| **Title:** | Cold-water immersion decreases cerebral oxygenation but improves recovery after intermittent-sprint exercise in the heat. |

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**Running Title:** Cooling for recovery in the heat.

**Abstract**

This study examined the effects of post-exercise cooling on recovery of neuromuscular, physiological and cerebral haemodynamic responses following intermittent-sprint exercise in the heat. Nine participants underwent three post-exercise recovery trials including a control (CONT), mixed-method cooling (MIX) and cold-water immersion (10°C; CWI). Voluntary force and activation were assessed simultaneously with cerebral oxygenation (near-infrared spectroscopy) pre- and post-exercise, post-intervention, 1h post- and 24h post-exercise. Measures of heart rate, core temperature, skin temperature, muscle damage and inflammation were also collected. Both cooling interventions reduced heart rate, core and skin temperature post-intervention ($P<0.05$). CWI hastened the recovery of voluntary force by 12.7±11.7% (mean±SD) and 16.3±10.5% 1h post-exercise compared to MIX and CONT, respectively ($P<0.01$). Voluntary force remained elevated 16.1±20.5% 24h post-exercise after CWI compared to CONT ($P<0.05$). Central activation was increased post-intervention and 1h post-exercise with CWI compared to CONT ($P<0.05$), without differences between conditions 24h post-exercise ($P>0.05$). CWI reduced cerebral oxygenation compared to MIX and CONT post-intervention ($P<0.01$). Further, cooling interventions reduced cortisol 1h post-exercise ($P<0.01$), though only CWI blunted creatine kinase 24h post-exercise compared to CONT ($P<0.05$). Accordingly, improvements in neuromuscular recovery after post-exercise cooling appear disassociated with cerebral oxygenation; rather, reflecting reductions in thermoregulatory demands to sustain force production.

**Introduction**

Neuromuscular functioning and exercise capacity are inversely associated with an elevated core temperature ($T_c$), as the recruitment of motor units during voluntary activation of skeletal muscle is reduced under heat stress (Cheung, 2007). Concomitantly, increasing thermal strain reduces cerebral blood flow velocity and oxygenation (Nybo & Nielsen, 2001; González-Alonso et al., 2004), also contributing to declines in motor outflow and exercise performance (Rasmussen et al., 2010). Despite such findings, the recovery of cerebrovascular regulation in relation to voluntary force production after exercise- and environment-induced heat stress remains equivocal. Where repeated bouts of exercise in the heat are required, post-exercise cooling is demonstrated to alleviate high $T_c$ and hasten the recovery of voluntary force, central activation (Pointon et al., 2012), and ensuing exercise performance (Vaile et al., 2008a; Peiffer et al., 2010). These findings may demonstrate post-exercise cooling to mitigate thermally-inhibited central nervous system (CNS) drive after hyperthermic exercise (Cheung, 2007). However, whether rapidly reducing the thermal strain developed during intermittent-sprint exercise in the heat might also ease cerebrovascular perturbations affecting corticomotor function is unknown.

González-Alonso et al. (2004) previously reported heat stress to reduce cerebral oxygenation during maximal exercise, as declines in middle cerebral artery blood flow velocity reflect a lowered mean arterial pressure (MAP) and cardiac output (Q). Such reductions in cerebral oxygenation are suggested to alter central motor output (Amman & Kayser, 2009), though it is yet to be determined whether these effects may be reversed with aggressive reduction of endogenous thermal strain. Indeed, hypoxic models indicate exercise capacity to return once oxygen delivery and uptake in the brain is restored (Nielsen et al., 1999). While

unsubstantiated under heat stress, it could be speculated that central blood volume shifts achieved via post-exercise cooling may regain cardiocirculatory homeostasis (Vaile et al., 2011), as greater MAP and Q increase cerebral perfusion, and presumably oxygenation, to improve compromised motor output to active musculature (Périard et al., 2012). This may be particularly prudent in hot conditions as declines in cerebral oxygenation that precipitate reduced exercise performance (Smith & Billaut, 2010) are likely exacerbated (González-Alonso et al., 2004; Rasmussen et al., 2010).

Therefore, the present study aimed to examine the effects of post-exercise cooling on physiological, neuromuscular, biochemical and perceptual measures of recovery following intermittent-sprint exercise in the heat. A further aim was to determine whether these interventions affected cerebral oxygenation and subsequent neuromuscular function. While the efficacy of cold-water immersion (CWI) in treating heat stress is unparalleled (Casa et al., 2007), we have previously shown a positive relationship between the magnitude of pre-cooling, voluntary force and intermittent-sprint performance in the heat (Minett et al., 2011). Given recent concerns surrounding the practical application of CWI in the field (Barwood et al., 2009), a dose response for post-exercise cooling was achieved via the use of: (1) whole-body CWI; and (2) mixed-method cooling (MIX) designed to maximise surface area coverage and maintain logistical practicality. We hypothesized that the larger dosage effects of CWI would improve the recovery of cerebral oxygenation and neuromuscular function compared to MIX and control (CONT), respectively.

**Methods**

Participants

Nine moderate- to well-trained, male team-sport athletes volunteered to participate in this study (mean ± standard deviation (SD): age 21 ± 2 y; stature 183.3 ± 7.0 cm; body mass 78.7 ± 8.1 kg). Participants were sub-elite, amateur level team-sport athletes and at the time of testing were undergoing in-season training, reporting ≥ 3 training sessions and competition on a weekly basis. Participants provided written and verbal informed consent and all experimental procedures were approved by the Ethics in Human Research Committee of the University.


Overview

Participants were familiarized with all equipment and procedures under experimental conditions before reporting to the laboratory for three separate experimental trials that were separated by 5 – 7 days. Each testing session included an intermittent-sprint exercise protocol (2 x 35 min bouts) completed on a 20 m tartan indoor running track in the heat. Environmental conditions were controlled using a customized gas heating system and four 2000 W electric heaters (Kambrook, Port Melbourne, VIC, Australia), with mean ± SD ambient temperatures maintained at 32.4 ± 1.0°C and 42.4 ± 6.1% relative humidity. Fluid intake was restricted to a standardized 350 mL of water ingested ad libitum throughout the exercise protocol, with complete consumption ensured during each trial. Upon completion, participants underwent a post-exercise recovery intervention administered using a randomized, repeated-measures crossover design.  Physiological, neuromuscular and perceptual responses were obtained pre-, post-, 1 h post- and 24 h post-exercise. Dietary intake and physical activity records were maintained 24 h pre-exercise and during the 24 h recovery period for the initial experimental trial and replicated thereafter. Participants

abstained from strenuous activity and alcohol consumption 24 h pre- and caffeine and food substances 3 h pre-exercise.

Exercise protocol

After a standardized 5 min warm-up consisting of progressively increasing shuttle running speeds and 6 maximal sprint efforts, participants completed 2 x 35 min bouts (Bout 1 and 2) of intermittent-sprint exercise, interspersed by a 15 min mid-exercise passive recovery period. The exercise protocol was adapted from that reported in a companion paper as part of a series of investigations incorporating the movement patterns of cricket fast bowlers (Minett et al., 2011). In brief, each 35 min bout was identical, both involving five sets of 6 x 15 m sprints every 30 s as per a 6-ball cricket over. Sets of sprints were separated by 5 min periods of 15 m shuttles at intensities (hard run, jog and walk) instructed on a minute-by-minute basis. Participants returned to their starting positions at 50 s of each self-paced minute to commence the subsequent exercise intensity. Shuttle-run distances were recorded during the initial testing session and matched in the remaining trials to standardize individual workloads. Maximal 15 m sprint times were assessed with an infra-red timing system (Speedlight; Swift, Wacol, QLD, Australia), while sub-maximal shuttle distances were calculated using 1 m markings adjoining the running track. The reliability of sprint time variables in the present study demonstrate the Intraclass Correlation Coefficient (ICC) as r= 0.50 – 0.70 and Coefficient of Variation (CV) as 1.6 – 2.6%. Accordingly, participants completed 10 sets of sprints and eight periods of sub-maximal shuttle running, representing 2 x 5 over spells of fast bowling.

Cooling interventions

Participants underwent one of three 20 min recovery interventions (CWI, MIX or CONT) within 10 min of exercise completion. CWI involved submersion to the mesosternale in 10.0 ± 0.4°C cold water (Halson et al., 2008; Pointon et al., 2012). MIX was performed using a cold, wet towel positioned over the head, neck and shoulders, with an ice-vest covering the torso (Arctic Heat, Brisbane, QLD, Australia) and ice-packs applied to the hamstrings and quadriceps (Techni Ice, Frankston, VIC, Australia) (Minett et al., 2012). The towel was soaked in cold water (5.0 ± 0.5°C) and the ice-vest and ice-packs were stored at -20°C before application. Participants received no cooling during the CONT and sat passively in 32°C and 42% relative humidity throughout all treatments.

Measures

Neuromuscular function

Maximal voluntary contraction (MVC) and central activation of the right knee extensors were assessed pre- and post-exercise, post-intervention, 1 h post-exercise and 24 h post-exercise using a custom-built isometric dynamometer. Participants sat in an upright position (trunk-thigh angle of 100°) on a modified leg extension bench (York Barbell Co., Toronto, ON, Canada), secured with an adjustable lap sash and padded ankle cuff superior to the lateral malleolus. A calibrated load cell (Model No. UU-K200; Dacell Co., Ltd., Cheongwon-gun, Chung-buk, Korea) was fixed between the moveable lever arm and the steel bench frame and connected to a BNC2100 terminal block and signal acquisition system (PXI1024; National Instruments, Austin, TX, USA). The MVC protocol involved 20 x 5 s isometric efforts completed using a work-to-rest ratio of 1:1. Force outputs were corrected for gravitation

effects and torque values quantified in relation to lever arm length during analyses using the methods of Cannon et al. (2008).

Muscle activation

Supramaximal stimulation of the femoral nerve was applied during contractions 1 – 4 and 17 – 20 of each MVC protocol. Reusable self-adhesive gel electrodes (90 x 50 mm; Verity Medical Ltd., Stockbridge, Hampshire, England) were positioned on the anterior thigh 3 cm below the inguinal fold and on the medio-posterior aspect of the upper thigh below the gluteal fold, serving as the cathode and anode respectively. The current applied to the femoral nerve was delivered by a Digitimer DS7 stimulator (Digitimer Ltd., Welwyn Garden City, Hertfordshire, England) using a single square-wave pulse with a width of 200 μs (400 V with a current of 100-450 mA) and customized LabVIEW software (version 8.0; National Instruments, North Ryde, NSW, Australia). Maximal peak twitch torque and M-wave amplitude were identified using incrementally increasing stimuli, and then increased by 10% to ensure supra-maximal stimulation. The central activation ratio (CAR) was calculated as the ratio between voluntary muscle torque and superimposed muscle torque as described by Kent-Braun and Le Blanc (1996).

Surface electromyography (EMG)

Knee extensor EMG was recorded during the MVC protocol, with differential surface electrodes (Bagnoli-16, Delsys Inc., Boston, MA, USA) positioned over the vastus medialis (VM) and vastus lateralis (VL) on the right thigh. EMG electrodes were positioned at the visual mid-point of the muscle belly and a self-adhesive reference electrode was attached to

the patella on the left leg. All placement sites were shaved, exfoliated and cleaned with an alcohol swab and electrodes and cables were taped to avoid any movement artifact. Further, a permanent marker was used to outline electrode placement to ensure consistency within and between testing sessions. Raw electrode output was pre-amplified and bandpass filtered, with a bandwidth frequency ranging from 20 to 450 Hz (common mode rejection ratio > 90dB; impedance input = 100MΩ; gain = 1000).

EMG amplitudes were determined via the root mean square (RMS) of the 300 ms following supramaximal stimulation and the MVC offset. Repolarisation of the superimposed M-wave and restoration of voluntary EMG signals were observed in all data sets by 300 ms post-stimulation, thus eliminating twitch artifact from contaminating the signal. Mean amplitude was quantified for both VM and VL; however, a mean value was expressed as an overall indication of neural drive to the knee extensors. EMG signals were normalized with respect to the RMS of M-wave amplitude and presented as percentage of the mean VM and VL M-wave amplitude. Processing of all neuromuscular data was performed using MATLAB software (R2010a; The MathWorks Inc., Natick, MA, USA).

Near-infrared spectroscopy (NIRS)

A continuous-wave NIRS instrument (Oxymon MKIII, Artinis Medical Systems B.V., Zetten, The Netherlands) was used to examine changes in oxygenated ($[O_2Hb]$), deoxygenated ($[HHb]$), and total ($[THb]$) cerebral haemoglobin concentrations throughout the MVC protocols. The NIRS probe was placed over the left prefrontal lobe, between Fp1 and F3 (international EEG 10-20 system) (Perrey, 2008) and adjusted by < 5 mm to optimize

signal strength (Billaut et al., 2010). Optode placement sites were cleaned with an alcohol swab, marked with an indelible pen and then photographed to standardize positioning within and between sessions. Inter-optode distance was set at 3.5 cm using a black, plastic spacer and was affixed to the skin with double-sided self-adhesive disks. A black, elastic headband was worn over the probe to further secure placement and minimize the effects of ambient light. Changes in $[O_2Hb]$ and $[HHb]$ concentrations were calculated using a modified Beer-Lambert law based on the absorption coefficient of continuous wavelength infra-red light (856 and 764 nm) and age-dependant differential path-length factors (range: 5.76 – 5.85) (Duncan et al., 1996; Billaut et al., 2010). The $[THb]$ was calculated as the sum of $[O_2Hb]$ and $[HHb]$ to provide an indicative measure of regional blood volume (Van Beekvelt et al., 2001). In addition, the tissue saturation index (TSI; quantified as the ratio between $[O_2Hb]$ and $[THb]$), which reflects the dynamic balance between $O_2$ supply and $O_2$ consumption and is independent of near-infrared photon pathlength in tissue, was calculated as an additional index of tissue oxygenation (Boushel et al., 2001). NIRS data were recorded at 10 Hz and averaged over the last second of each isometric contraction throughout the MVC protocol. These data were then normalized against a 120 s baseline value collected before the commencement of each session while subjects sat quietly on the isometric dynamometer with their eyes closed. The reliability of baseline TSI in the present study demonstrate the ICC as r= 0.86 – 0.88 and CV as 1.8 – 2.0%.

Venous blood collection and analyses

The effects of cooling interventions on muscle damage, inflammation and anabolic/catabolic responses were quantified from venous blood samples collected pre-, post-, 1 h post- and 24 h post-exercise. Samples were drawn from an antecubital vein using an evacuated venipuncture

assembly and serum separator tubes (BD Vacutainer, North Ryde, NSW, Australia), allowed to clot at room temperature before centrifugation (4000 rpm for 10 min at 4°C) and serum storage at -20°C. Creatine kinase (CK) was determined using an enzymatic method and bichromatic rate technique (CV= 2.8%), while C-reactive protein (CRP) was quantified according to the particle enhanced turbidimetric immunoassay methods (CV= 6.2%; Dimension Xpand spectrophotometer, Dade Behring, Atlanta, GA, USA). Testosterone and cortisol were assessed using a solid-phase, competitive chemiluminescent enzyme immunoassay (CV= 3.9% and 2.1% respectively; Immulite 2000, Diagnostic Products Corp., Los Angeles, CA, USA). Data are presented as circulating concentrations and no corrections were made for alterations in plasma volume.

Physiological measures

Hydration status was assessed on arrival to the laboratory via the provision of a mid-stream urine sample to measure urine specific gravity (USG) (PAL-10S; Atago Co. Ltd., Tokyo, Japan) and changes in pre- and post-exercise body mass recorded using calibrated scales (HW150 K; A&D, Adelaide, SA, Australia) as a measure of non-urinal fluid loss. Heart rate (HR) values were determined with a chest strap and wrist watch receiver (FS1; Polar Electro Oy, Kempele, Finland) and $T_c$ was monitored through a telemetric capsule (VitalSense; Mini Mitter, Bend, OR, USA) ingested 5 h pre-exercise. HR and $T_c$ were recorded at 5 and 10 min intervals respectively during exercise, and every 5 min throughout the intervention period. The reliability (ICC= 0.99) and validity (r= 0.98) of $T_c$ capsules have previously been reported as acceptable by Gant et al. (2006). Skin temperature ($T_{sk}$) was measured at four sites (sternum, mid-forearm, mid-quadriceps and medial calf) pre- and post-exercise, post-intervention, 1 h post- and 24 h post-exercise using an infra-red thermometer (ThermoScan

3000; Braun, Kronberg, Germany) as per the methods of Burnham et al. (2006) (ICC= 0.96; r= 0.92). A weighted-mean $T_{sk}$ was calculated using the Ramanathan (1964) formula and mean body temperature ($T_b$) was determined as described by Schmidt and Brück (1981).

Perceptual measures

Rating of perceived exertion (RPE; Borg CR-10 scale) was recorded every 5 min during the exercise protocol. Perceived thermal sensation (0= unbearably cold – 8= unbearably hot) was measured at 5 min intervals throughout exercise and intervention periods, whilst muscle soreness (0= normal – 10= extremely sore) was assessed pre-, post-, 1 h post- and 24 h post-session pre- and post-exercise, post-intervention, 1 h post- and 24 h post-exercise.

Statistical analyses

Data are presented as mean ± SD. A two-way (condition x time) repeated-measures analysis of variance (ANOVA) was performed to determine differences between cooling conditions (CWI vs. MIX vs. CONT). Unprotected pairwise comparisons (Protected Fisher's LSD) were applied to determine the source of significance, which was accepted when $P < 0.05$. Analysis was performed using the Statistical Package for Social Sciences (SPSS v 17.0, Chicago, IL, USA).

**Results**

Intermittent-sprint exercise performance

Shuttle-run efforts were standardized between sessions to match individual workloads, with a mean total distance covered during the exercise protocol of 4159 ± 270 m. Specifically, 3496 ± 240 m, 833 ± 99 m and 449 ± 43 m were accumulated at hard running, jogging and walking intensities, respectively. Mean 15 m sprint times were not significantly different between conditions during the exercise protocol (CWI 2.73 ± 0.08 s vs. MIX 2.74 ± 0.07 s vs. CONT 2.76 ± 0.12 s; $P$= 0.30 – 0.63).

MVC and CAR

Mean MVC and CAR were significantly reduced pre- to post-exercise in all conditions ($P$= 0.001 – 0.05; Figure 1), without differences between conditions ($P$= 0.52 – 0.98). However, CWI hastened the return of neuromuscular function towards pre-exercise values, with higher mean MVC evident post-intervention, 1 h post-exercise and 24 h post-exercise compared to CONT ($P$= 0.002 – 0.05). Further, mean MVC was greater 1 h post-exercise after CWI compared with MIX ($P$= 0.006). While mean MVC was significantly lower than pre-exercise values for the duration of the 24 h recovery period in MIX and CONT conditions ($P$= 0.001 – 0.01), no differences were evident compared to pre-exercise measures 24 h post-exercise in CWI trials ($P$= 0.41). Moreover, CAR was significantly higher post-intervention and 1 h post-exercise in CWI trials compared to CONT ($P$= 0.007 – 0.03), and 1 h post-exercise compared to MIX ($P$= 0.01), respectively. No significant differences were observed in CAR between conditions 24 h post-exercise ($P$= 0.09 – 0.53), although CAR remained significantly lower than pre-exercise measures ($P$= 0.002).

Voluntary EMG

Mean RMS of VM and VL were higher post-intervention after CWI compared to CONT ($P=$ 0.005; Figure 1C). Further, CWI demonstrated a greater mean RMS of VM and VL 1 h post-exercise than MIX ($P=$ 0.01) and CONT ($P=$ 0.003), respectively. No differences were evident in the change in mean RMS of VM and VL in any condition over time ($P=$ 0.13 – 1.00).

Potentiated M-wave properties

M-wave amplitude was reduced pre- to post-exercise in all trials ($P=$ 0.03 – 0.04; Table 1). The exercise protocol demonstrated no significant effects on M-wave latency or duration ($P=$ 0.07 – 0.91). However, M-wave amplitude was significantly higher with CWI post-intervention compared to CONT ($P=$ 0.01). No significant differences were evident in any remaining M-wave variables ($P=$ 0.08 – 0.98).

NIRS

Regional cerebral blood volume and oxygenation were significantly decreased in all trials pre- to post-exercise (↓ [O$_2$Hb], ↑ [HHb], ↓ [THb] and ↓ TSI; $P=$ 0.001 – 0.05; Figure 2). An exacerbated reduction in [O$_2$Hb] was evident post-intervention in both CWI and MIX compared to CONT ($P < 0.001$), albeit with greater reductions noted in CWI than MIX trials ($P=$ 0.02). CWI increased [HHb] ($P=$ 0.04) whilst lowering [THb] and TSI compared to CONT post-intervention ($P=$ 0.001 – 0.01). Despite a reduced [THb] after the MIX intervention ($P=$ 0.001), TSI remained unchanged compared to CONT ($P=$ 0.99), though was significantly higher than CWI ($P=$ 0.01). Nevertheless, suppressed [THb] indicates a reduced regional cerebral blood volume after CWI to be sustained 1 h post-exercise compared to

remaining conditions ($P= 0.03$). Similarly, [$O_2$Hb] and [HHb] were reduced with CWI compared to CONT ($P= 0.04 - 0.05$); however, TSI was not significantly altered between conditions 1 h post-exercise ($P= 0.08 - 0.91$). All variables had returned to pre-exercise values by 24 h post-exercise and no differences were present between conditions ($P= 0.35 - 0.95$).

Hydration, HR, $T_c$ and $T_{sk}$

No significant differences were observed in pre-exercise USG between conditions (CWI $1.015 \pm 0.006$ vs. MIX $1.015 \pm 0.008$ vs. CONT $1.014 \pm 0.007$; $P= 0.66 - 1.00$). Nude body mass was significantly reduced pre- to post-exercise (CWI $1.86 \pm 0.33$ kg vs. MIX $1.82 \pm 0.33$ kg vs. CONT $1.83 \pm 0.25$ kg; $P < 0.001$), with no differences between conditions ($P= 0.60 - 0.85$). No significant differences were demonstrated in HR, $T_c$, $T_{sk}$ and $T_b$ between conditions during exercise ($P= 0.09 - 0.98$; Figure 3). Increased HR, $T_c$ and $T_b$ were evident pre- to post-exercise ($P < 0.001$), despite no change in $T_{sk}$ over time in all conditions ($P= 0.33 - 0.79$). Both CWI and MIX cooling significantly reduced $T_c$ and $T_b$ during the intervention period compared to CONT ($P= 0.001 - 0.03$), with a lower $T_c$ and $T_b$ still evident 1 h post-exercise ($P= 0.001 - 0.009$). Cooling reduced $T_{sk}$ post-intervention ($P < 0.001$), with larger reductions apparent following CWI compared to MIX ($P < 0.001$). However, this lowered $T_{sk}$ had dissipated by 1 h post-exercise ($P= 0.41 - 0.67$), which was also reflected in the similar $T_b$ values observed between conditions ($P= 0.16$). Both MIX and CWI reduced HR after 15 min and 20 min of post-exercise cooling compared to CONT ($P= 0.03 - 0.04$).

Venous blood

No significant differences were observed between conditions in pre- and post-exercise concentrations of CK, CRP, testosterone or cortisol ($P$= 0.07 − 0.99; Table 2). However, cortisol responses were lower 1 h post-exercise in both MIX ($P$= 0.006) and CWI conditions ($P$= 0.003) compared to CONT. CWI blunted CK 24 h post-exercise ($P$= 0.047). CRP and testosterone responses were not significantly affected in recovery following either cooling intervention ($P$= 0.08 − 0.82). CK was elevated at all time-points in each trial following intermittent-sprint exercise ($P$= 0.001 − 0.01), though this was not reflected in any change in CRP compared to pre-exercise values ($P$= 0.07 − 0.92). Testosterone increased over time in CONT trials ($P$= 0.01 − 0.04), with no time effects apparent for MIX or CWI conditions ($P$= 0.08 − 0.99). Finally, cortisol concentrations were higher compared to pre-exercise values at all time points in CONT ($P$= 0.001 − 0.03), post-exercise and 1 h post-intervention in MIX ($P$= 0.003 − 0.02), and post-exercise only in CWI trials ($P$= 0.003).


Perceptual

Mean RPE was not significantly different between conditions (CWI 5.9 ± 1.4 vs. MIX 6.0 ± 1.6 vs. CONT 6.3 ± 1.5; $P$= 0.36 − 0.64); nor were differences in RPE detected at any time-point throughout the exercise protocol ($P$= 0.18 − 1.00; Figure 4A). While thermal sensation was reduced during the intervention period with CWI and MIX compared to CONT ($P <$ 0.001; Figure 4B), CWI remained lower than CONT 1 h post-exercise ($P$= 0.03). Perceived muscle soreness was increased after exercise ($P$= 0.001 − 0.003; Figure 4C), remaining elevated for the duration of the 24 h recovery period ($P$= 0.001 − 0.007). Further, CWI reduced perceived muscle soreness at all time points post-intervention compared to CONT

($P$= 0.001) and MIX 24 h post-exercise ($P$= 0.02). Lower muscle soreness was also reported with MIX compared to CONT 24 h post-exercise ($P$= 0.01).

**Discussion**

The present findings provide further insight into the benefits of CWI in improving acute recovery of neuromuscular contractile function after intermittent-sprint exercise in the heat. While both post-exercise cooling interventions reduced thermal strain (as evident via reductions in $T_c$, $T_{sk}$, $T_b$, HR and thermal sensation), only CWI hastened the recovery of MVC, central activation and motor unit recruitment (RMS). Novel to this investigation, we report the effects of post-exercise cooling on cerebral haemodynamics, demonstrating reduced [$O_2$Hb] and [THb] after both CWI and MIX interventions. Importantly, greater deoxygenation of the prefrontal cortex apparent with a lower TSI after CWI appeared disassociated with subsequent improvement in neuromuscular function. Thus, enhanced recovery of MVC is more likely attributable to the faster return of central activation achieved via larger acute reduction in $T_c$ post-intervention or the decreased muscle soreness and blunted CK response evident at 24 h. Although MIX may assist physiological, thermoregulatory and perceptual recovery following exercise in hot conditions, the greater physiological perturbations achieved with CWI likely accelerated the recovery of disruptions to neuromuscular function as indicated by maintenance of contractile force that were not observed in MIX or CONT.

Post-exercise cooling techniques in hot conditions aim to rapidly reduce elevated thermoregulatory and cardiovascular strain, alleviating impaired neuromuscular function and

facilitating the maintenance of subsequent exercise performance (Wilcock et al., 2006). That is, a return of voluntary force and activation has been shown upon reversal of high thermal strain after both passive heating/cooling (Morrison et al., 2004; Thomas et al., 2006) and exercise models (Pointon et al., 2012). The present findings indicate that intermittent-sprint exercise in the heat leads to prolonged reductions in MVC torque and CAR (Figure 1). However, the greater reduction in $T_b$ over the duration of the post-exercise intervention(CWI -2.18 ± 0.36°C·min$^{-1}$ vs. MIX -1.14 ± 0.42°C·min$^{-1}$ vs. CONT -0.31 ± 0.15°C·min$^{-1}$; $P <$ 0.001) might reflect the faster rate of neuromuscular recovery and return of voluntary force. Considering the increased RMS values (Figure 1C), the ergogenic benefits of CWI may owe to greater central activation and subsequent gain in skeletal muscle recruitment (Pointon et al., 2012). These findings corroborate previously reported benefits of CWI on MVC recovery in temperate conditions (Bailey et al., 2007; Vaile et al., 2008b; Ingram et al., 2009; Peiffer et al., 2009); further, demonstrating the influence of cooling modes on the recovery of voluntary force, particularly given negligible improvements in MVC were noted in MIX trials. Consequently, the recovery of neuromuscular function could be speculated to be influenced by the magnitude of cooling administered (i.e. reductions in $T_c$, $T_{sk}$ and $T_b$; Figure 3), with the superior conductance properties and larger surface area coverage of CWI (Casa et al., 2007) likely to explain the performance outcomes.

Cooling-induced changes to circulatory characteristics, such as altered central blood volume or regional blood flow, are thought to protect post-exercise contractile function (Wilcock et al., 2006; Leeder et al., 2012); though it is unknown how these mechanisms operate, particularly in light of the observed improvements in voluntary activation and CNS drive (Pointon et al., 2011). A reasonable postulate is that reduced thermal load and increased centralized blood volume achieved with post-exercise cooling might increase cerebral oxygen

availability and augment exercise capacity by maintaining neuronal activity (Rupp & Perrey, 2008; Billaut et al., 2010). However, we observed a post-intervention reduction in an index of cerebral blood volume ([THb]) in both cooling trials, though TSI was only reduced after CWI (Figure 2). Although decreased oxygenation of the prefrontal cortex has been linked with reduced voluntary force and power output during exercise in hypoxic conditions (Nybo & Rasmussen, 2007; Smith & Billaut, 2010), these detrimental effects to central drive may not always be replicated in hyperthermic conditions (Morrison et al., 2009). In fact, humans exhibit a large tolerance to changes in cerebral oxygenation in normoxic conditions, and are able to up-regulate neural drive (assessed via EMG measurements) and strenuous voluntary performance in the face of deoxygenation (Billaut et al., 2010). Underlying mechanisms to explain the divergent relationship between TSI and neuromuscular function observed here are speculative and require further investigation.

Traditionally, reduced thermal loads ($T_c$, and $T_{sk}$) and resultant peripheral vasoconstriction achieved using cold therapies have been suggested to increase central blood volume, and through an increased MAP and Q, enhance muscle blood flow to maintain performance during repeated exercise bouts (Vaile et al., 2008a). Provided the larger centralized blood volume achieved with CWI as suggested in the present study and elsewhere (Peiffer et al., 2009; Gregson et al., 2011; Vaile et al., 2011), it is noteworthy that no differences in HR were observed post-intervention between cooling trials (i.e. CWI vs. MIX) (Figure 3A). Given the improvements in MVC torque, CAR and RMS apparent only after CWI, these data may suggest the absence of cardiodynamic contributions to neuromuscular recovery after intermittent-sprint exercise in the heat. Interestingly, these events seemingly differ to the findings of Périard et al. (2012) who linked declining cerebral oxygenation with higher HR during hyperthermic exercise. Intuitively then, narrowing the $T_c - T_{sk}$ gradient via post-

exercise cooling should reduce peripheral blood flow required for heat loss, increasing MAP and Q ($\downarrow$ HR), and so cerebral perfusion, highlighted to influence aerobic performance under heat stress (Cheuvront et al., 2010). Regardless, our data indicates moderate reductions in cerebral oxygenation after post-exercise cooling in the heat as unconstraining of CNS drive, perhaps indicating the magnitude of cooling-induced reductions in $T_c$ and $T_{sk}$ as mediators of neuromuscular recovery and not lowered cerebral perfusion as related to cardiovascular strain.

Regardless of the centrally-mediated mechanisms discussed above, an alternative proposition pertaining to the benefits of post-exercise cooling relates to the blunted inflammatory responses often observed with such recovery techniques (Gregson et al., 2011). Interstitial protein and muscle enzyme release in addition to hormonal profiles may characterize exercise-induced muscle damage, inflammation and stress, indirectly highlighting the functional status of skeletal muscle (Halson et al., 2008; Pournot et al., 2011). Corroborating previous reports (Ingram et al., 2009; Pournot et al., 2011), CK efflux was attenuated with CWI 24 h post-exercise (Table 2). Despite this, post-exercise cooling demonstrated no effect on CRP at any time-point, though lower cortisol might reflect an ameliorated anti-inflammatory response 1 h post-exercise in MIX and CWI trials. Cooling-induced vasoconstriction is suggested to aid the maintenance of cellular integrity by decreasing circulatory and lymphatic permeability, easing interstitial fluid gain and blunting inflammatory events following exercise-induced muscle damage (Wilcock et al., 2006). Thus, while the efficacy of post-exercise cooling in improving biochemical perturbations incurred during exercise is contentious (Leeder et al., 2012), the maintenance of MVC torque in the CWI trial 24 h post-exercise, irrespective of similar CAR and RMS, may reflect sustained skeletal muscle structure, and so improved peripheral contractile force.

Further to the observed reduction in physiological strain, the present findings also highlight lowered subjective perceptions of soreness following post-exercise cooling (Figure 4). The analgesic effects of cooling are well documented (Leeder et al., 2012), potentially easing ratings of muscle soreness by mitigating acute tissue oedema and ensuing inflammatory responses to muscle damage (Bailey et al., 2007; Vaile et al., 2008b). Interestingly, CWI lowered reported muscle soreness at both acute (post-intervention and 1 h post-exercise) and delayed-onset time points (24 h post-exercise), though only 24 h post-exercise in the MIX trial. Considering the minimal changes observed in inflammatory markers (CRP) and the absence of any measurement of swelling, potential placebo effects of post-exercise cooling cannot be discounted (Leeder et al., 2012). However, when coupled with the greater reductions in thermal strain ($T_c$, $T_{sk}$ and thermal sensation) and the hydrostatic pressure associated with CWI (Wilcock et al., 2006), differences in muscle soreness between recovery methods might reflect the greater magnitude of cooling stimulus incurred. Still, if and/or how improved perceptual ratings of muscle soreness may influence subsequent neuromuscular performance is yet to be identified.

Finally, although these findings add novel insight into the effects of post-exercise cooling and neuromuscular recovery under heat stress, it is prudent that several limitations are acknowledged. Hyperthermic exercise reportedly reduces cerebral oxygenation (Nybo & Nielsen, 2001; Rasmussen et al., 2010; Périard et al., 2012) and is proposed as a contributing modulator of central fatigue (Nybo & Rasmussen, 2007). Our premise was that the removal of high thermal loads following exercise in the heat might alleviate these cerebrovascular perturbations and so hasten the recovery of CNS drive. Inherently, this implies the

improvement of cerebral blood flow characteristics otherwise compromised with hyperthermia (Nybo & Nielsen, 2001). The NIRS technique utilised here provides a non-invasive measure of cerebrovascular function, though underestimates cerebral blood flow values in comparison with direct (e.g. transcranial doppler) or anatomical measures (e.g. positron emission tomography) (Perrey, 2008). For this reason, we have presented changes in cerebral oxygenation in relation to baseline measures. Nevertheless, despite the high reliability of baseline values reported here, the absence of any direct blood flow assessment remains a limitation and warrants further inquiry. Future study would benefit from examining cerebrovascular and metabolic activity in the brain during recovery following post-exercise cooling in the heat as an index of neuronal activation.

In summary, these findings highlighted the physiological, cerebral haemodynamic and perceptual effects of post-exercise cooling and subsequent influence on neuromuscular recovery following intermittent-sprint exercise in the heat. Importantly, CWI hastened the recovery of voluntary force, increasing central activation and easing thermal strain. A novel finding is the reduction in cerebral oxygenation and improved CNS drive after CWI, suggesting the blood volume shift to be a reflex response to greater heat removal with post-exercise cooling. Thus, these changes in cerebral haemodynamics after post-exercise cooling do not appear to present a regulatory pathway for the recovery of MVC and CAR. Instead, corresponding differences in cooling magnitude between conditions (CWI > MIX > CONT) point to thermoregulatory and cardio-circulatory perturbations incurred during post-exercise cooling as reflective of the physiological and perceptual recovery achieved.

**Perspectives**

Post-exercise cooling is of benefit to athletic recovery following sustained self-paced intermittent-sprint exercise in hot conditions. Although field-based implementation can be problematic, the larger cooling stimulus of CWI appears most effective in alleviating heat strain, expression of muscle damage and perceptual soreness to maintain neuromuscular function. Mixed-method cooling strategies may still be relevant in applied settings, particularly where access to CWI techniques is limited or scenarios where the recovery of exercise performance is of lesser importance. However, practitioners should be aware of the influences of cooling magnitude on neuromuscular recovery and design cooling approaches to suit the individual constraints of each field-based scenario.

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**Table 1.** Mean ± SD poteniated M-wave properties of mean vastus medialis and vastus lateralis between conditions and time. * Significant difference compared to Control condition (P < 0.05). ^ Significant difference compared to pre-exercise (*P* < 0.05).

| | Pre-Ex | Post-Ex | Post-Int | 1 h Post-Ex | 24 h Post-Ex |
|---|---|---|---|---|---|
| *Latency (ms)* | | | | | |
| CONT | 5.1 ± 1.5 | 6.1 ± 2.1 | 4.7 ± 1.9 | 5.3 ± 2.1 | 5.3 ± 1.7 |
| MIX | 5.2 ± 2.2 | 6.1 ± 1.9 | 5.1 ± 2.4 | 5.2 ± 1.8 | 5.5 ± 1.8 |
| CWI | 5.1 ± 2.2 | 6.2 ± 2.0 | 5.2 ± 2.3 | 5.1 ± 2.3 | 5.4 ± 2.3 |
| *Duration (ms)* | | | | | |
| CONT | 7.1 ± 2.4 | 7.7 ± 2.2 | 7.6 ± 2.4 | 6.9 ± 2.5 | 7.6 ± 3.1 |
| MIX | 6.9 ± 2.6 | 7.8 ± 2.3 | 7.9 ± 2.7 | 6.8 ± 2.1 | 7.6 ± 2.4 |
| CWI | 7.2 ± 2.4 | 7.8 ± 2.3 | 9.7 ± 2.3 | 7.0 ± 1.9 | 7.5 ± 2.1 |
| *Amplitude (mV)* | | | | | |
| CONT | 7.5 ± 2.1 | 5.7 ± 1.5^ | 5.8 ± 2.1 | 7.3 ± 2.2 | 7.6 ± 2.3 |
| MIX | 7.7 ± 2.2 | 5.5 ± 1.5^ | 7.3 ± 2.5 | 7.3 ± 1.5 | 7.4 ± 0.9 |
| CWI | 7.5 ± 1.9 | 5.7 ± 0.8^ | 8.6 ± 1.8* | 7.8 ± 2.1 | 7.6 ± 1.7 |

**Table 2.** Mean ± SD biochemical data comparison between cooling modes. * Significant difference compared to Control condition ($P < 0.05$). ^ Significant difference compared to pre-exercise ($P < 0.05$).

| | Pre-Ex | Post-Ex | 1 h Post-Ex | 24 h Post-Ex |
|---|---|---|---|---|
| *Creatine Kinase ($U \cdot L^{-1}$)* | | | | |
| CONT | 218 ± 147 | 451 ± 270^ | 465 ± 271^ | 741 ± 504^ |
| MIX | 268 ± 220 | 498 ± 358^ | 567 ± 383^ | 656 ± 585^ |
| CWI | 240 ± 90 | 463 ± 203^ | 495 ± 220^ | 513 ± 235*^ |
| *C-Reactive Protein ($mg \cdot L^{-1}$)* | | | | |
| CONT | 1.41 ± 0.37 | 1.98 ± 1.42 | 1.89 ± 1.36 | 2.41 ± 1.44 |
| MIX | 1.49 ± 0.38 | 1.67 ± 0.34 | 1.71 ± 0.37 | 2.64 ± 1.94 |
| CWI | 1.63 ± 0.51 | 1.62 ± 0.36 | 1.79 ± 0.41 | 2.10 ± 0.93 |
| *Testosterone ($ng \cdot dL^{-1}$)* | | | | |
| CONT | 314 ± 81 | 412 ± 114^ | 390 ± 113^ | 392 ± 138^ |
| MIX | 316 ± 102 | 361 ± 88 | 311 ± 71 | 316 ± 89 |
| CWI | 319 ± 71 | 347 ± 49 | 319 ± 67 | 347 ± 72 |
| *Cortisol ($nmol \cdot L^{-1}$)* | | | | |
| CONT | 287 ± 109 | 500 ± 213^ | 658 ± 220^ | 419 ± 126^ |
| MIX | 262 ± 63 | 507 ± 184^ | 465 ± 241*^ | 317 ± 176 |
| CWI | 286 ± 78 | 500 ± 121^ | 348 ± 47* | 279 ± 107 |

**Figure 1.** A Mean ± SD peak torque, B mean ± SD central activation ratio, and C mean ± SD root mean square for combined mean vastus medialis and vastus lateralis for CWI, MIX and CONT trials. *Significant difference between CWI and CONT ($P < 0.05$). ^Significant difference between CWI and MIX ($P < 0.05$).
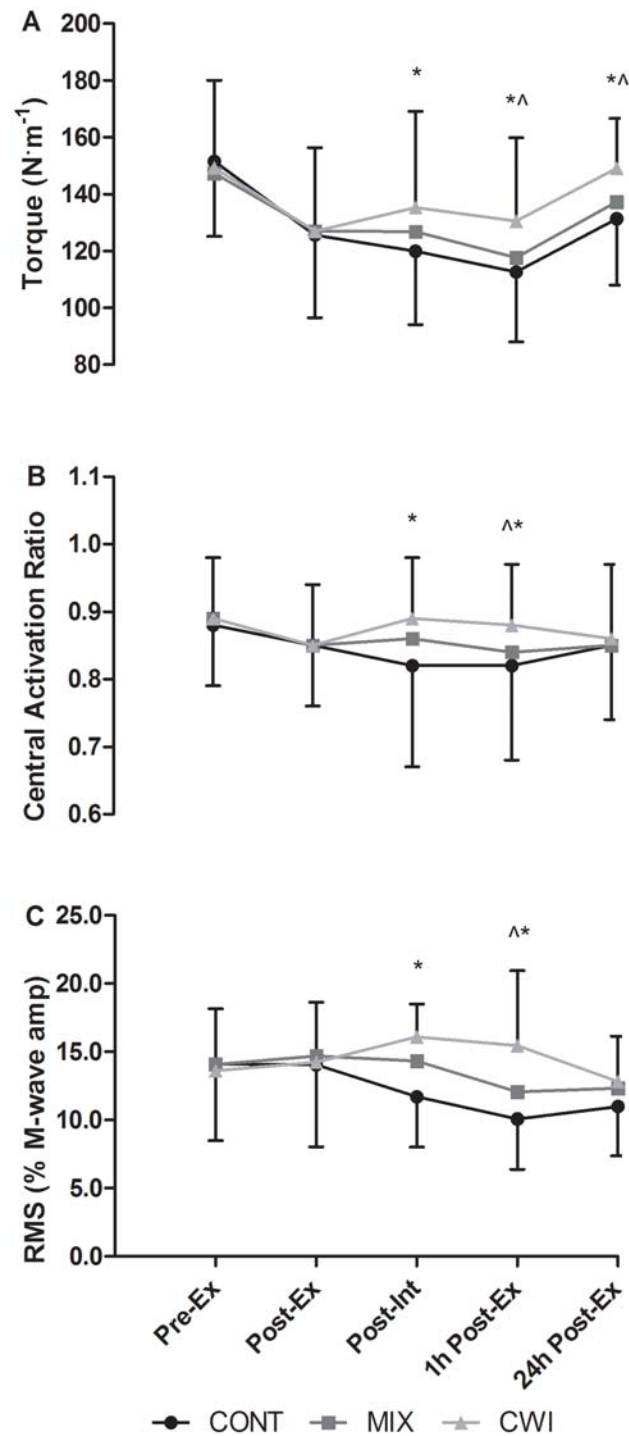
**Figure 2.** Changes in oxygenation of the prefrontal cortex between cooling modes. A Mean ± SD [$O_2Hb$], B mean ± SD [HHb], C mean ± SD [THb], and D mean ± SD TSI. *Significant difference between CWI and CONT ($P < 0.05$). ^Significant difference between CWI and MIX ($P < 0.05$). +Significant difference between MIX and CONT ($P < 0.05$).
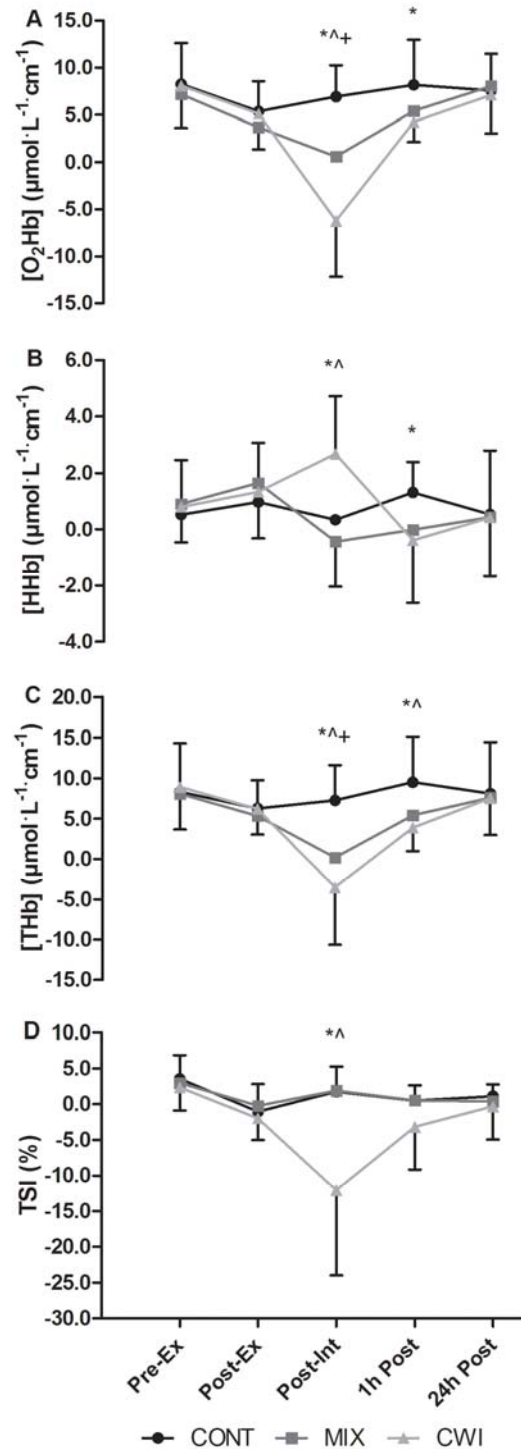
**Figure 3.** A Mean ± SD heart rate, B mean ± SD core temperature, C mean ± SD skin temperature and D mean ± SD body temperature for CWI, MIX and CONT trials. *Significant difference between CWI and CONT ($P < 0.05$). ^Significant difference between CWI and MIX ($P < 0.05$). +Significant difference between MIX and CONT ($P < 0.05$).
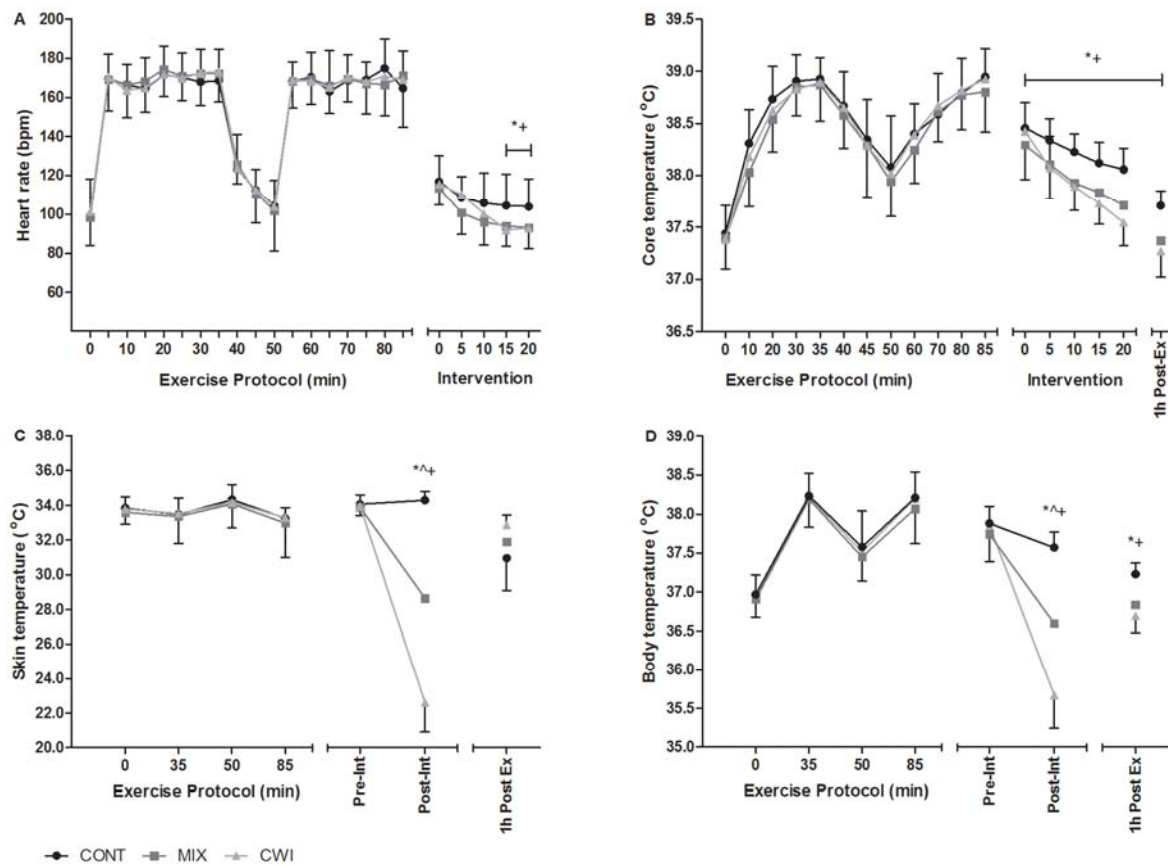
**Figure 4.** A Mean ± SD rating of perceived exertion, B mean ± SD thermal sensation, and C mean ± SD muscle soreness between cooling modes. *Significant difference between CWI and CONT ($P < 0.05$). ^Significant difference between CWI and MIX ($P < 0.05$). [+]Significant difference between MIX and CONT ($P < 0.05$).