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ORIGINAL ARTICLE

The effect of various cold-water immersion protocols on exercise-induced inflammatory response and functional recovery from high-intensity sprint exercise

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Abstract
Purpose The purpose of this study was to investigate the effects of different cold-water immersion (CWI) protocols on the inflammatory response to and functional recovery from high-intensity exercise.
Methods Eight healthy recreationally active males completed five trials of a high-intensity intermittent sprint protocol followed by a randomly assigned recovery condition: 1 of 4 CWI protocols (CWI-10 min × 20 °C, CWI-30 min × 20 °C, CWI-10 min × 10 °C, or CWI-30 min × 10 °C) versus passive rest. Circulating mediators of the inflammatory response were measured from EDTA plasma taken pre-exercise (baseline), immediately post-exercise, and at 2, 24, and 48 h post-exercise. Ratings of perceived soreness and impairment were noted on a 10-pt Likert scale, and squat jump and drop jump were performed at these time points.
Results IL-6, IL-8, and MPO increased significantly from baseline immediately post-exercise in all conditions. IL-6 remained elevated from baseline at 2 h in the CWI-30 min × 20 °C, CWI-10 min × 10 °C, and CWI-30 min × 10 °C conditions, while further increases were observed for IL-8 and MPO in the CWI-30 min × 20 °C and CWI-30 min × 10 °C conditions. Squat jump and drop jump height were significantly lower in all conditions immediately post-exercise and at 2 h. Drop jump remained below baseline at 24 and 48 h in the CON and CWI-10 min × 20 °C conditions only, while squat jump height returned to baseline in all conditions.
Conclusions Cold-water immersion appears to facilitate restoration of muscle performance in a stretch–shortening cycle, but not concentric power. These changes do not appear to be related to inflammatory modulation. CWI protocols of excessive duration may actually exacerbate the concentration of cytokines in circulation post-exercise; however, the origin of the circulating cytokines is not necessarily skeletal muscle.

Keywords Exercise-induced inflammation · Recovery · Cryotherapy · Cytokines · Skeletal muscle stress

Abbreviations

1 h	1 hour
2 h	2 hour
24 h	24 hour
48 h	48 hour
CWI	Cold-water immersion
GM-CSF	Granulocyte macrophage colony-stimulating factor
IFNγ	Interferon gamma
IL-1β	Interleukin 1 beta
IL-6	Interleukin 6
IL-8	Interleukin 8
IL-10	Interleukin 10
IL-12 p70	Interleukin 12 p70
MPO	Myeloperoxidase

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Pre	Pre-exercise
Post	Post-exercise
SSC	Stretch-shortening cycle
TNFα	Tumor necrosis factor alpha

Introduction

High-intensity exercise including eccentric activity, unaccustomed activity, and exercise of long duration and/or high intensity has been shown to induce an acute inflammatory response (Tee et al. 2007; Bleakley and Davison 2010; Leeder et al. 2012). In response to stress and/or muscle damage induced by exercise, muscle fibers and associated cells release signaling molecules (cytokines) into the circulation (Tidball and Villalta 2010; Paulsen et al. 2012; Suzuki et al. 2002; Peake et al. 2005b; Nieman et al. 2012), which subsequently influence the trafficking of inflammatory cells (Adams et al. 2011; Stacey 2010; Butterfield et al. 2006). This process initiates a positive feedback mechanism characteristic of the inflammatory response, resulting in further up-regulation of signaling molecules and activation/infiltration of inflammatory cells into muscle fibers that have been stressed or damaged during the exercise bout (Pedersen 2011). The inflammatory response is necessary for the resolution of any structural damage that may have occurred, and may be important for the adaptive response of skeletal muscle to exercise (Tidball and Villalta 2010; Pizza et al. 2002). However, if prolonged or excessive, the inflammatory response can cause secondary damage to surrounding cells and contribute to oxidative stress of the muscle fibers (Tidball and Villalta 2010; Carvalho et al. 2010; Puntel et al. 2011), thereby compounding the stress/damage the muscle fibers experience. For this reason, the inflammatory response has been implicated in delayed onset muscle soreness (DOMS), edema, reduced performance capacity, and fatigue commonly experienced following various forms of strenuous exercise (Smith 1991).

Cold-water immersion (CWI) is a popular form of cryotherapy that has been identified as a potentially effective anti-inflammatory intervention (Pournot et al. 2010; Clarke et al. 1958; Swenson et al. 1996; White and Wells 2013). CWI combines the compressive hydrostatic forces of water with low temperatures to reduce local metabolism and induce vasoconstriction (Thorsson et al. 1985; Wilcock et al. 2006). Following the stress of exercise, cold-induced hypometabolism reduces muscle fibers' O_2 usage (Yanagisawa et al. 2007; Yanagisawa and Fukubayashi 2010), thereby reducing metabolic stress and the release of signaling molecules from the tissue (Swenson et al. 1996). This in turn blunts the positive feedback, whereby these molecules attract and activate immune cells, which release more inflammatory mediators. As cold has been shown to induce vasoconstriction (Gregson et al. 2011; Yanagisawa et al. 2010; Thorsson et al. 1985), it may attenuate fluid accumulation at the site of stressed muscle fibers and inflammatory cell trafficking and signaling.

CWI is the most commonly used recovery modality (Barnett 2006) and is used by a wide variety of athletes to enhance recovery post-exercise; however, the protocols used in the field are largely based on the anecdotal experience not evidence-based research. Although many studies have investigated the cytokine response to exercise, few have validated the anti-inflammatory effects of CWI as the mechanism through which it may affect recovery from intense exercise in normothermic conditions (Wilcock et al. 2006). Further, while CWI is widely used by athletes, there continues to be ongoing debate in the literature regarding its efficacy as a post-exercise strategy to facilitate recovery from intense exercise. This is largely due to a high variability in methodology used to study CWI as a recovery modality. Therefore, the objectives of this study were threefold: (1) to measure the influence of CWI on exercise-induced inflammation and muscle performance; (2) to investigate inflammation as a mechanism for any recovery effect of CWI; and (3) to identify the most effective CWI protocol for restoring muscle function and/or attenuating exercise-induced inflammation. We hypothesize that CWI will result in lower circulating cytokine concentrations, which will be related to improved muscle performance and that these effects will be dose-dependent, such that the coldest condition (10 °C water for 30 min) will be associated with the largest effects.

Materials and methods

Eight recreationally active males (defined as 'participating in structured exercise 3–6 h per week', 23.6 ± 3.7 years, 180.8 ± 8.1 cm, 76.1 ± 8.6 kg, skin fold thickness 11.6 ± 3.5 mm, VO_{2peak} 50.7 ± 5.1 ml·kg^{-1} min^{-1}) volunteered to participate in the study. A power calculation conducted using IL-6 (average within subject minimum detectable difference of 1.72 ± 0.68 pg/ml) (Stacey 2010; Peake et al. 2005b; Halson et al. 2008), the most commonly studied exercise-induced cytokine, resulted in a minimum $n = 6$ to attain statistical power of 0.8 (G-Power Statistical Analysis, Germany). Participants were not taking any medications, did not report history of anti-inflammatory/antioxidant use, were free of cardiopulmonary and/or inflammatory conditions, and had no history of adverse reactions to cold, including chilblains, frostbite, Reynaud's phenomenon, or cold-induced urticaria. The study was approved by the University of Toronto Research Ethics Board and each participant provided written informed consent.

Experimental overview

In a randomized crossover design, participants completed a high-intensity intermittent sprint protocol followed immediately by one of five recovery conditions assigned in a random order over the 5 weeks of testing. Trial days were separated by 1 week. Moderate to high-intensity exercise (>12 on the Borg 20 point scale), alcohol, and caffeine were restricted 24 h prior to any blood collection. Nonsteroidal anti-inflammatory drugs, steroidal anti-inflammatory drugs, and antioxidant nutritional supplements were prohibited for the duration of the study. Drop jump and squat jump height, and ratings of perceived soreness and impairment were taken pre-exercise ('pre'), immediately post-exercise ('post'), and at 1, 2, 24, and 48 h post-exercise, while inflammatory markers were taken at these same time points excluding the 1 h time point. Anthropometric measures, VO_{2peak}, and familiarization with the exercise protocol, immersion in 10 °C water, and functional testing procedures was conducted 1 week prior to commencing the experimental protocol. All test procedures were completed at the University of Toronto Athletic Center. No food was consumed during the experimental trials. Water was allowed ad libitum (Fig. 1).

Exercise protocol

The exercise protocol was performed on a 200-m indoor track in the University of Toronto Athletic Center between the hours of 9 a.m. and 5 p.m. Participants attended sessions at the same time of day for each of the five trial weeks. 120 m was marked, and included one bend in the track, and two straight aways. Following pre-exercise blood sampling, participants completed a standardized dynamic warm-up consisting of 800-m of jogging (four laps of the track), a series of active stretches conducted on a 20-m sideline of the track and a 5-min free stretch. The sprint-protocol required the participants to complete 12 maximal sprints of 120 m, performed every 3 min. This exercise protocol was chosen to engage a large mass of muscle in high-force, repeated stretch–shortening contractions over a period of 20–25 s (sufficient to deplete phosphocreatine stores). A rest period of ~2.5 min was given to allow full replenishment of phosphocreatine stores to support maximal effort on subsequent bouts. This protocol was chosen to impose both mechanical and metabolic stress, as opposed to predominantly mechanical and damaging (eccentric overload) which would be expected to elicit a repeated-bout effect, and therefore not appropriate for a crossover design.

A research assistant stood at the starting point to collect pre-sprint heart rate (RS800CX, Polar, Finland). A second research assistant stood at the finish point to collect post-sprint heart rate acquired approximately 5 s following the completion of the sprint. Both research assistants recorded the time taken to complete the sprint and gave standardized verbal encouragement. The participant then walked back to the starting point and continued to walk along the side

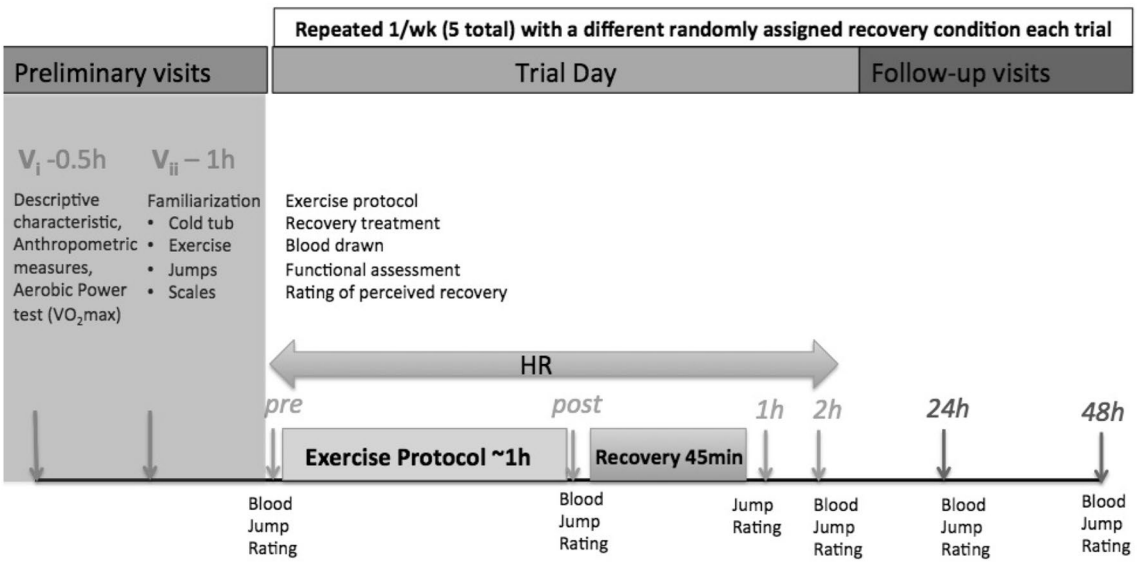

Fig. 1 Schematic representation of relative timing of experimental procedures. Muscle performance and ratings of perceived soreness and impairment were conducted pre-, immediately post-, and at 1, 2, 24, and 48 h post-exercise. Blood samples were taken at each time point excluding 1 h post-exercise. The exercise protocol, including warm-up and cool-down, was roughly 1 h in duration. Following a 15 min lapse in which participants returned to the laboratory, completed post-exercise testing, and changed into swim shorts, the recovery period commenced, lasting 45 min for each experimental condition (immersion time plus passive rest on a chair up to 45 min), such that the next time point was 1 h post-exercise (15 min lapse plus 45 min recovery period)

of the track until 3 min had elapsed since the beginning of the previous sprint. Upon completion of the 12 sprints repetitions, the participant completed a 400-m cool-down at a self-selected pace and returned to the laboratory for post-exercise performance and blood sampling, followed by the commencement of their randomly assigned recovery condition.

Recovery protocol

Following post-exercise blood sampling, ratings of perceived soreness and impairment, and changing into swim shorts (15 min following cool-down on the track), participants began a 45 min recovery intervention including the specified time immersed and the remainder of the 45 min spent in passive seated rest in a standard upright chair. Immersion protocols were 10 °C water for 10 min (CWI-10 min × 10 °C), 10 °C water for 30 min (CWI-30 min × 10 °C), 20 °C water for 10 min (CWI-10 min × 20 °C), 20 °C water for 30 min (CWI-30 min × 20 °C), or passive rest seated on a chair in the laboratory for 45 min (CON). Recovery protocols were assigned randomly by drawing a slip of paper with a letter corresponding to one of the five conditions from an envelope prior to each trial week. Participants were blinded to their condition by covering the settings on the automated regulator and withholding time information. The principal researchers were blinded to the conditions by having a third party code the conditions and set the temperature on the regulator. Immersion took place in the laboratory in a portable ice bath (iCool Sport, Australia), in which participants were immersed to the iliac crest and water was continually circulated by the ice bath's automated regulator. When the prescribed duration in the bath was completed in the immersion conditions, the remainder of time until the 2-h blood sampling point was spent quietly sitting on a chair in the laboratory.

Muscle function

Muscle function was assessed using a squat jump and drop jump performed on a force plate (Kistler, Switzerland) with interfacing software (Quattro Jump 1.08). Jump tests were conducted pre-exercise (baseline), immediately post-exercise, and at 1, 2, 24, and 48 h post-exercise. Force generation is a well-established indirect indicator of muscle damage (Paulsen et al. 2012; Warren et al. 1999). Squat jump was used as a measure of maximal voluntary concentric power, as it does not include pre-stretch activation (Byrne and Eston 2002). As such, it gives an indirect measure of muscle fiber activation, excitability, and functionality of contractile elements. Participants started in a standing position on the force plate with their hands on their hips. When the computer indicated initiation of recording by an auditory signal, the participant was instructed to squat down to 90°, pause for a second, and jump straight up maximally, landing on the force plate and returning to a standing position, where they remained until a second auditory signal from the computer indicated the completion of recording. Participants completed three squat jumps at each time point and the mean of the best two from each time point was used for analysis to eliminate the erroneous effect of a submaximal effort.

Drop jump was used as a measure of maximal voluntary power in a stretch–shortening cycle (SSC) with the eccentric phase emphasized. As this jump includes a SSC, the muscles derive force from pre-stretch activation including elastic forces of passive tissues associated with the neuromuscular system, stretch–reflex activation, and/or altered sarcomere length (Byrne and Eston 2002). Participants started in a standing position on the force plate with their hands on their hips. When the computer gave an auditory signal indicating initiation of recording, the participant was instructed to step on to the 40 cm box, turn, and step-off the box, landing on the force plate, and immediately performing a maximal vertical jump. Three drop jumps were performed at each time point and the mean of the best two jumps was used for analysis to eliminate the erroneous effect of a submaximal effort.

Participants were familiarized with these two jumps prior to commencing experimental trials to account for any learning that may affect jump height, and to ensure correct technique for the jumps. Scripted instructions were given by a research assistant to the participants for each jump, and participants were reminded to jump maximally for each jump.

Blood sampling

Two, 4.0-ml vacutainer tubes treated with 7.2 mg of EDTA (BD™ Vacutainer, USA) were collected from the antecubital vein while the participant lay supine on an examination table pre-, post-, 2, 24, and 48 h post-exercise. Baseline samples were taken 10 min prior to starting the exercise protocol (allowing time for functional testing and ratings of perceived soreness and impairment following blood sampling). Post-exercise samples were taken immediately following the cool-down protocol on the track, and later samples were taken 2, 24, and 48 h from the baseline sampling time. The tubes were spun in a centrifuge (Universal 320 R, Hettich, Germany) for 15 min at 4 °C at 1,560*g*, and the plasma was aliquotted into 3, 0.5 ml Eppendorf tubes and frozen at −80 °C until the time of assaying. The blood sample for a given time point was taken prior to the bout of muscle performance testing for that time point.

Inflammatory assays

Samples were analyzed for total plasma concentration using electrochemiluminescence-based solid phase sandwich immunoassays (Meso-Scale Discovery, Gaithersbury, MD). A 9-plex multiarray assay (Human, Ultrasensitive Pro-inflammatory 9-plex, MSD, USA) for interleukin (IL)-1, IL-2, IL-6, IL-8, IL-10, IL-12 p70, interferon gamma (IFNer), tumor necrosis factor alpha (TNFαN), and granulocyte macrophage colony-stimulating factor (GM-CSF) was used. A single-plex assay for myeloperoxidase (MPO) (Human, Ultrasensitive Myeloperoxidase, MSD, USA) was conducted. Samples and provided standards were analyzed in duplicate according to manufacturers' instructions. Inter-assay coefficients of variance (CV) ranged from 0.6 to 3.4 %. Minimal detectable concentrations for the analytes were 0.35 pg/ml for IL-2, 0.090 pg/ml for IL-8, 1.4 pg/ml for IL-12 p70, 0.36 pg/ml for IL-1β, 0.20 pg/ml for GM-CSF, 9.53 pg/ml for IFNγ, 0.27 pg/ml for IL-6, 0.21 pg/ml for IL-10, 0.50 pg/ml for TNFα, and for 33 pg/ml MPO.

Ratings of perceived soreness and perceived impairment

Ratings of perceived soreness and perceived impairment were recorded pre-, post-, 1, 2, 24, and 48 h post-exercise. Rating of perceived impairment was recorded on a 10-point Likert numerical rating scale where "1" was "Fully Functional" and "10" was "Debilitated". Rating of perceived soreness was recorded on a similar 10-point Likert scale where "1" was "No soreness" and "10" was "Agonizing". Perceived soreness was rated during an eccentric contraction (sitting into a chair) and a concentric contraction (standing out of the chair), with anatomical location of soreness indicated on a diagram.

Statistical analyses

Data in text and figures represent the mean ± SD with a 95 % confidence interval, expressed as change from baseline to account for inter-individual variation. A two-way (five sample times × five recovery condition) repeated-measures analysis of variance (ANOVA) was used to determine main and interaction effects for sample time and recovery condition for mean plasma cytokine concentration change from baseline (pg/ml) using SPSS™ version 21 (IBM, USA). A two-way (six sample times × five recovery conditions) repeated measures ANOVA was used to determine main and interaction effects for sample time and recovery condition for mean jump height change from baseline (cm) and mean rating on a 10-point Likert scale change from baseline using SPSS™ version 21 (IBM, USA). The assumption of sphericity was tested by Mauchley's W. In the case that sphericity was violated, the Greenhouse–Geisser correction was used. Tukey's least significant difference was used to post hoc significant main and interaction effects using GraphPad Prism software. Relationships between average plasma cytokine concentration changes from baseline, jump height, and perceptions of recovery were analyzed using Pearson's product correlations on SPSS™ version 21 (IBM, USA).

The standardized difference in means or effect size of changes between each experimental group and control for each post-intervention time point (1, 2, 24, and 48 h) was calculated using pooled standard deviation. Cohen's effect size statistics were used as threshold values for effects of a given experimental condition when compared with control at each of the above time points, such that a beneficial effect (greater than the smallest practically relevant change), unclear, or harmful effect was assessed qualitatively according to the magnitude-based inference model outlined by Hopkins et al. (2009). The inference was generated from the confidence limits derived from the *p* value associated with the effect and the magnitude of the difference in means such that a 0 % probability that the true value falls within the confidence limits corresponds with a qualitative inference of a "most unlikely" effect; 0.5 % "very unlikely"; 5 % "unlikely"; 25 % possibly; 75 % likely; 95 % likely; 99.5 % very likely; 100 % most likely. The effect was considered "unclear" if the confidence interval of the effect was found to be both beneficial and harmful (i.e. substantially positive/beneficial and negative/harmful effect). Experimental mean ± SD, control mean ± SD, difference in means ± 90 % CL and the associated qualitative inference are reported.

Results

All participants completed the intermittent sprint protocol on all five occasions. No significant differences between conditions were observed on measures of exertion during the exercise protocol or plasma cytokine concentrations at the pre-exercise time point. A significant main effect for time was observed in plasma IL-6, IL-8, and MPO concentration change from baseline. No other inflammatory markers showed significant changes from baseline at any time point.

Plasma IL-6 concentration change from baseline

A significant main effect for time was observed for plasma IL-6 ($F_{(1.67,6.65)} = 9.434$, $p < 0.05$). Plasma IL-6 was significantly greater than baseline post-exercise ($p < 0.05$) in all experimental conditions. At 2 h post-exercise plasma IL-6 concentration remained significantly elevated from baseline in the CWI-30 min × 20 °C (1.118 ± 0.247 pg/ml), the

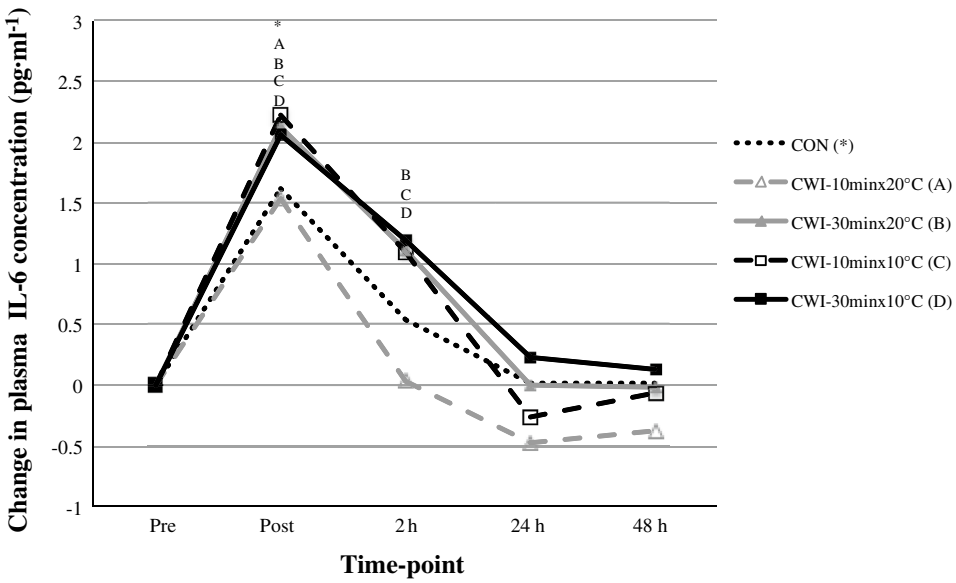

Fig. 2 Mean plasma IL-6 change from baseline (pre) following sprint exercise (post) and 1 of 4 different CWI protocols or passive rest (CON) at 2, 24, and 48 h post-exercise. Significant change in mean plasma concentration from baseline at a given time point is indicated by the *letter* corresponding to CON or 1 of 4 CWI conditions listed in the legend

CWI-10 min × 10 °C (1.091 ± 0.532 pg/ml), and the CWI-30 min × 10 °C conditions (1.191 ± 0.638 pg/ml). Plasma IL-6 concentrations returned to baseline in all conditions at 24 and 48 h post-exercise (Fig. 2).

Plasma IL-8 concentration change from baseline

A significant main effect for time ($F_{(4,16)} = 13.635$, $p < 0.01$) and interaction effect for time and recovery condition ($F_{(16,64)} = 2.079$, $p < 0.05$) were observed for plasma IL-8 concentration change from baseline. Plasma IL-8 was significantly greater than baseline post-exercise ($p < 0.01$) in all experimental conditions, except CWI-10 min × 20 °C. At 2 h post-exercise, plasma IL-8 concentration remained significantly elevated from baseline following CWI-30 min × 20 °C (1.350 ± 0.701 pg/ml) and CWI-30 min × 10 °C (2.569 ± 0.841 pg/ml). Further CWI-10 min × 10 °C was associated with significantly lower plasma IL-8 (0.127 ± 0.340 pg/ml) compared with CWI 30 min × 20 °C ($p < 0.05$) and CWI 30 min × 10 °C ($p < 0.05$) at the 2 h time point. Plasma IL-8 concentrations returned to baseline in all conditions at 24 and 48 h post-exercise (Fig. 3).

Plasma MPO concentration change from baseline

A significant main effect for time ($F_{(4,16)} = 11.744$, $p < 0.01$) and interaction effect for time and recovery condition ($F_{(16,64)} = 1.915$, $p < 0.05$) were observed for plasma MPO concentration change from baseline. Plasma MPO was significantly greater than baseline post-exercise ($p < 0.05$) in all conditions and continued to increase, peaking at 2 h post-exercise following CWI-30 min × 20 °C (1.492 ± 0.619 pg/ml, $p < 0.01$) and CWI-30 min × 10 °C (2.028 ± 0.709 pg/ml, $p < 0.01$). CWI-30 min × 10 °C was associated with significantly higher plasma MPO when compared with CWI-10 min × 10 °C (0.395 ± 0.121 pg/ml, $p < 0.05$) and CON (0.557 ± 0.235 pg/ml, $p < 0.05$) at 2 h post-exercise. Plasma MPO concentrations returned to baseline in all conditions at 24 and 48 h post-exercise (Fig. 4).

Squat jump

A significant main effect for time was observed ($F_{(5,20)} = 49.434$, $p < 0.01$) for squat jump height change from baseline. Squat jump height was significantly lower than pre-exercise at all time points in all conditions, with the greatest decrement in jump height observed at 1 h post-exercise in all conditions. Although squat jump height was below pre-exercise values in all of the conditions and 24 and 48 h post-exercise, it was significantly below pre-exercise values at 48 h in CWI-10 min × 20 °C (−3.49 ± 2.3 cm) and CWI-30 min × 10 °C (−2.10 ± 2.4 cm). No CWI condition was more effective than passive rest for recovery of squat jump performance at any time point (Fig. 5).

Magnitude-based inferences were used to make qualitative deductions about the effect of the respective CWI protocols on recovery of squat jump height when compared with control according to Hopkins et al. (2009). A likely to very likely effect was found for the CWI protocols to induce a negative effect on squat jump height at 1 h post-exercise, with the most substantially negative effects observed for CWI-10 min × 10 °C and CWI-30 min × 10 °C. CWI-30 min × 10 °C was associated with substantially negative effects on squat jump height at 2 h post-exercise, while the other conditions were associated with unclear effects. No clear inferences could be made on

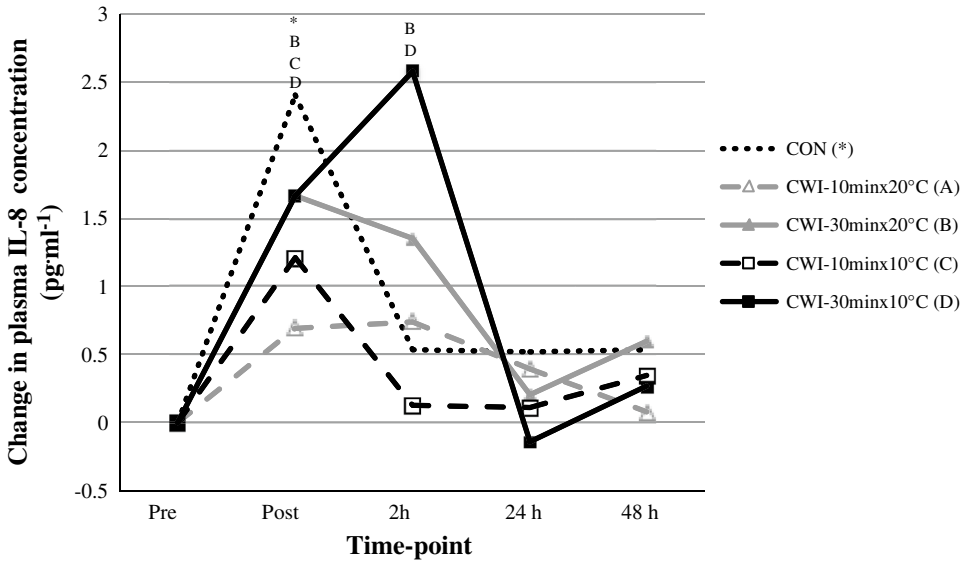

Fig. 3 Mean plasma IL-8 change from baseline (pre) following sprint exercise (post) and 1 of 4 different CWI protocols or passive rest (CON) at 2, 24, and 48 h post-exercise. Significant change in mean plasma concentration from baseline at a given time point is indicated by the *letter* corresponding to CON or 1 of 4 CWI conditions listed in the legend

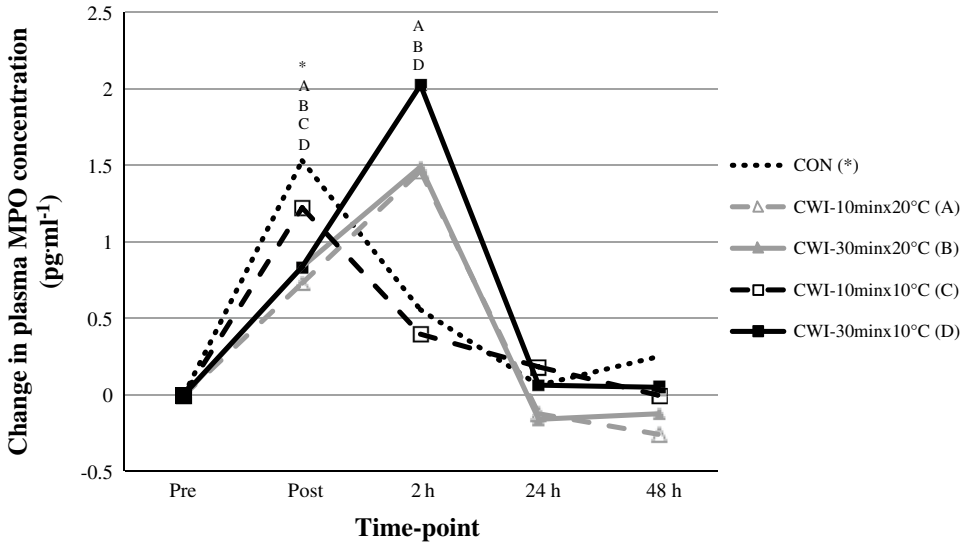

Fig. 4 Mean plasma MPO change from baseline (pre) following sprint exercise (post) and 1 of 4 different CWI protocols or passive rest (CON) at 2, 24, and 48 h post-exercise. Significant change in mean plasma concentration from baseline at a given time point is indicated by the *letter* corresponding to CON or 1 of 4 CWI conditions listed in the legend

the effects of any CWI condition at 24 and 48 h post-exercise. Inferences of effects for the respective CWI conditions when compared with CON at each post-cooling time point are reported in Table 1.

Drop jump

A significant main effect for time was observed ($F_{(5,20)} = 29.366$, $p < 0.01$) for drop jump height change from baseline. Drop jump height was significantly lower than pre-exercise immediately post-exercise, and at 1 and 2 h post-exercise in all conditions. Drop jump height remained below pre-exercise values at 24 h in CON (-4.91 ± 3.0 cm, $p < 0.05$), CWI-10 min × 20 °C (-2.78 ± 2.9 cm, $p < 0.05$), and CWI-30 min × 20 °C (-2.47 ± 0.9 cm, $p < 0.05$). At 48 h post-exercise, drop jump height remained below pre-exercise values in all conditions except CWI-10 min × 10 °C (1.96 ± 3.3 cm, $p < 0.05$), which was associated with significantly greater jump height than CON (-4.63 ± 1.6 cm, $p < 0.01$). CWI-10 min × 10 °C was the only condition associated with mean jump height values greater than baseline at any time point, with positive change in mean jump height observed at 24 and 48 h post-exercise (Fig. 6).

Magnitude-based inferences were used to make qualitative deductions about the effect of the respective CWI protocols on recovery of drop jump height when compared with control according to Hopkins et al. (2009). A likely beneficial effect was found for CWI-10 min × 10 °C, a possible beneficial effect was found or CWI-10 min × 20 °C, and unclear effects were found for the 30 min CWI protocols, although no harmful effect is expected for

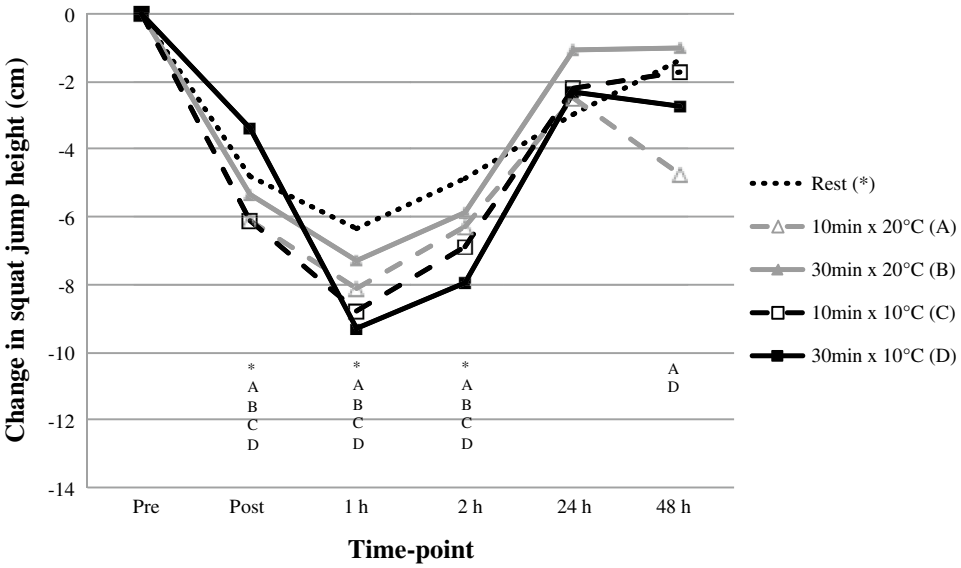

Fig. 5 Mean squat jump height change from baseline (pre) following sprint exercise (post) and 1 of 4 different CWI protocols or passive rest (CON) at 1, 2, 24, and 48 h post-exercise. Significant change in jump height from baseline at a given time point is indicated by the *letter* corresponding to CON or 1 of 4 CWI conditions listed in the legend

Table 1 Mean change in squat jump height change from baseline for each CWI condition and control with qualitative inferences for the effect of each condition on squat jump recovery following sprint-based exercise

Time point	Control mean (cm) ± SD	Experimental condition	Experimental mean (cm) ± SD	Mean difference (cm)	±90 % CL	Qualitative inference
1 h	CON −6.2 ± 1.95	10 min × 20 °C	−6.2 ± 4.8	2.1	±3.6	Unclear (73 % likely to harm)
		30 min × 20 °C	−7.4 ± 2.9	2.0	±3.6	Unclear (76 % likely to harm)
		10 min × 10 °C	−8.9 ± 3.3	3.5	±3.6	Likely harmful
		30 min × 10 °C	−10.7 ± 2.8	4.5	±3.6	Very likely harmful
2 h	CON −4.71 ± 1.58	10 min × 20 °C	−5.2 ± 3.2	2.6	±3.2	Unclear (likely harmful, unlikely beneficial)
		30 min × 20 °C	−4.7 ± 3.4	0.8	±3.2	Unclear
		10 min × 10 °C	−5.7 ± 3.9	1.9	±3.2	Unclear
		30 min × 10 °C	−7.4 ± 1.7	3.4	±3.2	Likely harmful, very unlikely beneficial
24 h	CON −2.36 ± 0.37	10 min × 20 °C	−1.0 ± 2.1	−0.1	±2.4	Unclear
		30 min × 20 °C	−0.1 ± 2.8	−1.8	±2.4	Unclear
		10 min × 10 °C	−0.7 ± 1.8	−1.5	±2.4	Unclear
		30 min × 10 °C	−1.7 ± 1.8	−1.1	±2.4	Unclear
48 h	CON −1.37 ± 2.59	10 min × 20 °C	−2.3 ± 4.3	2.1	±3.7	Unclear
		30 min × 20 °C	−0.2 ± 2.7	−1.1	±3.7	Unclear
		10 min × 10 °C	−0.2 ± 5.6	−0.8	±3.7	Unclear
		30 min × 10 °C	−2.3 ± 3.3	1.0	±3.7	Unclear

90 %CL, add/subtract this value to the mean effect for the 90 % confidence limits of the population effect positive mean difference values reflect a smaller negative effect on jump height in the control condition negative mean difference values reflect a smaller negative effect on jump height in the experimental condition

CWI-30 min × 10 °C. CWI-30 min × 10 °C was associated with substantially negative effects on squat jump height at 2 h post-exercise, while the other conditions were associated with unclear effects. Beneficial effects were associated with all CWI conditions at 24 h post-exercise with very likely beneficial effects associated with both 10 °C conditions. Unclear effects of CWI were observed at 48 h post-exercise, except for CWI-10 min × 10 °C, which was associated with a very likely beneficial effect. Inferences of effects for the respective CWI conditions compared

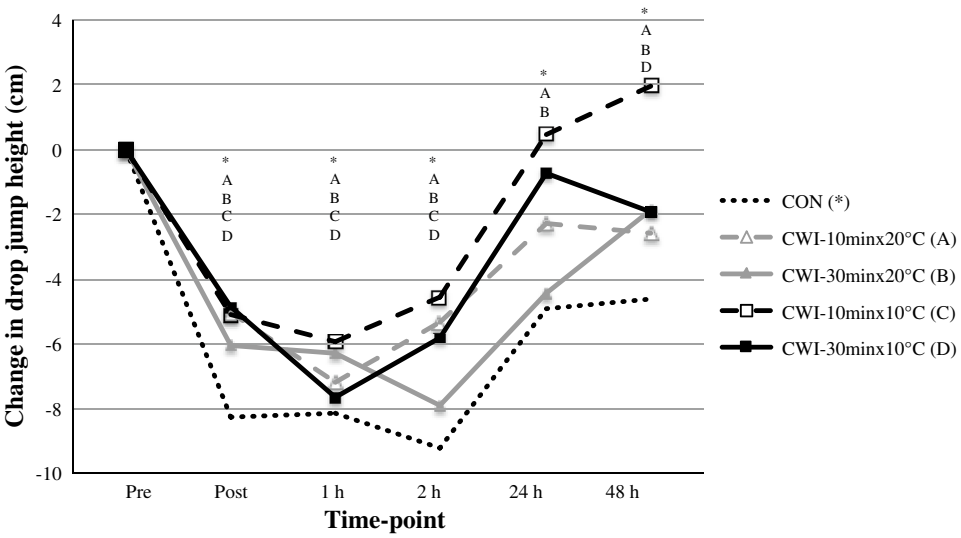

Fig. 6 Mean change from pre-exercise (baseline) performance for drop jump height. Significant change in jump height from baseline at a given time point is indicated by the letter corresponding to CON or 1 of 4 CWI conditions listed in the legend

Table 2 Mean change in drop jump height change from baseline for each CWI condition and control with qualitative inferences for the effect of each condition on drop jump recovery following sprint-based exercise

Time point	Control mean (cm) ± SD	Experimental condition	Mean (cm) ± SD	Mean difference (cm)	±90 % CL	Inference
1 h	CON −6.28 ± 6.81	10 min × 20 °C	−6.4 ± 3.6	0.2	±4.6	Unclear
		30 min × 20 °C	−7.4 ± 5.9	0.2	±4.6	Unclear
		10 min × 10 °C	−6.6 ± 7.1	0.4	±4.6	Unclear
		30 min × 10 °C	−8.8 ± 2.4	1.8	±4.6	Unclear (possible harmful)
2 h	CON −7.38 ± 3.43	10 min × 20 °C	−4.6 ± 3.4	−2.1	±4.4	Unclear (possibly beneficial)
		30 min × 20 °C	−7.5 ± 7.5	−0.9	±4.4	Unclear
		10 min × 10 °C	−2.5 ± 5.2	−3.9	±4.4	Likely beneficial, unlikely harmful
		30 min × 10 °C	−5.9 ± 3.0	−1.7	±4.4	Unclear (unlikely harmful)
24 h	CON −3.77 ± 1.45	10 min × 20 °C	−1.8 ± 2.8	−8.8	±8.9	Likely beneficial, very unlikely harmful
		30 min × 20 °C	−3.6 ± 7.6	−8.8	±8.9	Likely beneficial, very unlikely harmful
		10 min × 10 °C	+2.3 ± 5.9	−13.2	±8.9	Very likely beneficial, very unlikely harmful
		30 min × 10 °C	−0.6 ± 2.8	−10.8	±8.9	Very likely beneficial, very unlikely harmful
48 h	CON −4.39 ± 2.26	10 min × 20 °C	−2.3 ± 1.4	−1.8	±4.6	Unclear
		30 min × 20 °C	−2.1 ± 4.8	−2.3	±4.6	Unclear
		10 min × 10 °C	+2.0 ± 6.4	−6.7	±4.6	Very likely beneficial, most unlikely harmful
		30 min × 10 °C	−2.2 ± 3.9	−2.7	±4.6	Unclear (80 % likely to be beneficial)

90 % CL, add/subtract this value to the mean effect for the 90 % confidence limits of the population effect positive mean difference values reflect a smaller negative effect on jump height in the control condition, negative mean difference values reflect a smaller negative effect on jump height in the experimental condition

with CON at each post-cooling time point are reported in Tables 2, 3.

Ratings of perceived soreness and perceived impairment

Muscle soreness was rated lowering into a chair (eccentric) and standing (concentric) out of a chair. A significant main effect for time was observed for rating of concentric soreness ($F_{(4,16)} = 11.860$, $p < 0.01$), eccentric soreness ($F_{(4,16)} = 13.709$, $p < 0.01$), and perceived impairment ($F_{(4,10)} = 13.536$, $p < 0.01$).

Concentric soreness

Rating of perceived concentric soreness peaked post-exercise (2.4 ± 0.4 points, $p < 0.01$) in all recovery conditions

Table 3 Change from baseline of mean ratings of perceived soreness and perceived impairment following sprint exercise and 1 of 4 CWI protocols or passive seated rest (CON)

	Recovery condition				
	CON	CWI-10 min × 20 °C	CWI-30 min × 20 °C	CWI-10 min × 10 °C	CWI-30 min × 10 °C
Concentric soreness					
Baseline	0	0	0	0	0
Post-ex	2.1 ± 1.0	3.1 ± 1.1	3.0 ± 1.0	1.9 ± 0.5	2.4 ± 0.6
2 h	1.4 ± 0.8	2.1 ± 0.6	1.6 ± 0.5	1.6 ± 0.5	1.1 ± 0.6
24 h	1.4 ± 0.4	2.1 ± 0.6	1.9 ± 0.6	1.4 ± 0.5	1.6 ± 0.7
48 h	0.7 ± 0.3	2.7 ± 0.9	0.6 ± 0.4	0.9 ± 0.4	0.9 ± 0.4
Eccentric soreness					
Baseline	0	0	0	0	0
Post-ex	1.4 ± 0.7	2.2 ± 0.9	2.0 ± 0.5	1.7 ± 0.5	1.7 ± 0.6
2 h	1.2 ± 0.8	1.4 ± 0.5	0.8 ± 0.4	1.2 ± 0.5	1.0 ± 0.4
24 h	0.9 ± 0.3	1.4 ± 0.5	1.4 ± 0.4	1.2 ± 0.3	1.3 ± 0.6
48 h	1.2 ± 0.8	3.0 ± 2.7	0.8 ± 1.5	1.2 ± 1.3	1.0 ± 1.6
Perceived impairment					
Baseline	0	0	0	0	0
Post-ex	3.3 ± 0.9	2.8 ± 1.1	3.6 ± 1.0	2.9 ± 0.8	3.4 ± 0.9
2 h	1.7 ± 0.6	2.5 ± 0.8	1.3 ± 0.6	2.1 ± 0.6	2.1 ± 0.9
24 h	1.4 ± 0.4	2.2 ± 0.8	1.5 ± 0.4	1.6 ± 0.6	2.2 ± 0.7
48 h	0.9 ± 0.5	2.6 ± 1.0	0.9 ± 0.5	1.3 ± 0.5	0.9 ± 0.5

Values are mean change from baseline on a 10-point Likert scale ± SD

and remained above baseline at 24 h (1.6 ± 0.6 points, $p < 0.05$). At 48 h rating of concentric soreness returned to baseline (0.8 ± 0.4 points). No differences were observed between conditions.

Eccentric soreness

Rating of perceived eccentric soreness peaked post-exercise (1.9 ± 0.3 points, $p < 0.05$) in all recovery conditions and remained above baseline at 24 h (1.2 ± 0.4 points, $p < 0.05$). At 48 h rating of eccentric soreness returned to baseline (0.9 ± 0.4 points). No differences were observed between conditions.

Perceived impairment

Rating of perceived impairment peaked post-exercise (4.4 ± 0.3 points, $p < 0.05$) in all recovery conditions and remained above baseline at 24 (2.8 ± 0.6 points, $p < 0.05$) and 48 h (1.4 ± 0.9 points). No differences were observed between conditions.

Discussion

CWI is used widely by athletes of all types, however, the protocols used are generally anecdotal and vary widely with regards to duration and temperature. The present study is the first to systematically compare different protocols of CWI for their purported anti-inflammatory and subsequent muscle performance recovery effects following intense exercise (Peterson and Pizza 2008). The main findings of this study were that CWI following high-intensity intermittent sprint exercise does not significantly reduce plasma markers of inflammation or perceptions of soreness and impairment. Further, CWI in both cold (10 °C) and cool (20 °C) temperatures for 30 min can exacerbate the response of inflammatory markers in the blood following exercise. However, CWI-10 min × 10 °C appears to be the most effective for restoring force production in a SSC. The inflammatory response to intermittent sprint exercise returned to baseline by 24 h, while perceptions of soreness and impairment returned to baseline by 48 h and muscle performance remained below baseline at 48 h.

The effect of CWI as a recovery modality remains controversial, and although it is thought to function by blunting the exercise-induced inflammatory response, this mechanism is not well supported in the literature (Smith, 1991). CWI has been shown to reduce intramuscular temperature (Peiffer et al. 2009; Costello et al. 2012; Rupp 2012), subsequently reducing muscle perfusion (Gregson et al. 2011; Yanagisawa et al. 2007) and O_2 usage (Yanagisawa et al. 2007). Reduced blood flow to the muscle is thought to attenuate the potential for muscle fiber edema,

while reducing the metabolic rate of stressed muscle fibers is thought to reduce stress signaling and the ensuing inflammatory response to intense exercise (Pournot et al. 2010). Decrements in muscle force generating capacity and increased soreness experienced following intense exercise are commonly attributed to edema and inflammation inducing secondary damage to muscle fibers and other structures of the musculoskeletal system (Wilcock et al. 2006). Hence, by reducing muscle perfusion and oxygen demand of muscle following exercise, CWI is thought to attenuate decrements in muscle performance and increased soreness by reducing edema and inflammatory signaling.

Recovery of muscle performance

CWI is used by athletes exposed to varying physical demands. The efficacy of CWI is equivocal, likely due to high variability in the exercise stress and CWI protocols studied. Our findings suggest that CWI may be effective for restoring muscle performance in a stretch–shortening movement in the days following exercise, but does not significantly effect the restoration of muscle performance in a purely concentric movement, nor is it appropriate for short-term (<2 h) restoration of force generation. Squat jump performance was negatively affected by CWI at 1 and 2 h post-exercise when compared with CON. This is likely due to reduced intramuscular temperature following the four CWI protocols, slowing motor and sensory neural conductance velocity (Herrera et al. 2010) and contraction kinetics (Bergh and Ekblom 1979). Although intramuscular temperature was not measured in the current study previous studies have shown significant reductions in intramuscular and subcutaneous temperature with similar temperatures and durations as the current study (Yanagisawa et al. 2007). No CWI was more effective than CON for squat jump performance at 24 or 48 h. Pointon et al. (2011) found voluntary concentric force to be impaired 11 % at 24 h following two repetitions of CWI for 9 min at 9 °C after intermittent sprint exercise in the heat (Pointon et al. 2011). Similarly, Bailey et al. (2007) found no effect of CWI for 10 min at 10 °C for squat jump performance recovery 24 and 48 h following shuttle running, despite improvements in maximum voluntary contraction force when compared with passive rest (Bailey et al. 2007). Thus, although squat jump is a valid measure of voluntary muscle activation, it may not be sensitive enough to detect subtle changes in specific force generation of muscle. This may also raise the issue of internal versus external validity of muscle performance tests as those with greater internal validity (i.e., MVC) may not translate to more applied muscle performance tests (i.e., squat jump). Magnitude-based inferences indicate that CWI is likely to harm squat jump performance at 1 and 2 h post-exercise, and should thus be used with caution for "pre-cooling" or between bout techniques prior to activities requiring concentric power. Recommendations for the use of CWI for recovery on concentric power 24–48 h post-exercise are unclear.

In contrast to the absence of effect of CWI on squat jump performance, drop jump height change from baseline was significantly smaller at 2, 24, and 48 h following CWI-10 min × 10 °C when compared with CON, as well as following CWI-30 min × 10 °C when compared with CON at 24 h. CWI-10 min × 10 °C was the only condition associated with positive change from baseline at 24 and 48 h. The major difference in force production between squat jump and drop jump is the derivation of force from pre-stretch activation in drop jump, as it contains a SSC. Pre-stretch activation contributes force to a movement by a combination of utilizing elastic energy of structures of the musculature, activating proprioceptive reflexes, increasing the time to produce force and/or by potentiating sarcomere length (Ettema 2001; Horita et al. 1996). The discrepancy observed between squat jump and drop jump recovery suggest that following sprint exercise, performance reduction may be a result of neuromuscular impairment rather than structural disruption due to the inflammatory process.

It has been previously shown that concentric and stretch–shortening movements follow different recovery time courses owing to impairment in stretch reflex activation (Nicol et al. 1996). Activation of types III and IV muscle afferents may reduce the facilitation of the stretch reflex and its subsequent contribution to force production. Because CWI has been shown to alter neural conductance velocity (Herrera et al. 2010), it is possible that it may be preserving stretch–reflex sensitivity in the days following high-intensity sprint exercise. Previous studies have also shown CWI to be effective at facilitating force production in a stretch–shortening movement (i.e. counter-movement jump) (Pournot et al. 2010), which may be of particular importance for athletes requiring recovery of muscle's ability to produce force in change of direction movements. Our results suggest that CWI for 10 min at 10 °C was the most effective for promoting recovery of force generation in this type of movement. Magnitude-based inferences indicate that CWI-10 min × 10 °C is likely beneficial for recovery of power production in a SSC 2 h post-exercise. The effects of all CWI protocols are likely to be beneficial on recovery of drop jump performance 24 h post-exercise compared with control. 10 °C conditions for 10 or 30 min are very likely to have a beneficial effect on drop jump performance and are very unlikely to have a harmful effect. CWI-10 min × 10 °C is very likely to also have a beneficial effect at 48 h post-exercise when compared with passive rest and, is thus, recommended for the use

for recovery of stretch–shortening force production 24 and 48 h post-exercise.

Effect on post-exercise inflammation

IL-6 is classically up-regulated in skeletal muscle during exercise (Peake et al. 2005a; Nieman et al. 2012; Bruunsgaard et al. 1997; Suzuki et al. 2002), and may also be produced and released by other cells, including leukocytes, fibroblasts, and endothelial cells (Tidball and Villalta 2010; Pedersen et al. 1998). Although several studies have investigated post-exercise IL-6 responses, few have studied the effects of CWI following exercise in normothermic conditions. Nemet et al. (2009) found an increase in plasma IL-6 following 250 m intermittent sprints, and no effect of local ice application to the hamstring muscles, despite seeing reduced circulation of other cytokines and hormones (Nemet et al. 2009). In the present study, plasma IL-6 concentration similarly increased following intermittent sprint exercise. In contrast to our hypothesis, no CWI condition was associated with reduced IL-6 concentration when compared with CON. Indeed, plasma IL-6 concentration was significantly elevated in both 30-min conditions when compared with CON and CWI-10 min × 10 °C at 2 h post-exercise. The exacerbation of the cytokine response suggests that 30 min immersed in cold or cool water imposes a compound stress on the body. A similar increase in IL-6 has been observed following CWI for recovery from exercise (Lee et al. 2012a; Vaile et al. 2007). Although the duration of time was not held constant between conditions and no control condition was used, Lee et al. (2012) observed an increased IL-6 response after immersion in 11.7 °C water compared with a decrease in IL-6 after immersion in 23.5 °C water (Lee et al. 2012b). This response is further supported by human studies of exercise fatigue during short- or long-term cold exposure, indicating immunostimulatory effects of cold (Castellani et al. 2002).

IL-6 is classically up-regulated and released from skeletal muscle during exercise of high intensity (Nieman et al. 2012) and is also dependent on the mass of muscle engaged (Peake et al. 2005b). Because it is released from inflammatory cells and endothelium during injury or infection, the IL-6 response to exercise is thought to exert pro-inflammatory effects and has been associated with exercise-induced inflammation, muscle damage (Bruunsgaard et al. 1997), and delayed onset muscle soreness (Tomiya 2004). More recently, the effects of IL-6 released from skeletal muscle as a 'myokine' have suggested that it is released from the muscle to act in a hormone-like manner (Pedersen et al. 2003). Interestingly, IL-6 released from the muscle is also shown to inhibit production of TNFα and enhance IL-1ra and IL-10 anti-inflammatory cytokine production (Pedersen 2007), suggesting a net anti-inflammatory effect of IL-6 when released from the muscle. Although the elevated IL-6 observed in these CWI conditions and the potential role for muscle-derived IL-6 to act as an anti-inflammatory cytokine may suggest an anti-inflammatory activity of CWI, this is not consistent with the findings for IL-8 and MPO, which are pro-inflammatory and not pleiotropic.

An alternative explanation may be impaired clearance of IL-6 due to cold-induced vasoconstriction. Arterio-venous difference studies have shown the plasma IL-6 changes during exercise are almost entirely due to release from contracting skeletal muscle (Steensberg et al. 2000). Thus, the sustained elevation observed in plasma IL-6 concentration observed following both 30 min conditions and both 10 °C conditions may be a result of reduced blood flow to the muscle during the recovery period. It has been previously shown that CWI induces significant reductions in intramuscular blood flow (Gregson et al. 2011). It may be that the reduction in blood flow to the muscle impedes the clearance of IL-6 produced in the muscle, resulting in sustained circulation in the blood during the acute recovery phase (0–2 h post-exercise). However, without tissue specific measurement of IL-6 to directly measure production and clearance at skeletal muscle, this is merely speculation. Owing to previous reports of cold-induced upregulation of IL-6, it is likely that IL-6 measured in the plasma at 2 h in the present study represents IL-6 production from multiple cell types.

IL-8 is a known neutrophil chemoattractant (Baggiolini and Clark-Lewis 1992) that is up-regulated in skeletal muscle during intense exercise (Nieman et al. 2012) and MPO is an enzyme implicit in neutrophil cytotoxicity, thus is indicative of neutrophil activation (Klebanoff 2005). IL-8 is commonly up-regulated and released from skeletal muscle during intense exercise (Nieman et al. 2012). As neutrophil trafficking, thus presence in circulation, can be enhanced in the absence of increased activity, MPO was used to indirectly indicate the change in neutrophil activity associated with exercise and subsequent CWI. This was chosen in lieu of more general tissue oxidation detection to connect the specific chemoattraction induced by IL-8 changes for neutrophil trafficking and subsequent activity of neutrophils, rather than oxidative agents in general. Significant increases from baseline in plasma IL-8 and MPO concentration were observed following high-intensity intermittent sprint exercise in all experimental conditions, except IL-8 in the CWI-10 min × 20 °C condition. Furthermore, plasma IL-8 and MPO concentrations remained elevated or continued to increase beyond post-exercise concentrations following CWI-30 min × 20 °C and CWI-30 min × 10 °C at 2 h post-exercise, suggesting an exacerbation of the inflammatory response to exercise when prolonged cold exposure is applied. Although plasma cytokines were not measured, Stacey (2010) observed increased trafficking of neutrophils

following CWI-10 min × 20 °C following intermittent all out exercise compared with control (Stacey 2010). As IL-8 is a neutrophil chemoattractant and was observed to be up-regulated post-exercise, it is possible that neutrophil trafficking was also altered; however, neutrophil counts were not measured in the current study. While IL-8 returned to near baseline in CON, CWI-10 min × 20 °C, and CWI-10 min × 10 °C, it continued to increase following CWI-30 min × 20 °C and CWI-30 min × 10 °C, suggesting that while stress signals abated in the 10 min CWI conditions and control, the stress response persisted in the 30 min CWI conditions. This finding is supported by studies showing cold changes in neutrophil activation and adhesion to the vascular endothelium in association with increases in IL-8 production (Bøkenes et al. 2004). Given the similar pattern observed in plasma MPO in the 30-min conditions, neutrophil functional activity appears to have been up-regulated, as well as chemotactic signaling. Although it was not significant, both of the 30-min CWI conditions were associated with a latent increase in ratings of soreness and impairment at 24 h post-exercise. It may be that the acute inflammatory response observed at 2 h may have contributed to secondary muscle damage resulting in enhanced perceptions of soreness and impairment. It seems likely that prolonged exposure to CWI may enhance the acute (0–2 h post-exercise) inflammatory response following a bout of high-intensity intermittent exercise. An unexpected finding was the non-significant increase in IL-8 observed post-exercise in the CWI-10 min × 20 °C. This may reflect the variability in the exercise-induced cytokine response, as well as the complexity of the response of chemokines released from multiple cell types in response to a common stressor. IL-8 mRNA has been shown to increase significantly in muscle, but is not released to the same extent as IL-6 from skeletal muscle (Pedersen et al. 2007), suggesting that the increase in the plasma may be more attributable to inflammatory or endothelial perturbation. Nevertheless, the pattern remains consistent of elevated IL-8 at 2 h when compared with post-exercise and sustained or enhanced elevation of MPO at 2 h at this time point, supporting our suggestion that these observations are related to neutrophil trafficking and activation.

As blood samples were drawn from the anticubital veins, we cannot infer where the additional cytokine response originated. It may be that blood flow to the muscles was reduced for a long enough period of time that upon exiting the bath, the reperfusion of the muscles might have resulted in an increase in inflammatory marker production and release. Alternatively, it may be a similar ischemia/reperfusion response from the cutaneous tissue exposed to cooling. Muscle biopsy samples could help resolve these uncertainties in future studies. In addition, intramuscular temperatures were not measured, therefore, the specific relationship between muscle temperature per se and post-exercise inflammatory response remains unexplored.

Although participants and researchers were largely blinded to the specifics of the study protocol, the nature of the experimental conditions and control conditions were such that it is very difficult to truly double-blind this type of study.

Conclusion

CWI for 10 min at 10 °C appears very likely to be more effective than passive recovery at restoring force generating capacity of muscle in a SSC, but no CWI protocol used in the current study was effective at restoring performance in a purely concentric movement. CWI does not attenuate the inflammatory response to an acute bout of normothermic high-intensity intermittent sprint exercise when compared with passive recovery. 30-min immersions to the iliac crest in both cool (20 °C) and cold (10 °C) water appear to exacerbate specific aspects of the exercise-induced inflammatory response. Performance effects CWI used following normothermic sprint exercise are not likely a result of attenuation of the inflammatory response to this type of exercise.

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Conflict of interest The authors report no potential conflicts of interest.

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