimulation of the femoral nerve was

administered via a reusable gel adhesive electrode (diameter 10 mm; MEDI-TRACE™ Mini 100 Pediatric Foam Electrodes, Covidien, Mansfield, MA, USA) located on the anterior thigh 3 cm below the inguinal fold. An additional reusable gel adhesive electrode (90 x 50 mm; Verity Medical Ltd., Stockbridge, Hampshire, England) positioned on the medio-posterior aspect of the upper thigh below the gluteal fold acted as the anode. A single square-wave pulse with a width of 200 μ s (400 V with a current of 100-450 mA) was delivered by a Digitimer DS7 stimulator (Digitimer Ltd., Welwyn Garden City, Hertfordshire, England) connected to a BNC2100 terminal block and signal acquisition system (PXI1024; National Instruments, Austin, TX, USA). Peak twitch force was detected with incremental increases in stimulus intensity and final levels were amplified by 10% to ensure attainment of supramaximal stimulation. Five pulses separated by 20 s were delivered in a rested state to assess resting evoked twitch properties. Subsequently, participants completed a maximal voluntary contraction (MVC) protocol involving 5 x 5 s isometric trials using a work to rest ratio of 1:6. The MVC was defined as the mean peak torque value attained during voluntary contractions. A superimposed twitch was manually triggered during each MVC within 1 – 2 s after commencement to coincide with an observed plateau in peak torque. An additional stimulus was delivered to the resting muscle immediately post-

contraction to calculate potentiated twitch properties. Voluntary activation (VA) was determined using the twitch interpolation technique (1). Time to peak torque (TPt) was calculated using the time from evoke force onset to peak potentiated twitch torque. Analyses of neuromuscular data

were performed using MATLAB software (R2009b 7.9.0.529, The Mathworks Inc., Natick, MA, USA).

Physiological measurements

Paragraph Number 11 Pre- and post-exercise towed dry measures of nude body mass were recorded using calibrated scales (HW 150 K, A & D, Australia) to estimate sweat loss. A mid-stream urine sample was collected pre-exercise to assess urine specific gravity (USG; Refractometer 503, Now. Nippon Optical, Works Co, Tokyo, Japan). Heart rate was measured using a chest transmitter and wrist watch receiver (FS1, Polar Electro Oy, Finland) at 5 min intervals during respective intervention and exercise protocols. Core temperature (T_c) was measured using a telemetric capsule (VitalSense, Mini Mitter, USA), ingested at a standardized 5 h pre-exercise to ensure passing into the gastrointestinal tract. The T_c was measured pre-intervention and at 5 min intervals throughout the cooling intervention and exercise protocol, respectively. For detailed discussion of the reliability (ICC= 0.99) and validity ($r= 0.98$) of ingestible T_c capsules see Gant, Atkinson and Williams (18). Skin temperatures (T_{sk}) were assessed at the sternum, mid-forearm, mid-quadriceps and medial calf by an infra-red thermometer (ThermoScan 3000, Braun, Germany) at 5 min intervals during the pre-cooling intervention, mid-protocol break and post-exercise. This technique has been reported to be a reliable (ICC= 0.96) and valid ($r= 0.92$) measure of T_{sk} (4). Mean T_{sk} was calculated according to Ramanathan (35).

Blood collection and biochemical analysis

Paragraph Number 12 A 100 μ L pre-exercise sample of capillary blood was collected from a hyperaemic earlobe to measure pH, glucose, lactate [La⁻] and bicarbonate (HCO₃) (ABL825 Radiometer, Copenhagen, Denmark). Additional capillary blood samples were collected mid-exercise and immediately post-exercise. To determine the effects of pre-cooling on muscle damage, inflammation and generic stress responses, venous blood was collected from an antecubital vein pre- and 30 min post-exercise and analysed for creatine kinase (CK), C-reactive protein (CRP), testosterone (TEST), cortisol (CORT) and insulin (INS). Using an evacuated venipuncture system and serum separator tubes (Monovette, Sarstedt, Numbrecht, Germany), samples were allowed to clot at room temperature prior to centrifugation for 10 min at 4000 rpm. Supernatant was then extracted and stored at -20°C until analysis. Before analysis the serum was allowed to reach room temperature and mixed via gentle inversion. CK and CRP were analyzed according to manufacturer's instructions provided in the respective assay kits (Dimension Xpand spectrophotometer, Dade Bearing, USA). CK concentrations were determined using an enzymatic method and bichromatic rate technique. CRP samples were manually diluted according to manufacturer's instructions and analyzed with the particle enhanced turbidimetric immunoassay technique (PETIA). INS, TEST and CORT levels were detected using a solid-phase, competitive chemiluminescent enzyme immunoassay (Immulite 2000, Diagnostic Products Corp., Los Angeles, CA). To avoid inter-assay variations, all samples for each subject were analysed in the same assay run. Intra-assay Coefficients of Variance were < 5% for all venous blood analyses. Serum hormone concentrations were not corrected for plasma volume shifts, thus all statistical analyses were performed on hormone values based on actual measured circulating concentrations.

Perceptual Measures

Paragraph Number 13 Rating of perceived exertion (RPE) and thermal sensation scale (TSS) were recorded at 5 min intervals throughout pre-cooling and exercise protocols. RPE was determined according to the Borg CR-10 scale, where ranking ranged from 0 (nothing at all) to 10 (maximal). TSS was assessed using an 8-point Likert scale, ranging from 0 (unbearably cold) to 8 (unbearably hot).

Statistical analysis

Paragraph Number 14 Data are reported as mean \pm standard deviation (SD). A repeated-measures ANOVA was performed to detect within treatment differences. Unprotected pairwise comparisons (Protected Fisher's LSD) were applied to determine the source of significance, which was accepted when $P \leq 0.05$. Analysis was performed using the Statistical Package for Social Sciences (SPSS v 16.0, Chicago, IL). Standardised effect sizes (ES; Cohen's d) analyses were used in interpreting the magnitude of differences between conditions. An ES was classified as trivial (<0.20), small ($0.20-0.49$), moderate ($0.50-0.79$) or large (>0.80).

RESULTS

Performance

Paragraph Number 15 Mean and peak \pm SD speed and % decline in speed for Spell 1 and Spell 2 respectively are presented in Table 1, whilst mean \pm SD individual sprint times are presented in Figure 1A. No significant differences were detected between cooling procedures for mean sprint times or peak sprint speeds during Spell 1 or Spell 2, respectively ($P= 0.08 - 0.91$). Large ES

indicated faster mean sprint times with WB ($d= 0.94$) and HH cooling ($d=1.07$) compared to CONT during Spell 2. The % decline during Spell 2 was attenuated with WB cooling compared to control ($P= 0.04$). Large ES were apparent for a smaller % decline during Spell 1 in H ($d= 0.92$), HH ($d= 1.26$) and WB ($d= 0.87$) compared with CONT conditions.

Paragraph Number 16 Results for mean and total distance covered during sub-maximal exercise bouts including hard running, jogging and walking are presented in Table 1. Mean \pm SD individual self-paced hard running efforts are presented in Figure 1B. Overall, total distances accumulated were significantly higher with WB (4833 ± 380 m) and HH cooling (4644 ± 360 m) compared to H (4602 ± 448 m) and CONT conditions (4413 ± 545 m), respectively ($P= 0.001 - 0.04$; $d= 0.70 - 1.26$). No significant differences and moderate ES continued a dose-response effect between remaining total distance comparisons ($P= 0.06 - 0.12$; $d= 0.53 - 0.72$). Significant differences and large ES data indicated greater mean and total distances covered in the WB condition for hard running compared to CONT ($P= 0.001$; $d= 1.49$), H ($P= 0.001$; $d= 0.86$) and HH cooling ($P= 0.02$; $d= 0.83$) conditions. There were also significant increases in hard running distances in a dose-response fashion following H ($P= 0.02$; $d= 0.62$) and HH cooling ($P= 0.001$; $d= 0.81$) compared to the CONT. Moreover, a significantly increased mean and total hard running completed in Spell 1 was observed with HH cooling data compared to CONT ($P= 0.01$). Similarly, Spell 2 H ($P= 0.04$) and HH sessions ($P= 0.00$; $d= 0.87$) resulted in significantly higher mean and total hard running work completed compared with CONT, whilst still less than WB cooling.

[INSERT FIGURE 1]

Paragraph Number 17 There were significant increases in mean and total jogging distances covered during Spell 1 and Spell 2 in WB ($P= 0.03$ and 0.02) and HH sessions ($P= 0.01$ and 0.01) compared with CONT. Greater mean and total values for jogging distances covered were evident between WB and H cooling sessions during Spell 1 ($P= 0.02$). No other significant differences were noted among conditions for all remaining mean and total distances for jogging or walking variables during Spell 1 and Spell 2 ($P= 0.08 - 0.88$).

[INSERT TABLE 1]

Physiological responses

Paragraph Number 18 Significant reductions and large ES were apparent for T_c (Figure 2A) at the end of the intervention period for WB ($P= 0.03$; $d= 1.62$) and HH cooling sessions ($P= 0.04$; $d= 1.37$) compared with H. Similarly, T_c was reduced (Figure 2A) at the end of the intervention period for WB ($P= 0.003$; $d= 1.72$) and HH cooling sessions ($P= 0.04$; $d= 1.46$) compared to CONT. This trend was maintained throughout, with large ES ($d= 0.82 - 1.41$) indicative of a reduced T_c following WB cooling. Significant reductions in T_c with HH cooling compared to CONT were also noted during the protocol ($P= 0.01$; $d= 0.85$). In comparison with the WB method, T_c response for H cooling and CONT was elevated during the exercise protocol ($d= 0.85 - 1.41$). Finally, HH cooling did not differ from the H cooling condition; rather was increased compared to the WB cooling specifically between the 10th – 35th ($d= 0.81 - 1.19$) and 80th – 85th min ($d= 0.81 - 0.85$).

Paragraph Number 19 Significant differences and large ES indicated a lower T_{sk} throughout the WB intervention period ($P= 0.001$; $d= 2.54 - 4.62$) compared to all other conditions (Figure 2B). Large reductions in T_{sk} were also apparent for H ($d= 1.00 - 1.85$) and HH cooling sessions ($d= 0.99 - 1.74$) during the intervention period compared to CONT. During the exercise protocol, WB cooling resulted in a reduced T_{sk} compared to all other conditions ($P= 0.001 - 0.03$; $d= 1.02 - 5.71$); however T_{sk} did not differ between any other conditions.

Paragraph Number 20 Significant differences and large ES data indicated a lower HR post-intervention within WB ($P= 0.03$; $d= 1.83$) and HH cooling sessions ($P= 0.02$; $d= 1.75$) compared to CONT conditions (Figure 2C). Moreover, reduced HR values with WB ($d= 1.07$) and HH cooling ($d= 1.01$) over H cooling interventions were evident post-intervention. HR responses following WB cooling were significantly reduced ($P= 0.05$; $d= 1.07$) compared to CONT during the exercise protocol. Large ES indicated reduced HR values following WB cooling compared to H ($d= 1.25$) and HH cooling ($d= 1.34$) during the exercise protocol.

[INSERT FIGURE 2]

Paragraph Number 21 Pre-exercise USG was not significantly different between WB cooling ($1.018 \pm .006$), HH cooling (1.017 ± 0.005), H (1.017 ± 0.008) or CONT conditions (1.017 ± 0.008 ; $P= 0.70 - 0.84$; $d= 0.06 - 0.22$). Body mass changes from non-urine fluid loss post-exercise were significantly less with WB cooling compared to CONT (1.8 ± 0.2 v 2.2 ± 0.4 ; $P= 0.01$; $d= 1.58$). Large ES data indicated less sweat losses from changes in body mass with WB cooling compared to HH (2.0 ± 0.4 ; $d= 0.82$) and H cooling (2.1 ± 0.5 ; $d= 1.00$) respectively.

Neuromuscular

Paragraph Number 22 No significant differences and trivial to moderate ES (Table 2; $P= 0.07 - 0.87$; $d= 0.03 - 0.68$) were observed between respective cooling conditions for mean MVC at all time points. Mean MVC was significantly reduced pre- to post-exercise in H cooling ($P= 0.04$; $d= 1.09$) and CONT conditions ($P= 0.01$; $d= 1.46$). Pre- to post-exercise differences in mean MVC were not significantly different, with trivial to small ES data following WB and HH cooling ($P= 0.432 - 0.925$; $d= 0.04 - 0.35$). No significant differences were evident between respective cooling volumes for post-intervention TPt (Table 2; $P= 0.52 - 0.92$). However, large ES were evident for faster TPt mid-exercise with faster responses evident in the WB cooling compared to H ($d= 1.13$) and CONT sessions ($d= 1.07$). Changes in pre- to post-exercise TPt were not significant, though moderate ES were detected for reduced TPt in CONT ($P= 0.39$; $d=0.79$), H ($P= 0.34$; $d=0.74$) and HH ($P= 0.47$; $d=0.65$). Conversely, a large ES depicts slower TPt pre- to post-exercise for WB ($P= 0.11$; $d=1.16$). No significant differences and trivial to moderate ES (Table 2; $d= 0.03 - 0.45$) were detected between VA at all time points. Finally, no significant change, though moderate to large ES were detected pre- to post-exercise for reduced VA ($P= 0.06 - 0.47$; $d= 0.72 - 2.03$).

[INSERT TABLE 2]

Biochemical analyses

Paragraph Number 23 No significant differences ($P= 0.06 - 1.00$) were evident between conditions in pH, glucose, $[La^-]$ or HCO_3 markers pre- or post-exercise (Figure 3). Significant

differences and large ES indicated reduced $[La^-]$ concentrations mid-exercise with WB cooling compared to CONT ($P= 0.02$; $d= 1.64$). Further, lower $[La^-]$ values were evident mid-exercise with WB compared to H cooling conditions ($d= 1.36$). Large ES indicated reduced glucose concentrations post-exercise between HH cooling with H cooling ($d= 0.82$) and CONT sessions ($d= 1.03$). No significant differences and trivial to moderate trends ($P= 0.25 - 1.00$; $d= 0.00 - 0.59$) were determined for all pre-exercise CK, CRP, TEST, INS and CORT concentrations (Table 3). Large ES indicated reduced post-exercise CK measures with WB ($d= 0.82$) and H cooling ($d= 0.83$) compared to CONT conditions. Large ES data were detected for CORT levels within the WB cooling condition and were increased compared to CONT ($d= 1.06$) post-exercise. No significant change and trivial to moderate ES were detected for all remaining post-exercise venous blood variables of CK, CRP, TEST, INS and CORT ($P= 0.07 - 0.99$; $d= 0.00 - 0.58$).

[INSERT FIGURE 3]

[INSERT TABLE 3]

Perceptual measures

Paragraph Number 24 Significantly reduced mean RPE values were evident for WB (4.2 ± 0.8 ; $P= 0.03$; $d= 1.17$), HH (4.6 ± 0.7 ; $P= 0.04$) and H cooling conditions (4.6 ± 0.8 ; $P= 0.02$) compared to CONT condition (4.9 ± 1.0). Significant differences and large ES data indicate reduced mean TSS with WB cooling (5.2 ± 0.5) compared to HH (5.7 ± 0.4 ; $P= 0.00$; $d= 1.69$), H (5.8 ± 0.5 ; $P= 0.01$; $d= 1.95$) and CONT conditions (6.3 ± 0.6 ; $P= 0.00$; $d= 2.93$). Moreover,

significantly reduced TSS values during HH ($P= 0.01$; $d= 1.87$) and H cooling ($P= 0.00$; $d= 1.28$) were evident compared to CONT sessions.

DISCUSSION

Paragraph Number 25 The aim of this investigation was to determine the effects of volume-dependent pre-cooling on physiological and performance outcomes for self-paced intermittent-sprint exercise in the heat. In addition we attempted to determine the related volume effect on neuromuscular function as related to intermittent-sprint exercise performance in the heat. The novel finding of this study is that performance appears to be effected by the dose of pre-cooling, in that larger surface area coverage resulted in increased work capacity, with greater suppression of physiological load. Most salient, was that pre-cooling resulted in the maintenance of MVC even though there was increased work completed across the respective cooling interventions. These findings indicate that enhanced thermoregulatory control following pre-cooling may negate the down-regulation of exercise performance in the heat, and hence allows for the maintenance of skeletal muscle recruitment and work output.

Paragraph Number 26 Exercise and environmentally induced heat stress may severely impair exercise capacity through reduced time to fatigue and an inability to maintain desired intensities (20,30). Results from the current investigation are consistent with previous pre-cooling studies of attenuated thermoregulatory strain and improved intermittent-sprint exercise performance in the heat (8,11,12). A common feature of the present study and previous work (11) is the pre-cooling induced improvements in self-paced, sub-maximal distances covered between maximal sprint exercise bouts. The step-wise response to cooling resulted in the reduction in distance

covered as the volume of cooling was reduced. However, through the use of WB cooling, participants covered greater hard running and jogging distances in Spells 1 and 2 respectively (Table 1, Figure 1B). Similarly, larger cooling application was associated with greater maintenance of maximal sprint times (Table 1, Figure 1A). Hence, the current study corroborates previous research which shows performance benefits for free-paced intermittent-sprint exercise in the heat (8,11,12).

Paragraph Number 27 Unique to the current intermittent-sprint pre-cooling study was the collection of neuromuscular data at set time points during the testing session, rather than purely pre- and post-exercise. The attenuated thermoregulatory loads observed with WB cooling maintained MVC mid- and post-exercise (Table 2). These changes are apparent despite the absence of condition-specific differences in % VA (Table 2). Exercise and environmentally induced hyperthermia seemingly reduce skeletal muscle force output through the impairment of VA (28,30,36,37). However, an inverse relationship between muscle function and T_{e} is apparent as isometric MVC and % VA returns to baseline following the resumption of normative T_{e} values (28,36). Hence it is postulated that neuromuscular responses to exercise in the heat are directly linked to elevated thermal load in addition to any protocol-specific fatigue (28,36). Accordingly, the blunting of T_{e} during exercise and subsequent preservation of MVC and VA with more extensive cooling (Figure 2A) may account for the relationship between cooling volumes and self-paced running workloads. That said, an inability to directly assess muscle function during the exercise protocol mean factors relating to physiological strain or anticipatory regulation of CNS responses cannot be overlooked (14,26).

Paragraph Number 28 Given the lack of difference in VA between cooling volumes, the increased distances covered during self-paced exercise bouts may not be completely accounted for solely by peripheral or central factors. Rather, it is plausible that an interaction of afferent and efferent stimuli, combined with a reduced perceptual strain following pre-cooling may augment muscle recruitment during exercise, which manifests as a sustained MVC mid- and post-exercise, despite the increased workloads performed (14,38). Although electromyographic responses were not measured in the present study, a faster TPt mid-exercise was evident with WB cooling. The faster Tpt may highlight the protective properties of pre-cooling for exercise in the heat (13). Accordingly, pre-cooling may improve the adopted pacing strategy during self-selected work in the heat (14,22), essentially due to a greater preservation of MVC via maintenance of muscle recruitment (21) and subsequent curbing in the down-regulation of exercise intensity in the heat (as noted in the control condition).

Paragraph Number 29 While WB cooling is often reported as the gold-standard of cooling interventions, part-body cooling techniques may also have favorable physiological and performance benefits (3,8). Results from the present study demonstrate cooling volume to seemingly influence both the physiological response and ensuing intermittent-sprint exercise performance. Maintenance of repeated sprint time was evident in all pre-cooling conditions in Spell 1, though this trend was only continued in the WB condition during Spell 2 following the re-application of cooling procedures (Table 1; Figure 1A). Further, increased hard running and jogging distances covered during self-paced exercise bouts imply the presence of improved maintenance of exercise intensities (14,22) following WB in contrast to part-body or no cooling conditions (Table 1; Figure 1B). Further, as with the maintenance of MVC, a step-wise

interaction seemed evident, in that; the use of HH cooling indicated some performance benefits over the CONT condition. Conversely, reduced surface area coverage with H cooling and CONT provided little or no performance improvement, resulting in either the slowest sprint performances, or least distance covered. These findings support the existence of a dose-dependent response to cooling application and performance outcomes (8).

Paragraph Number 30 The attenuation of thermal load, through the application of pre-cooling, and subsequent improvements in heat storage and dissipation capacities, increases the period for optimal exercise performance prior to the attainment of critical T_c in lab settings (20). Accordingly, WB cooling was most effective in reducing T_c and T_{sk} pre-exercise and continuing to blunt the rise in body temperature for the duration of the exercise protocol. Increased cooling volumes may result in greater physiological perturbations, or the prolonged suppression of increased physiological loads (9,11); hence the volume effect of cooling observed in the present investigation is not surprising. Nevertheless, an attenuated thermoregulatory response during exercise in the heat may delay redistribution of cardiac output to the periphery and sweat responses for heat dissipation (6). Moreover, the maintenance of central blood volume protects against reduced stroke volume and associated cardiovascular strain (19). Pre-cooling induced reductions in cardiovascular strain are observed during set-paced exercise protocols (3,24), yet higher workloads during self-paced exercise appear to conceal this effect (11,12,22).

Paragraph Number 31 In contrast, marked reductions in HR were observed following WB cooling, in spite of higher workloads completed. Given the 20 – 30 min time frame during which the effects of pre-cooling remain evident (39), the length of spells and cooling reapplication mid-

session might overstate the volume effects in this case. However, limited cardiovascular drift may reflect improved blood volume status owing to decreased sweating demands (29). Restricted fluid intake and sweat loss alterations in nude body mass in the present study between 2.4 – 2.9% exceed levels contributing to heightened thermal strain and aerobic performance decrements (7). Nevertheless, differences in sweat rate (~400 mL) between WB cooling and CONT conditions may not adequately explain HR variance, nor augmented shuttle-running performance (19). Thus, it is likely multiple afferent and efferent indicators owing to reduced physiological (sweat loss, T_{re} and HR) and perceptual loads (RPE and TSS) promote continued maintenance of exercise intensity, muscle recruitment and voluntary force production (14,38).

Paragraph Number 32 In accordance with previous reports, analysis of blood markers of anaerobic metabolites and muscle damage demonstrated minimal differences between conditions (8,11,15,25). Given the well documented alterations in metabolism associated with exercise in the heat (17) and the reduced physiological responses to exercise with pre-cooling and/or augmented performance outcomes (14,27,34), the lack of differences in metabolic markers is not unexpected. Yet reduced [La⁻] mid- and post-exercise with greater cooling volumes are inconsistent with elevated self-paced running distances and maintenance of maximal sprints speeds. Similarly, reduced CK concentrations post-exercise in WB and HH cooling despite higher workloads may be a result of reduced levels of heat stress experienced during these conditions (2). Nevertheless, WB cooling appears to have stimulated sympatho-adrenal activity and subsequently increased CORT secretion as detected post-exercise. Whilst reduced sweat rates with more extensive pre-cooling may have assisted in explaining reduced [La⁻] and CK concentrations, elevated CORT may be attributed to higher workloads completed or the larger

cooling stimulus. As such, despite the reduced physiological strain evident during the (larger volume) cooling conditions, the increased work performed seems to still invoke greater generic stress responses.

Paragraph Number 33 In conclusion, this investigation highlights the dose-response relationship evident between pre-cooling volume and ensuing physiological, perceptual and performance outcomes in hot conditions (8,9,11). Improved performance responses may result from the maintenance of exercise intensities during self-paced exercise bouts, and potentially relate to the maintenance of MVC. Moreover, despite WB methods proving most effective, part-body techniques also offer a blunted thermoregulatory response, albeit to a lesser extent. These responses may owe to pre-cooling induced suppression of thermoregulatory responses to exercise in the heat allowing for enhanced physiological control and reduced perceptual efforts. Reduced levels of heat stress with pre-cooling may facilitate the maintenance of MVC through the maintenance of muscle recruitment thereby permitting higher work output. From a practical perspective, field-based practitioners should be aware of the inverse relationship between volume of cooling and performance benefits. In addition, practitioners should select interventions that allow for sufficient volume, yet within logistical constraints of their individual team contexts.

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CONFLICT OF INTEREST

1. MP is an employee of Cricket Australia. There are no conflicts of interest for any of the authors.
2. The results of the present study do not constitute endorsement by ACSM.

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FIGURE CAPTIONS

Figure 1. **A** Mean \pm SD individual 15-m sprint times (s) across all pre-cooling conditions. **B** Mean \pm SD individual hard running distances (m) covered across all pre-cooling conditions.

Figure 2. **A** Mean \pm SD core temperature, **B** mean \pm SD skin temperature and **C** mean \pm SD heart rate for Whole Body, Head + Hand, Head and Control conditions. ^a represents a significant difference between Whole Body and Control conditions ($P < 0.05$). ^b represents a significant difference between Whole Body and Head conditions ($P < 0.05$). ^c represents a significant difference between Whole Body and Head + Hand conditions ($P < 0.05$). ^d represents a significant difference between Head + Hand and Control conditions ($P < 0.05$). ^e represents a significant difference between Head + Hand and Head conditions ($P < 0.05$). ^f represents a significant difference between Head and Control conditions ($P < 0.05$). ¹ represents a large ES between Whole Body and Control conditions ($d > 0.8$). ² represents a large ES between Whole Body and Head conditions ($d > 0.8$). ³ represents a large ES between Whole Body and Head + Hand conditions ($d > 0.8$). ⁴ represents a large ES between Head + Hand and Control conditions ($d > 0.8$). ⁵ represents a large ES between Head + Hand and Head conditions ($d > 0.8$). ⁶ represents a large ES between Head and Control conditions ($d > 0.8$).

Figure 3. Mean \pm SD capillary blood comparison of anaerobic metabolites between cooling volume and time. ^a represents a significant difference between Whole Body and Control conditions ($P < 0.05$). ¹ represents a large ES between Whole Body and Control conditions ($d > 0.8$). ² represents a large ES between Whole Body and Head conditions ($d > 0.8$). ³ represents a large ES between Head + Hand and control conditions ($d > 0.8$). ⁴ represents a large ES between Head + Hand and Head conditions ($d > 0.8$). ⁵ represents a large ES between Head and Control conditions ($d > 0.8$).

TABLE CAPTIONS

Table 1. Mean \pm SD sprint time variables and sub-maximal running distances covered per session for whole body, head+hand, head and control conditions. ^a Significant difference compared to Control condition ($P < 0.05$). ^b Significant difference compared to Head condition ($P < 0.05$). ^c Significant difference compared to Head + Hand condition ($P < 0.05$). ¹ Large ES ($d > 0.8$) compared to Control condition. ² Large ES ($d > 0.8$) compared to Head condition. ³ Large ES ($d > 0.8$) compared to Head + Hand condition.

Table 2. Mean \pm SD mean peak torque, time to peak torque and voluntary activation (VA) level for pre-cooling methods pre-intervention, post-intervention, mid-exercise and post-exercise. ^a Significant difference compared to Control condition ($P < .05$). ^b Significant difference compared to Head condition ($P < .05$). ^c Significant difference compared to Head + Hand condition ($P < .05$). ¹ Large ES compared to Control condition ($d > .8$). ² Large ES compared to Head condition ($d > .8$). ³ Large ES compared to Head + Hand condition ($d > .8$). ^{*} Significant difference compared to pre-intervention values ($P < .05$). [†] Large ES compared to pre-intervention values ($d > .8$).

Table 3. Mean \pm SD biochemical comparison of between cooling volume and time.

^a Large ES compared to control ($d > 0.8$).

Figure 1

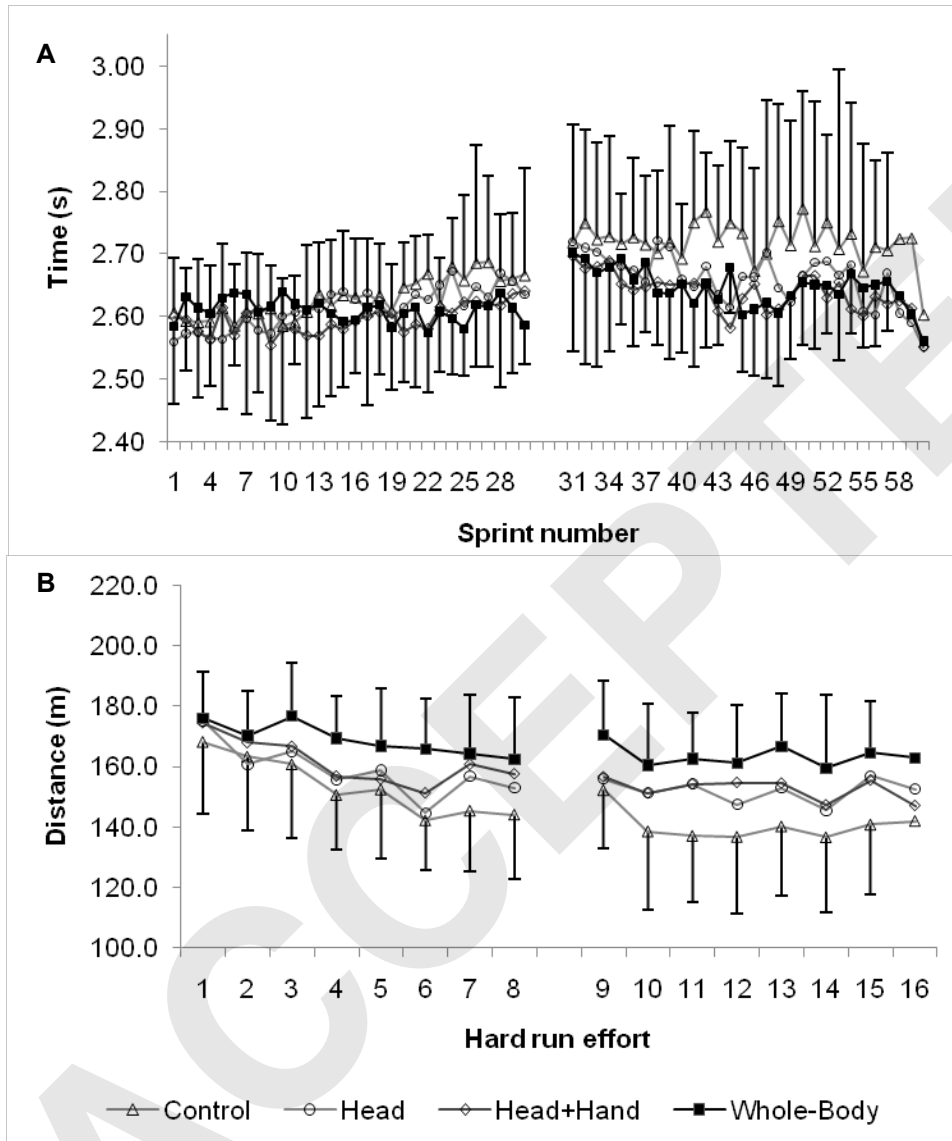


Figure 2

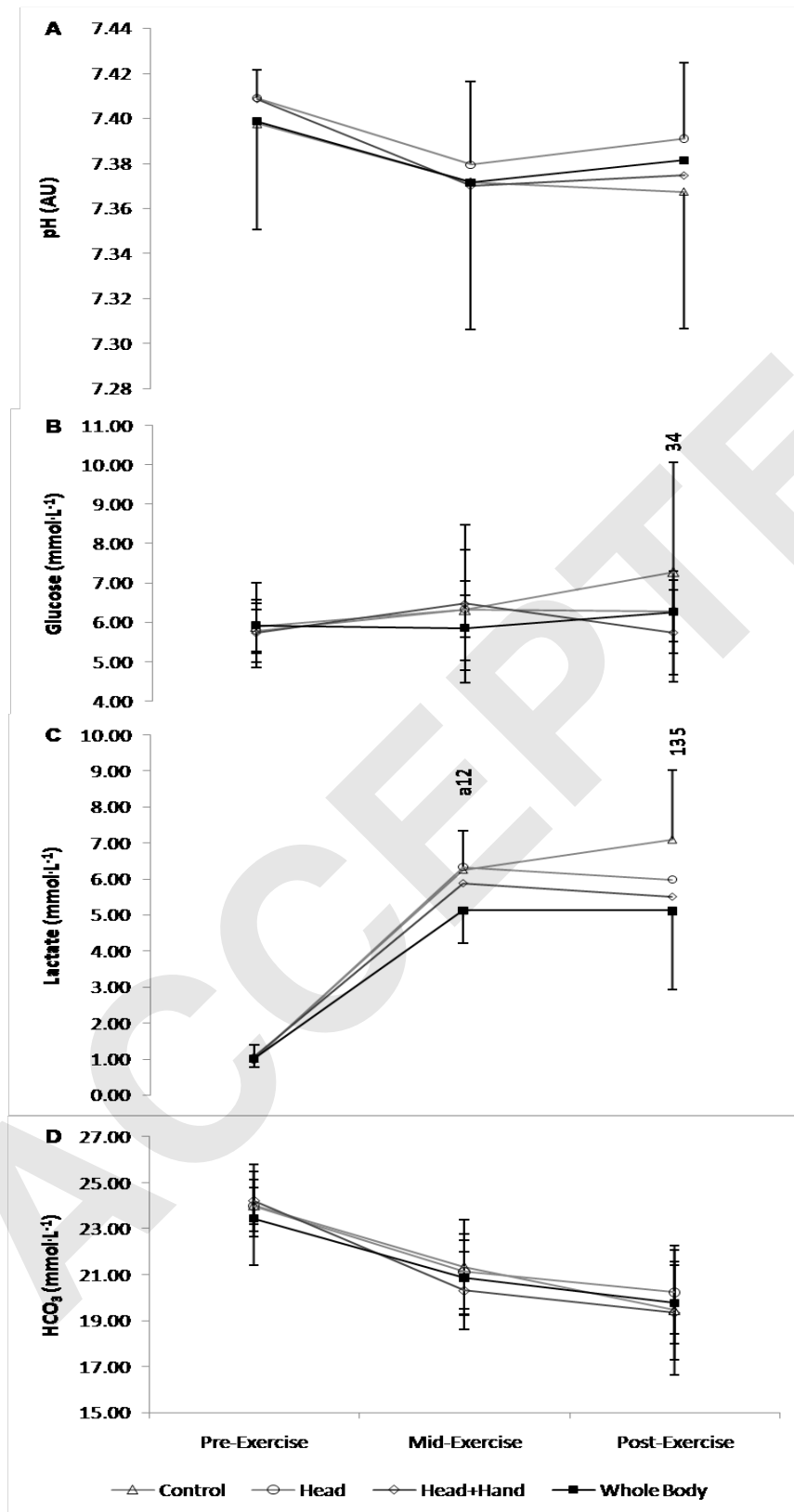
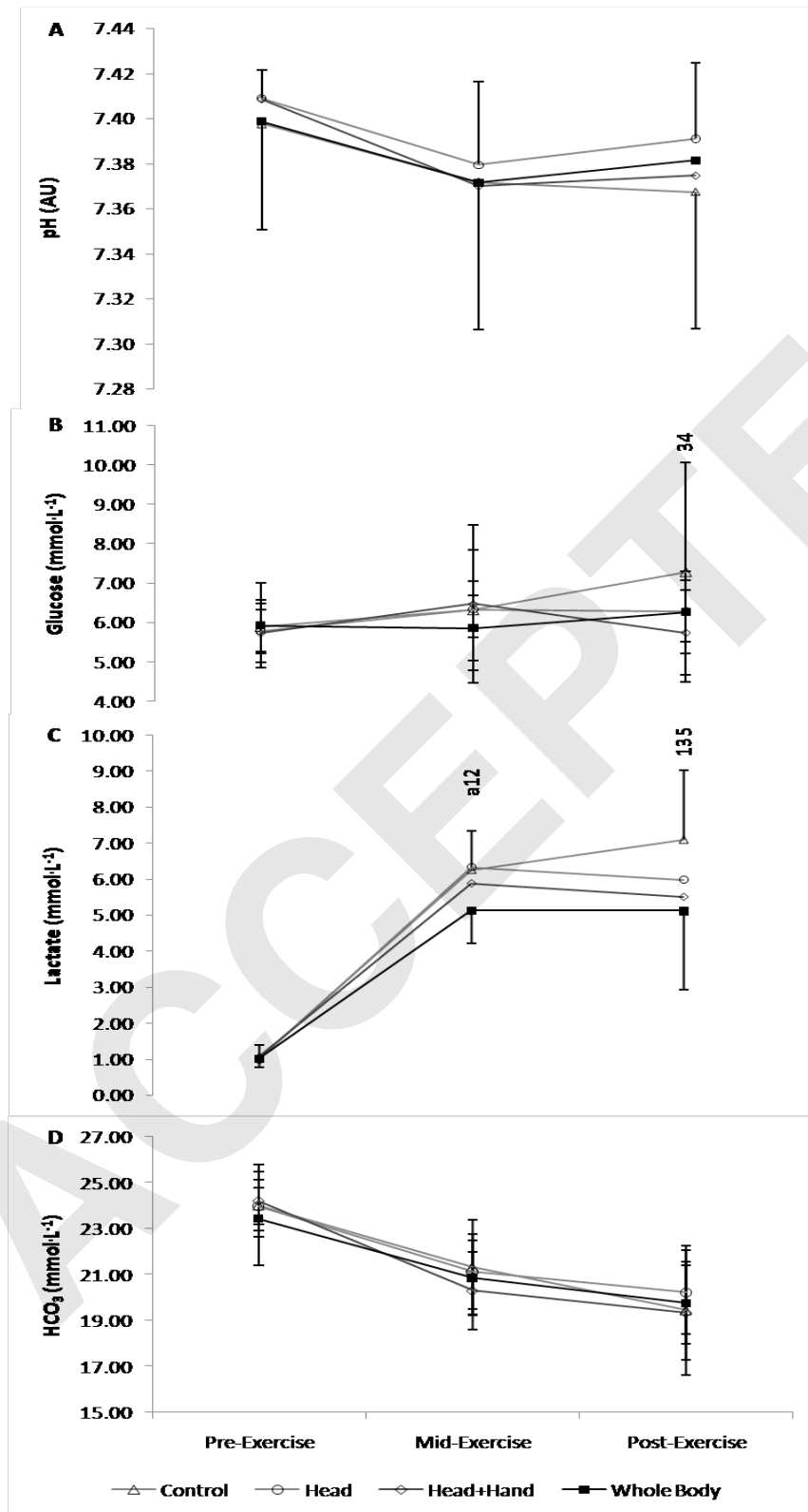


Figure 3



Activity	Control	Head	Head+Hand	Whole Body
<i>Sprint time variables</i>				
Mean Spell 1 sprint (s)	2.63 ± 0.06	2.61 ± 0.08	2.60 ± 0.07	2.61 ± 0.11
Mean Spell 2 sprint (s)	2.72 ± 0.13	2.66 ± 0.09	2.64 ± 0.08 ¹	2.65 ± 0.09 ¹
Spell 1 decline (%)	6.29 ± 2.10	4.91 ± 2.14 ¹	4.44 ± 2.04 ¹	5.00 ± 2.09 ¹
Spell 2 decline (%)	7.74 ± 4.49	7.18 ± 4.40	6.22 ± 3.87	5.69 ± 3.39 ^a
<i>Sub-maximal running distances</i>				
Mean Spell 1 hard run (m)	153.3 ± 19.7	158.7 ± 18.4	161.4 ± 12.7 ^a	168.9 ± 16.2 ^{ab12}
Mean Spell 2 hard run (m)	140.5 ± 21.8	152.1 ± 20.7 ^a	152.6 ± 17.2 ^{a1}	163.5 ± 17.6 ^{abc123}
Mean Spell 1 jog (m)	102.0 ± 14.9	103.3 ± 13.5	104.6 ± 11.8 ^a	107.6 ± 11.2 ^{ab}
Mean Spell 2 jog (m)	94.1 ± 18.4	99.8 ± 14.8	99.5 ± 13.5 ^a	100.7 ± 11.1 ^a
Mean Spell 1 walk (m)	63.3 ± 4.5	62.4 ± 6.9	64.1 ± 7.3	64.1 ± 6.4
Mean Spell 2 walk (m)	59.9 ± 9.3	60.5 ± 8.3	60.6 ± 7.4	62.8 ± 6.1
Total Spell 1 hard run (m)	1226.7 ± 157.1	1269.7 ± 147.5 ^a	1291.1 ± 101.4 ^a	1351.3 ± 129.9 ^{ab12}
Total Spell 2 hard run (m)	1124.3 ± 174.7	1216.6 ± 165.5	1220.7 ± 137.6 ^{a1}	1308.2 ± 141.0 ^{abc123}
Total Spell 1 jog (m)	816.2 ± 119.4	826.0 ± 108.3	837.0 ± 94.8 ^a	860.6 ± 89.9 ^{ab}
Total Spell 2 jog (m)	753.1 ± 147.0	798.0 ± 118.3	796.2 ± 108.2 ^a	805.7 ± 88.6 ^a
Total Spell 1 walk (m)	253.3 ± 18.1	249.4 ± 27.8	256.3 ± 29.1	256.3 ± 25.7
Total Spell 2 walk (m)	239.7 ± 37.4	242.1 ± 33.1	242.2 ± 29.4	251.0 ± 24.5

Table 1.

Variable	Control	Head	Head+Hand	Whole Body
<i>Pre-Intervention</i>				
Mean Peak Torque (Nm)	167.3 ± 37.0	163.1 ± 42.3	157.8 ± 39.2	162.3 ± 37.4
Time to Peak Torque (ms)	98.0 ± 11.1	96.6 ± 16.8	92.4 ± 16.8	88.1 ± 11.0
VA Level (%)	75.6 ± 8.9	74.7 ± 6.9	73.3 ± 9.5	78.4 ± 9.1
<i>Post-Intervention</i>				
Mean Peak Torque (Nm)	159.9 ± 47.7	162.7 ± 36.1	153.3 ± 27.4	144.2 ± 36.4
Time to Peak Torque (ms)	94.6 ± 12.0	97.1 ± 16.5	99.9 ± 18.1	96.7 ± 13.1
VA Level (%)	70.7 ± 17.1	75.8 ± 18.1	72.2 ± 11.6	68.4 ± 9.1
<i>Mid-Exercise</i>				
Mean Peak Torque (Nm)	142.9 ± 50.2	146.4 ± 26.2	145.1 ± 25.0	158.0 ± 21.9
Time to Peak Torque (ms)	93.3 ± 15.0	92.8 ± 12.1	90.1 ± 15.5	81.4 ± 16.3 ¹²
VA Level (%)	65.0 ± 9.8	66.3 ± 14.1	68.7 ± 15.4	69.6 ± 18.2
<i>Post-Exercise</i>				
Mean Peak Torque (Nm)	139.3 ± 54.0 ^{**}	139.7 ± 29.5 ^{**}	150.8 ± 46.1	151.1 ± 28.9
Time to Peak Torque (ms)	93.1 ± 14.0	90.1 ± 9.7	86.7 ± 12.5	95.2 ± 15.9
VA Level (%)	68.9 ± 15.9 [†]	68.6 ± 9.4 [†]	69.5 ± 12.2	67.5 ± 16.8 [†]

Table 2.

Variable		Control	Head	Head+Hand	Whole Body
CK (UL)	Pre	301 ± 193	231 ± 137	248 ± 169	252 ± 163
	Post	645 ± 594	392 ± 133 ^a	411 ± 187	390 ± 181 ^a
CRP (UL)	Pre	2.8 ± 4.4	2.8 ± 4.3	2.7 ± 4.1	2.4 ± 3.1
	Post	2.9 ± 4.5	3.0 ± 4.5	2.8 ± 4.3	2.8 ± 3.4
INS (μL·mL ⁻¹)	Pre	5.6 ± 1.7	6.5 ± 2.9	5.9 ± 1.0	6.2 ± 2.1
	Post	5.9 ± 2.4	5.7 ± 1.4	5.9 ± 0.6	5.4 ± 1.4
TEST (ng/dL)	Pre	401 ± 85	368 ± 50	385 ± 103	390 ± 99
	Post	524 ± 126	505 ± 99	471 ± 131	505 ± 85
CORT (nmol·L ⁻¹)	Pre	319 ± 115	299 ± 118	299 ± 140	332 ± 74
	Post	544 ± 211	611 ± 161	602 ± 161	694 ± 190 ^a

Table 3.