Cold water immersion enhances recovery of submaximal muscle function after resistance exercise

ABSTRACT

We investigated the effect of cold water immersion (CWI) on the recovery of muscle function and physiological responses following high-intensity resistance exercise. Using a randomized, cross-over design, 10 physically active men performed high-intensity resistance exercise, followed by one of two recovery interventions: 10 min of cold water immersion at 10°C, or 10 min active recovery (low-intensity cycling). After the recovery interventions, maximal muscle function was assessed after 2 h and 4 h by measuring jump height and isometric squat strength. Submaximal muscle function was assessed after 6 h by measuring the average load lifted during six sets of 10 squats at 80% 1RM. Intramuscular temperature (1 cm) was also recorded, and venous blood samples were analyzed for markers of metabolism, vasoconstriction and muscle damage. CWI did not enhance recovery of maximal muscle function. However, during the final three sets of the submaximal muscle function test, the participants lifted a greater load (p<0.05; 38%; Cohen's d 1.3) following CWI compared with active recovery. During CWI, muscle temperature decreased ~6°C below post-exercise values, and remained below pre-exercise values for another 35 min. Venous blood O_2 saturation decreased below pre-exercise values for 1.5 h after CWI. Serum endothelin-1 concentration did not change after CWI, whereas it decreased after active recovery. Plasma myoglobin concentration was lower, whereas plasma interleukin-6 concentration was higher after CWI compared with active recovery. These results suggest that cold water immersion after resistance exercise allow athletes to complete more work during subsequent training sessions, which could enhance long-term training adaptations.

Key words: cryotherapy, recovery, performance, thermoregulation, muscle damage, blood gases

INTRODUCTION

Recovery between exercise training sessions and competitive events is a key determinant of long-term training adaptation and successful performance. Inadequate recovery after exercise may prevent athletes from training at the required intensity, or completing the required loads during subsequent training sessions. It may also increase the risk of injury, illness and overtraining (1). Recognition of the importance of recovery for training adaptation and maintenance of performance has stimulated intense interest in the effectiveness of various strategies to promote faster recovery from intense exercise. Some of these recovery strategies are collectively referred to as 'cryotherapy', and include immersion in cold water/ice baths, topical application of ice, or brief exposure to extreme cold air. Numerous studies have examined the effects of various forms of cryotherapy on muscle function and indirect markers of muscle damage after intense eccentric exercise (6, 8, 9, 16, 18, 20, 36, 38, 39, 41, 44) and plyometric exercise (14, 19, 24). By contrast, fewer studies have assessed the potential benefits of cryotherapy after more traditional resistance exercise (10, 12, 13, 23), which is more typical of athletic training programs.

The findings from studies on the effects of cryotherapy on recovery from eccentrically-biased exercise are not directly applicable to traditional resistance exercise because eccentric exercise causes more severe muscle damage (27). Most of the studies described above assessed the effects of cryotherapy on recovery of muscle function after eccentric exercise by measuring maximal isometric strength from 24 h onwards. A few studies have assessed recovery by measuring jump or squat performance (9, 23, 39, 44) or voluntary muscle activation during maximal muscle contractions (38). No research to date has evaluated the effects of cryotherapy on volitional work capacity during subsequent submaximal exercise on the same day. This information is important, because recovery from exercise involves both peripheral and central factors (34), and athletes often need to recover quickly between training sessions on the same day.

The mechanisms by which cryotherapies such as cold water immersion can enhance post-exercise recovery are not yet well established—particularly in the context of recovery after resistance exercise. Studies on endurance exercise indicate that cold water immersion reduces blood flow to muscle and the limbs (31, 45). The vasoactive factors that mediate these changes in blood flow in response to cold water immersion after exercise are unknown. Endothelin-1 is a potent vasoconstrictor that has been implicated in vascular responses to cold exposure (30, 35), and may therefore regulate the decrease in muscle and limb blood flow that others have reported following cold water immersion (31, 45). Cold water immersion reduces muscle temperature after endurance exercise (15, 31) and intermittent sprint exercise (4, 21), but it is uncertain at present how long this response persists during recovery from exercise. This information is important, because changes in muscle temperature could influence local blood flow and metabolism in muscle. Ihsan et al (22) reported that cold water immersion after endurance exercise decreases muscle blood flow and metabolic activity. Changes in muscle oxygen saturation during exercise are accompanied by changes in venous blood O_2 saturation (7, 29). However, it is unknown whether changes in muscle oxygen saturation following cold water immersion are also associated with changes in venous blood O_2 saturation. This information is important to establish whether changes in O_2 supply/demand following cold water immersion are localized to skeletal muscle or whether they also occur systemically.

Cold water immersion has been proposed to benefit recovery from exercise by reducing inflammation (3). However, research into the effects of cryotherapy on systemic markers of inflammation (e.g., cytokines, C-reactive protein) after eccentric exercise (16, 38, 41, 44) and more traditional resistance exercise (10, 12, 13, 23) has produced inconsistent findings. These studies generally collected blood samples immediately, 1 h or 24 h post-exercise, and may therefore have overlooked peaks in the inflammatory response that occur between 1 and 24 h

after exercise (37). Further research is needed to understand the effects of cold water immersion on inflammation during the first few hours after resistance exercise.

The primary aim of this study was to compare the effects of cold water immersion versus active recovery on short-term restoration of maximal and submaximal muscle function after resistance exercise. A secondary aim of the study was to extend existing knowledge of the physiological mechanisms by which cold water immersion could enhance recovery from exercise. To do this, we measured muscle temperature during and after cold water immersion. We also assessed muscle soreness and swelling, and collected blood samples at regular intervals in the first few hours after cold water immersion to measure changes in venous blood O_2 saturation, endothelin-1 as a potential mediator of vasoconstriction, myoglobin as a marker of muscle damage, and lactate/pH and IL-6 as systemic markers of muscle metabolism and inflammation. We hypothesized that compared with active recovery, cold water immersion would (i) enhance recovery of maximal and submaximal muscle function, (ii) increase serum endothelin-1 concentration, and (iii) reduce muscle temperature, muscle soreness and swelling, venous blood O_2 saturation, plasma myoglobin and IL-6 concentrations.

METHODS

Subjects

Ten physically active young men (mean ± SD age: 21.3 ± 1.6 years, height 1.81 ± 0.06 m, body mass 84.7 ± 12.4 kg) volunteered to participate in this study. All participants were strength training 2–3 times a week at the time of the study, but were not accustomed to cold water immersion. The experimental procedures and potential risks were explained to the participants before they provided written informed consent. All participants were screened for

contraindications to resistance exercise. The study was approved by the Human Research Ethics Committee of the University of Queensland.

Study design

The participants first completed two familiarization sessions to introduce them to the exercise protocols that would be used in the experimental trials, followed by baseline testing to measure their strength. Two weeks after the familiarization sessions and baseline testing, the participants completed two experimental trials, separated by 14 d. They completed self-directed training in the 14 d preceding the baseline testing, and completed a training record covering this period. Training was replicated for the period between the first and second experimental trial. The order of the experimental trials was randomized and counterbalanced. These trials involved a single, standardized bout of high-intensity resistance exercise, followed by cold water immersion or active recovery. The participants refrained from consuming alcohol, caffeine and tobacco over the 24 h preceding both experimental trials. They also refrained from consuming any nutritional supplements between the baseline testing and the completion of their second experimental trial. They completed a 24 h nutritional record prior to the first experimental trial, which was photocopied and returned to them to replicate for the 24 h prior to the second trial. They refrained from participating in any lower body exercise training for 48 h prior to each experimental trial.

Pre-experimental sessions

Pre-experimental sessions were completed 14 d prior to the first experimental trial, and included a repetition maximum (RM) testing session, two familiarization sessions, and a baseline testing session. All pre-experimental sessions and experimental trials were performed within the same temperature controlled laboratory (temperature 24.3 ± 0.6^{o}C, humidity 48.6 ± 1.2%). On the first day, RM testing and a first familiarization session were performed. The

participants were tested to determine their RM for back squat, front squat and incline leg press. These RM data were used to set the workload for resistance exercise in the experimental trials. Following 5 to 10 min of stationary cycling, the participants performed a first repetition at an estimated 90% of RM for each exercise. If successful, the participants repeated each exercise with gradually increasing resistance until failure, or the loss of correct technique (as assessed by one of the investigators, L.A.R). A minimum of 3 min recovery was allowed between attempts. A successful RM was recorded as the greatest mass lifted with correct form, through a complete range of movement. Approximately 15–30 min later, the participants started the first familiarization session. The familiarization session involved the participants practicing the maximal muscle function tests that would be used during the experimental trials. On the second day, the participants repeated the familiarization session from the first day, before they practiced the submaximal muscle function test. More details of these tests are described below. On the third day, the participants were tested to determine their baseline maximal and submaximal muscle function. After these baseline measures were recorded, the participants were familiarized with the cold water immersion therapy to be used during the experimental trials.

Experimental trials

For each trial, the participants arrived at the laboratory at 8:30 am, having eaten breakfast at 7 am, and consumed 10 $ml \cdot kg^{-1}$ of water over the preceding 2 h. Resting venous blood samples were collected upon arrival. Maximal and submaximal muscle function were not measured on the morning of each experimental trial due to time constraints, and also to avoid any residual fatigue from the testing procedures that could affect performance during the subsequent resistance training session. Instead, maximal and submaximal muscle function recorded during the third day of the pre-experimental sessions was used to compare with

changes in maximal and submaximal muscle function measured during each trial (see details above).

After collecting blood samples, resting superficial muscle temperature (1 cm deep) was recorded and segmental limb volumes were calculated (described below). The participants then completed a high-intensity resistance training session, lasting approximately 1 h. This session consisted of the following: six sets of front- and back-squats at loads corresponding to 8-, 8-, 10-, 12-, 10- and 10-RM; three sets of 12 walking dumbbell lunges with a total mass corresponding to 40% of body mass; three sets of 12 counter-movement drop jumps from a height of 50 cm. Recovery time between sets was 90 s, with 120 s between exercises. Strong verbal encouragement was provided where required, to maintain repetition tempo, form, and recovery periods. The participants drank water *ad libitum* during the training session. A venous blood sample was collected, while muscle temperature, upper leg circumferences, muscle soreness and maximal muscle function were measured as soon as possible after the training session.

In the first 15 min following resistance exercise, the participants changed clothes and moved to a different room, where they started a 10 min recovery intervention consisting of cold water immersion or active recovery. After the participants completed these recovery interventions, they recovered for a period of 6 h. Maximal muscle function was assessed again at 2 and 4 h, while submaximal muscle function was measured 6 h following completion of the recovery interventions.

During the 6 h recovery period (i.e., after cold water immersion or active recovery), the participants remained in the laboratory (23–25°C), and were allowed to walk around at a low intensity. During the first 4 h of recovery they were given standardized meals that provided 1.2 $g \cdot kg \cdot h^{-1}$ carbohydrate and 0.4 $g \cdot kg \cdot h^{-1}$ protein.

Cold water immersion and active recovery

For the cold water recovery intervention, the participants sat in an inflatable bath (iBody, iCool Australia Pty Ltd, Miami, Australia) containing water at 10 ± 0.3°C. The participants immersed their body up to their clavicle continuously for 10 min in water. We chose 10°C as a suitable water temperature, because this has commonly been used in previous cold water immersion studies (2). Furthermore, recent studies indicate that 10–15 min of immersion in water at 8–10°C is effective for reducing tissue temperature, blood flow, microvascular blood volume and metabolic activity in skeletal muscle after exercise (4, 21, 22, 31). Water circulation and temperature in the bath was continuously maintained using a circulatory cooling unit (iCool Lite, iCool Australia Pty Ltd., Miami, Australia).

For the active recovery intervention, the participants exercised on a cycle ergometer (Wattbike®, Wattbike Ltd., Nottingham, UK) for 10 min at a low, self-selected intensity. We selected active recovery as a control condition or comparison for cold water immersion instead of passive recovery because in reality, it is unlikely that athletes would do no activity at all while recovering from prior exercise. The participants cycled a mean ± SD distance of 3.62 ± 0.25 km, at an average power output of 45 ± 7 W.

Maximal muscle function tests

The participants completed four tests of maximal muscle function, including a countermovement jump, an un-weighted squat jump, a weighted squat jump and isometric squat. They performed these tests in random order at 2 h and 4 h after the recovery interventions.

Countermovement jump performance was measured while the participants adopted a stance shoulder-width apart, holding a wooden bar in the conventional position for a back squat. On instruction, the participants maximally flexed their knees, prior to jumping vertically for maximum height. Hands remained in contact with the wooden bar at all times,

while the wooden bar remained in contact with the upper back. They performed three jumps, and data from the best jump were analyzed. The participants performed the un-weighted and weighted squat jumps while holding a wooden bar (un-weighted; ~500 g) or a barbell (Australian Barbell Company, Mordialloc, AU) loaded to 30% RM (weighted; 37 ± 9.8 kg) in the same position. They performed three maximal jumps, and the data from the best jump were recorded. For both jumps, the participants lowered their body to 90^{o} knee flexion and paused for 2 s, before jumping for maximum height.

Jump performance was measured using a portable force transducer (GymAware, Kinetic Performance Technology, Australia). Data were transmitted from the transducer to a hand-held personal digital assistant. For the countermovement jump and un-weighted squat, data were calculated for jump height, work, mean and peak velocity. For the weighted squat, mean and peak power was also calculated. These calculations were done using native GymAware software (Kinetic Performance Technology, Australia). The coefficient of variation for these tests was established during the baseline testing procedures. It was 1.9% for countermovement jump height, 2.8% for un-weighted squat jump height, and 2.9% for the weighted squat jump height.

Isometric squat strength was measured using a modified back squat. The participants adopted a standard squat position on a Smith machine at one third of maximum depth, and pressed upward against a stationary bar (25, 43). The participants positioned themselves on a force platform (Kistler, Ostfildern, Germany), while ground reaction force data was collected at 1,000 Hz using native software (BioWare V.5.1.3, Ostfildern, Germany). The participants were instructed to press upwards against the bar as quickly and as forcefully as possible without any prior flexion of the knees, spine or hips. They performed three efforts lasting 3 s each, and the data from the best effort was analyzed. Data were filtered with a Butterworth 4^{th} order digital low-pass filter with cut-off frequency of 10 Hz prior to analysis. Isometric squat characteristics comprised peak vertical force, and force development. Rate of force

development was calculated over 30, 50, 100 and 200 ms. The coefficient of variation for maximum isometric strength was established as 0.9% during the baseline testing procedures.

Submaximal muscle function test

The submaximal muscle function test was similar to that described previously (25). This test was designed to simulate a second training session on the same day (i.e., after the initial resistance training session). It consisted of six sets of back squat repetitions performed at 80% of RM, separated by 3 min passive recovery. The participants attempted to complete ten repetitions per set. If they were unable to complete 10 repetitions during any set, they stopped lifting, and rested for 3 min before beginning the next set. The participants continued this sequence until they had attempted to complete six sets. Repetitions were performed using a squat rack (Force Fitness Equipment, Baltimore, USA), and the number of successful repetitions and mass lifted per set were recorded, in addition to the total mass lifted over the six sets. The coefficient of variation for the total mass lifted during this high-intensity resistance exercise test was established as 0.7% during the baseline testing procedures.

Muscle temperature measurement

Muscle temperature was recorded before and after the training session, continuously throughout the recovery interventions, and over the following 2 h. It was measured by inserting an 18-gauge cannula into the vastus lateralis muscle to a depth of 1 cm. Thigh skinfold thickness was measured using Harpenden skinfold calipers (HSK BI, Baty International, West Sussex, UK), and divided by two to determine subcutaneous fat thickness (19). A fine-wire thermistor (T204E, Physitemp Instruments Inc, NJ, USA) was inserted through the cannula to the required depth, and removed once temperature had stabilized (~5 s; pre- and post-exercise). In a similar manner, a cannula was inserted prior to the recovery intervention, and secured with medical tape and waterproof dressing. Data were logged at 1

Hz using a portable logger (SQ2020, Grant instruments, UK), and averaged over 1 min intervals for the recovery intervention and 5 min intervals for the first 2 h of recovery.

Blood collection and analysis

Venous blood samples were collected into vacutainers containing serum, EDTA and lithium heparin. Serum vacutainers were left to clot at room temperature for 30 min prior to centrifugation. Vacutainers containing EDTA and lithium heparin were immediately put on ice, and then centrifuged at 1,000 g at 4^{o}C for 10 min. Plasma and serum were aliquotted and stored at –80^{o}C for later analysis. Samples were collected before and after the initial resistance exercise, and 15 min, 30 min, 45 min, 1 h, 1.5, 2 h, 4 h, and 6 h after the recovery intervention. A portion (100 μL) of fresh heparinized blood from the samples at all time points except 4 and 6 h after the recovery intervention was pipetted into CG4+ cartridges (Abbott Point of Care, NJ, USA). These cartridges were then immediately inserted into a portable point-of-care device (iSTAT, Abbott Point of Care, NJ, USA) to measure blood lactate concentration, pH, venous blood O_2 saturation and venous blood carbon dioxide saturation.

Plasma myoglobin concentration was measured using an immunoassay (Roche diagnostics GmbH, Germany) and an automated clinical analyzer (Cobas E411, Roche diagnostics GmbH, Germany). Plasma interleukin-6 (IL-6) concentration was measured by enzyme-linked immunosorbent assay (Quantikine® HS ELISA, R&D Systems, Minneapolis, USA). Serum endothelin-1 (ET-1) concentration was also measured by enzyme-linked immunosorbent assay (Quantikine® ELISA, R&D Systems, Minneapolis, USA). Myoglobin, IL-6 and ET-1 were analyzed in duplicate, with sample means taken as the result. The intra-assay coefficient of variation was 1.7% for myoglobin, 9.3% for IL-6 and 6.0% for ET-1.

Limb volume assessment

Segmental limb volume for the lower and upper thigh of the right leg was calculated based on three circumferences. Anthropometric tape was used to measure the circumference (i) above the knee, (ii) mid-thigh, and (iii) at the sub-gluteal fold. Positions were marked with a permanent marker for site identification during and between trials. Limb volume between circumferences 1 and 2 (lower thigh), and 2 and 3 (upper thigh) were calculated, based upon the formula proposed by Katch and Katch (15). The coefficient of variation for upper and lower limb volume assessment was 0.5 and 0.6% respectively.

Muscle soreness perception

Leg muscle soreness was assessed under two conditions: (i) standing with feet shoulder-width apart, (ii) squatting to a 90^{o} knee angle, so that the quadriceps muscles were under tension. Perceived soreness was rated on a horizontal visual analogue scale from 0 (no soreness) to 100 (maximal soreness).

Statistical analysis

Statistical analysis was conducted using the Statistical Package for Social Sciences program (V.19, IBM, New York, USA). With the exception of IL-6, all data were normally distributed (as confirmed using the Shapiro-Wilks test), and were analyzed using a two-factor repeated measure ANOVA. When significant trial and time×trial interaction effects were evident ($p < 0.05$), paired t-tests were used to compare changes over time and between trials. IL-6 data were analyzed using the Friedman's two-way ANOVA by ranks and Wilcoxon's signed rank test. The false discovery rate was used for multiple comparisons of time points within and between trials. Absolute values for intramuscular temperature, blood gases, pH and ET-1 that were recorded pre-exercise, 15 min, 30 min and 1 h post-exercise values were pooled. The pooled data were then used to calculate Pearson's correlations to examine the relationships between these variables. Cohen's effect size (d) was calculated to compare the magnitude of changes

over time and differences between the trials, and assessed as follows: 0.2 = small effect, 0.5 = moderate effect and 0.8 = large effect. All data except IL-6 are presented as means ± SD; IL-6 data are presented as means ± interquartile range. Significance was set at a level of $p < 0.05$.

RESULTS

Maximal muscle function

Performance in all jumps decreased after the resistance exercise ($p < 0.05$), but then progressively increased from 2–4 h after resistance exercise in both the cold water immersion and active recovery trials (Table 1). Countermovement and un-weighted squat jump height remained below pre-exercise values ($p < 0.05$) at 2 h and 4 h (approximately −3.5 cm or −11% for both countermovement and un-weighted squat jump height). Performance in all jumps was generally similar after cold water immersion and active recovery. Similar trends existed for changes in peak velocity, mean velocity, work, peak power and mean power during the recovery period for all jumps. Peak isometric strength followed a similar trend, decreasing post-exercise ($p < 0.05$) and recovering towards pre-exercise values by 4 h (Table 2). There were no significant differences in the rate of force development between the trials at any time point (Table 2).

Submaximal muscle function

Submaximal muscle function was assessed at 6 h after the recovery interventions by measuring the average and total load that the participants lifted during six sets of ten squats at 80% 1 RM. There were no significant differences in average or total load between the cold water immersion and active recovery trials during the first three sets of the submaximal function test ($p > 0.05$) (Figure 1). However, the average load ($p = 0.025$; d +1.3; +38%) and total load ($p = 0.021$; d +0.7; +16%) that the participants were able to lift during the final

three sets was significantly greater following cold water immersion compared with active recovery. The average load lifted during the final three sets was not significantly different following cold water immersion compared with the average load recorded during the baseline testing ($p > 0.05$).

Muscle temperature

Muscle temperature increased consistently in all participants after the resistance exercise ($p < 0.05$). By contrast, the changes in muscle temperature during and after cold water immersion and active recovery were more variable. Individual responses are presented in Figures 2A and 2B. During cold water immersion, muscle temperature decreased by up to 12°C below post-exercise values in some participants, while in others it only decreased by 1–2°C. During active recovery, muscle temperature increased by 1–2°C above post-exercise values in some participants. In other participants, muscle temperature was 3–4°C below post-exercise values at the start of active recovery, and then only increased by ~1°C. After active recovery, muscle temperature rose unusually high (i.e., 41°C) in some participants. These participants were therefore excluded from further analysis. Group data for five participants in the cold water immersion and active recovery trials are presented in Figure 2C. In these participants, muscle temperature did not change significantly following cold water immersion, whereas it increased significantly during active recovery ($p < 0.05$), and remained elevated for 2 h. Muscle temperature was significantly higher ($p < 0.05$) between the 10^{th} and 70^{th} minute of the recovery period after active recovery compared with cold water immersion.

Blood gases, pH and lactate

Blood lactate concentration was higher than pre-exercise values after the resistance exercise ($p < 0.05$). It returned to pre-exercise values within 15 min after active recovery,

whereas it remained significantly higher than pre-exercise values for 2 h after cold water immersion ($p < 0.05$) (Figure 3A). Blood pH was lower than pre-exercise values after resistance exercise ($p < 0.05$) (Figure 3B). It then increased above pre-exercise values after active recovery, but not after cold water immersion. Between 15 min and 2 h after cold water immersion, it was lower compared with active recovery. Venous blood CO_2 saturation followed a similar trend to blood pH (Figure 3D). Venous blood O_2 saturation did not change after the resistance exercise. However, it decreased below pre-exercise values after cold water immersion, and remained significantly lower compared with the active recovery trial for the next 2 h (Figure 3C).

Muscle damage, swelling and soreness

Plasma myoglobin concentration increased above pre-exercise values from 2–4 h after both the cold water immersion and active recovery trials ($p < 0.05$) (Figure 4A). It was significantly lower following cold water immersion compared with active recovery at each time point ($p < 0.05$; d –1.0 to –1.4; –51% to –139%).

Lower and upper thigh volume changed over time ($p < 0.001$) (see Table 3). Both lower and upper thigh volume had returned to pre-exercise values within 1 h following cold water immersion ($p > 0.05$), and remained stable over the remaining 5 h. By contrast, lower thigh volume remained above pre-exercise values following active recovery ($p < 0.05$). Upper thigh volume displayed a similar trend following active recovery. Lower thigh volume ($p < 0.05$; d –0.4; –2.2%; –0.1 cm) and upper thigh volume ($p < 0.05$; d –0.2; –2.7; –0.1 cm) were significantly lower from 2–6 h following cold water immersion compared with active recovery.

Muscle soreness while standing upright and squatting at 90^{o} changed significantly over time ($p < 0.001$), increasing pre- to post-exercise, before gradually decreasing towards pre-

exercise values over the 6 h. Soreness while squatting was significantly lower following cold water immersion after 5 h ($p = 0.036$; d –0.6; –31%) and 6 h ($p = 0.011$; d –0.6; –37%).

Endothelin-1 and IL-6

Serum endothelin-1 concentration decreased after the active recovery trial ($p < 0.05$), but did not change after the cold water immersion trial. It was below pre-exercise values at 0.25 h and 0.5 h after active recovery ($p < 0.05$). There were no differences in endothelin-1 between trials at any time point ($p > 0.05$) (Figure 4B).

Plasma IL-6 concentration was significantly higher than pre-exercise values from 0.25 h to 2 h after both the cold water immersion and active recovery trials ($p < 0.05$) (Figure 4C). It was significantly higher at 1.5 h ($p = 0.028$; d +1.5; +122%) and 2 h ($p = 0.038$; d +1.4; +98%) following cold water compared with active recovery.

DISCUSSION

In this study we investigated how cold water immersion influences the recovery of maximal and submaximal muscle function following high-intensity resistance exercise. Contrary to our hypothesis, compared with active recovery, cold water immersion did not alter recovery of maximal strength or countermovement jump performance. However, it did enhance recovery of submaximal muscle function during a high-intensity resistance exercise test. Cold water immersion also substantially reduced muscle temperature, muscle soreness and swelling, venous O_2 saturation and plasma myoglobin concentration compared with active recovery. Surprisingly, cold water immersion did not alter serum endothelin-1 concentration, whereas it is induced a greater increase in plasma IL-6 concentration compared with active recovery. These findings add to existing knowledge of the performance benefits and physiological effects of cold water immersion after exercise.

406 Contrary to our hypothesis, cold water immersion did not enhance recovery of maximal
407 muscle function, as measured by jump performance and isometric strength. Pointon et al. (38)
408 also reported no significant effects of applying ice packs on recovery of maximal isometric
409 torque 2 h after eccentric exercise. By contrast, Vaile et al. (44) observed that cold water
410 immersion enhanced recovery of peak force during isometric squats and peak power during
411 jump squats 24 h after eccentric exercise. Fonda and Sarabon (9) also noted that 3 min
412 exposure to extremely cold air (–140 to –190°C) assisted recovery of muscle power during
413 jump squats and countermovement jumps 1 h after eccentric exercise. All other studies have
414 failed to demonstrate any benefit of cryotherapy on recovery of maximal strength 24 h or
415 more after eccentric exercise (6, 8, 16, 18, 20, 36, 39) and plyometrics (14, 19, 24). The
416 results of the present study are not directly comparable with these other studies because the
417 exercise was not exclusively eccentric in nature and the participants were already familiar
418 with resistance exercise. Consequently, the muscle damage after exercise was probably less
419 severe in this study compared with studies described above. Nevertheless, our findings
420 suggest that cryotherapy generally does not influence any metabolic or neuromuscular factors
421 that reduce maximal strength and/or power after exercise (e.g., impaired Ca^{2+}-release from
422 sarcoplasmic reticulum (17)).

423 In support of our hypothesis, cold water immersion enhanced recovery of submaximal
424 muscle function. The participants in the present study were able to lift a greater average and
425 total load during the final three sets of the submaximal muscle function test after cold water
426 immersion compared with active recovery. The obvious implication of this finding is that cold
427 water immersion may assist athletes who sometimes need to train (or compete) twice within
428 the same day. In contrast with our findings, two other studies (13, 23) discovered that cold
429 water immersion did not enhance the total number of squats that participants could perform,
430 or the average power during each squat 24 h and 48 h after resistance exercise. There are two
431 the obvious differences that may account for these conflicting findings. Firstly, in these other

studies (13, 23), participants only performed 4 sets of up to 10 squat exercises, whereas the participants on our study performed 6 sets of up to 10 squat exercises. Secondly, in these other studies (13, 23), submaximal muscle function was tested at 24 h and 48 h after exercise, whereas we tested submaximal muscle function 6 hours after exercise. Therefore, the benefits of cold water immersion may depend on how and/or when submaximal muscle function is tested.

Cold water immersion elicited various physiological responses, some of which could explain the improvement in submaximal muscle function. We measured muscle temperature continuously during cold water immersion, and for a further 2 h. Intramuscular temperature increased by approximately 3–4°C after resistance exercise. After cold water immersion, it was ~4.0°C below pre-exercise values, and ~7°C below post-exercise values. Due to substantial inter-individual variation, these changes were not statistically significant. Muscle temperature then returned to pre-exercise values with 20–25 min after cold water immersion. The first 30 min following tissue injury is recognized as a potential window of opportunity to treat muscle injuries (32). A decrease in muscle temperature during this period can reduce secondary tissue damage (33). We did not assess secondary tissue damage directly. However, we found that cold water immersion significantly reduced plasma myoglobin concentration after exercise. This finding provides tentative evidence that cold water immersion may have minimized secondary tissue damage. This result contrasts with most other research indicating no significant effect (9, 13, 14, 16, 18, 19, 23, 24, 38, 39, 44) or an increase (12, 41) in plasma myoglobin concentration or creatine kinase activity in response to cryotherapy after eccentric or resistance exercise. Differences in the extent of muscle damage between resistance exercise and eccentric exercise, the timing of blood collection or the timing of cryotherapy treatments after exercise could partially account for this disparity. Cold water immersion also reduced muscle soreness and swelling, which may have alleviated feelings of discomfort, thereby

allowing the participants to perform better during the last three sets of the submaximal exercise test.

Cold water immersion may also benefit recovery from exercise by inducing vasoconstriction and restricting the infiltration of inflammatory cells into muscle (28). We measured the serum concentration of endothelin-1 to determine whether this potent vasoconstrictor might mediate explain previous observations that cold water immersion reduces blood flow to the limbs (45) and in skeletal muscle (22, 31). Contrary to our hypothesis, serum endothelin-1 concentration did not increase significantly after cold water immersion. This result was somewhat surprising, considering that other research has demonstrated that serum endothelin-1 concentration increases significantly immediately after immersing only the hand in cold water (≤13°C) (30, 35). Several factors could account for why serum endothelin-1 concentration did not increase in response to cold water immersion in the present study. Circulating endothelin-1 may have increased rapidly *during* cold water immersion, and then returned to baseline levels less than 15 min after cold water immersion when we collected a blood sample (35). Cold water immersion may also have increased the secretion of endothelin-1 in muscle tissue independently of any significant change in circulating endothelin-1 concentration (42). Alternatively, prior resistance exercise itself likely caused vasodilation in skeletal muscle (31), which may have attenuated endothelin-1 secretion into the circulation following cold water immersion. The decrease in serum endothelin-1 concentration that occurred following active recovery was probably due to the sustained vasodilation of skeletal muscle in response to the low-intensity cycling.

Several studies have investigated whether cryotherapy aids recovery by reducing inflammation. Tseng et al (41) observed that topical ice treatment did not influence plasma cytokine concentrations 1 h after eccentric exercise, whereas it reduced plasma IL-6 and TNF-α concentrations 24 h post-exercise. Most other studies have reported no effects of cold water immersion (13, 44), application of ice packs (38) or air-pulsed cryotherapy (–30°C) (16) on

systemic inflammatory mediators after resistance exercise or eccentric exercise. In contrast with these findings, we observed that plasma IL-6 concentration was higher after cold water immersion compared with active recovery. IL-6 has traditionally been used as a marker of inflammation after exercise, yet it is not consistently associated with greater muscle damage after exercise (37). It is therefore difficult to suggest with confidence that cold water immersion enhanced systemic inflammation after exercise in the present study. Instead, the higher plasma IL-6 concentration may reflect sustained release of IL-6 from skeletal muscle in response to glycogenolysis (26), which increases in response to cold water immersion (40). The rise in plasma IL-6 concentration after cold water immersion in the present study was unexpected; we are therefore uncertain if or how this response might influence recovery from exercise.

The decrease in microvascular perfusion in skeletal muscle following cold water immersion is accompanied by a decline in local metabolic activity (22). To determine if changes in muscle tissue oxygenation after cold water immersion alter systemic oxygen supply/demand, we measured changes in venous blood O_2 saturation. We observed that cold water immersion substantially reduced venous blood O_2 saturation to between 25 and 30%, while it raised venous blood CO_2 saturation. These effects persisted throughout the initial 2 h of recovery from exercise. The decrease in venous blood O_2 saturation that occurred after cold water immersion was similar in magnitude to the decrease that which occurs at the onset of exercise (7). This decline in venous blood O_2 saturation therefore likely represents a genuine physiological response to cold water immersion. For example, it might reflect an increase in O_2 extraction in skeletal muscle following cold water immersion. Whether this decline in venous blood O_2 saturation following cold water immersion might affect organs/tissues other than skeletal muscle is uncertain.

Perspectives and significance

In designing the present study, we aimed to address some of the knowledge gaps and limitations of previous research in the broader field of cryotherapy described previously. We did this through the following means: (i) using traditional resistance exercise as an exercise protocol to simulate common training practices of athletes; (ii) measuring both maximal and submaximal muscle function during the early recovery period; (iii) using active recovery as a control treatment, and (iv) using a randomized cross-over design to minimize any potential series order effects and inter-individual variation. We acknowledge some limitations to the present study. Firstly, we only recorded muscle temperature superficially within skeletal muscle (i.e., 1 cm). The reasons for the substantial individual variation in changes in muscle temperature during and after the recovery interventions are not immediately obvious. The intensity of active recovery and adipose tissue thickness at the site that we measured muscle temperature varied between individuals. However, muscle temperature did not correlate with either exercise intensity or adipose tissue thickness. Secondly, we did not assess muscle blood flow or O_2 saturation within the muscle. Lastly, we did not collect muscle tissue to examine in greater detail the local mechanism(s) by which cold water immersion may have enhanced recovery from exercise. Despite these limitations, our findings are strengthened by the nature of the research design. Our finding that cold water immersion allowed the participants to perform more volitional work hints at some central benefits of cold water immersion. Whether cold water immersion provides more than a simple ‘placebo’ effect remains a contentious issue (4, 5). ‘Central’ perceptions of better recovery may play a more dominant role than ‘peripheral’ physiological factors in the capacity for athletes to recover from exercise. If cold water immersion does allow athletes to undertake greater workloads during subsequent training sessions, then this may lead to better training adaptations. Alternatively, cold water immersion could also reduce training adaptations (11, 46) by attenuating some of the key biochemical and molecular processes that underpin local adaptations in skeletal muscle, including protein synthesis, mitochondrial biogenesis and angiogenesis. Future

studies in the field of cold water immersion should focus on addressing the central versus peripheral effects, and the acute versus chronic effects of cold water immersion.

Acknowledgements

The authors wish to thank the participants for their time and efforts during this study, and Vince Kelly and Gary Wilson for their technical assistance during the study. This study was supported by research grants from Sports Medicine Australia, and the Centre of Excellence for Applied Sport Science Research at the Queensland Academy of Sport, Brisbane. L. Roberts is supported by an International Postgraduate Research Scholarship at The University of Queensland.

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671

672 **Figure legends**

673 **FIGURE 1.** Mean load lifted per set (mean ± SD) over 6 sets for cold water immersion
674 (CWI) and active recovery (ACT) conditions, and baseline trial conducted during the
675 familiarization session (BAS). *Significant difference from set 1 ($p < 0.05$). #Significant
676 difference between CWI and ACT ($p < 0.05$). †Significant difference between ACT and BAS
677 trials ($p < 0.05$).

678

679 **FIGURE 2.** Individual data for muscle temperature during the cold water immersion (CWI)
680 trial (Panel A) and the active recovery (ACT) trial (Panel B); solid line represents the mean.
681 Panel C represents mean ± SD group data for n=5 (see Results for explanation). *Significant
682 difference from baseline ($p < 0.05$). #Significant difference between trials ($p < 0.05$).

683

684 **FIGURE 3.** Changes in blood lactate concentrations (A), blood pH (B), blood SO_2% (C),
685 blood CO_2% (D) and plasma myoglobin concentration (E) before (PRE), after (POST) and
686 0.25 to 6 hours after exercise for cold water immersion (CWI) and active recovery (ACT)
687 conditions. AU, arbitrary units. Data are expressed as mean ± SD *Significant difference from
688 pre-exercise ($p < 0.05$). #Significant difference between trials ($p < 0.05$).

689

690 **FIGURE 4.** Changes in plasma myoglobin concentration (A), serum endothelin-1
691 concentration (B) and plasma IL-6 concentration (C**)** before (PRE), immediately after (POST)
692 and 0.25 to 6 hours after exercise for cold water immersion (CWI) and active recovery (ACT)
693 conditions. Data are expressed as mean ± SD except for IL-6 where data are median ±
694 interquartile range. *Significant difference from pre-exercise ($p < 0.05$). #Significant
695 difference between trials ($p < 0.05$).

TABLE 1. Changes in countermovement jump, un-weighted squat jump and weighted squat jump performance before (PRE), immediately after (POST), 2 and 4 hours post-exercise for cold water immersion (CWI) and active recovery (ACT) conditions. Data are mean ± SD. *Significant difference from pre-exercise ($p < 0.05$). #Significant difference between trials ($p < 0.05$). N.B. Baseline strength and power data were recorded during the third day of pre-experimental sessions. See 'Experimental trials' in the Methods section for details.

			Time			
Task	**Variable**	**Condition**	**PRE**	**POST**	**2 h**	**4 h**
Counter movement jump	Jump height (cm)	CWI	33.7 ± 6.4	26.7 ± 8.2*	29.6 ± 7.1*	30.3 ± 6.5*
		ACT		27.6 ± 6.0*	30.6 ± 6.9	30.6 ± 6.3
	Peak velocity (m/s)	CWI	3.4 ± 0.5	3.1 ± 0.5*	3.2 ± 0.5	3.3 ± 0.6
		ACT		3.1 ± 0.5*	3.3 ± 0.3	3.3 ± 0.4
	Mean velocity (m/s)	CWI	2.0 ± 0.4	1.7 ± 0.2*	1.8 ± 0.2	1.8 ± 0.2
		ACT		1.7 ± 0.1*	1.8 ± 0.2	1.8 ± 0.2
	Work (kJ)	CWI	39.6 ± 8.7	32.1 ± 8.9*	35.0 ± 8.5	37.0 ± 9.6
		ACT		32.8 ± 6.9*	36.5 ± 7.6	35.3 ± 8.5
Un-weighted squat jump	Jump height (cm)	CWI	31.3 ± 6.7	24.9 ± 7.4*	27.0 ± 6.1* #	27.6 ± 6.5*
		ACT		23.7 ± 4.7*	28.3 ± 6.1* #	27.6 ± 6.6*
	Peak velocity (m/s)	CWI	3.1 ± 0.4	2.8 ± 0.3*	3.0 ± 0.3	3.0 ± 0.4
		ACT		2.9 ± 0.3*	3.0 ± 0.3	3.1 ± 0.4
	Mean velocity (m/s)	CWI	1.6 ± 0.2	1.5 ± 0.2*	1.6 ± 0.1	1.6 ± 0.2
		ACT		1.5 ± 0.1*	1.6 ± 0.1	1.6 ± 0.2
	Work (kJ)	CWI	35.7 ± 8.5	28.9 ± 8.0*	34.5 ± 10.9	33.7 ± 7.1
		ACT		30.1 ± 5.3*	34.3 ± 8.3	34.6 ± 6.4
Weighted squat jump	Jump height (cm)	CWI	16.3 ± 3.4	12.5 ± 3.1*	13.7 ± 3.1*	14.8 ± 3.9
		ACT		13.4 ± 3.6*	14.6 ± 3.5	14.6 ± 3.4
	Peak velocity (m/s)	CWI	2.4 ± 0.2	2.1 ± 0.2*	2.2 ± 0.2*	2.2 ± 0.2*
		ACT		2.1 ± 0.3*	2.1 ± 0.2*	2.2 ± 0.2*
	Mean velocity (m/s)	CWI	1.2 ± 0.1	1.0 ± 0.1*	1.1 ± 0.1*	1.1 ± 0.1*
		ACT		1.1 ± 0.1*	1.1 ± 0.1*	1.1 ± 0.1*
	Peak power (kW)	CWI	1.3 ± 0.4	1.2 ± 0.3*	1.2 ± 0.3*	1.2 ± 0.3*
		ACT		1.1 ± 0.4*	1.1 ± 0.3*	1.2 ± 0.3*
	Mean power (W)	CWI	503 ± 188	436 ± 137*	464 ± 156*	463 ± 139*
		ACT		443 ± 117*	454 ± 131*	453 ± 136*

700 **TABLE 2.** Changes in peak force and rate of force development (RFD) during isometric squats before (PRE), immediately after (POST), and 2 and
701 4 hours post-exercise for cold water immersion (CWI) and active recovery (ACT) conditions. Data are mean ± SD. *Significant difference from
702 pre-exercise ($p < 0.05$). N.B. Baseline strength and power data were recorded during the third familiarization session. See 'Experimental trials' in
703 the Methods section for details.
704
705

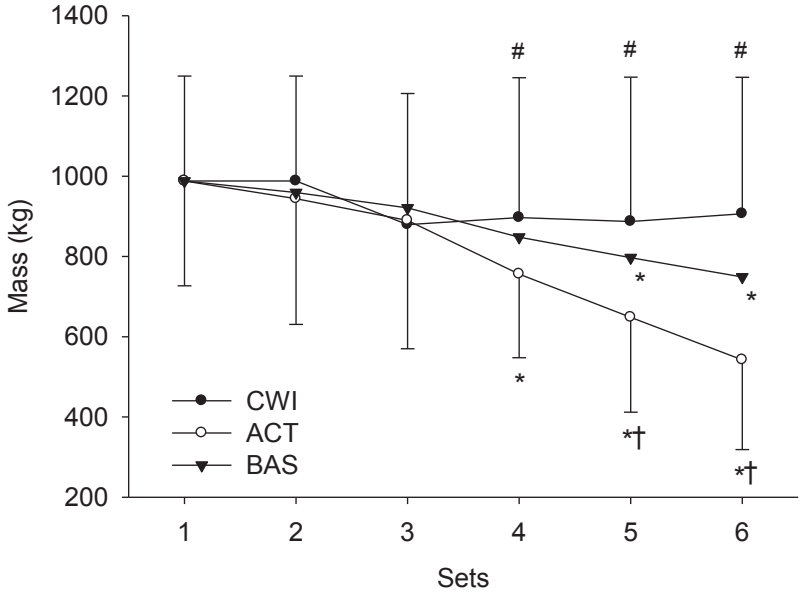
		Time			
Variable	**Condition**	**PRE**	**POST**	**2 h**	**4 h**
Peak force (kN)	CWI	2.3 ± 0.6	2.1 ± 0.4*	2.2 ± 0.7	2.2 ± 0.6
	ACT		2.1 ± 0.4*	2.3 ± 0.5	2.8 ± 0.68
RFD 0 – 30 ms (N/s)	CWI	972 ± 135	887 ± 151*	954 ± 243*	958 ± 199
	ACT		881 ± 128*	1026 ± 204*	1007 ± 202
RFD 0 – 50 ms (N/s)	CWI	1015 ± 128	938 ± 147	992 ± 246	996 ± 212
	ACT		1043 ± 165	1062 ± 211	1037 ± 194
RFD 0 – 100 ms (N/s)	CWI	1137 ± 151	1088 ± 195	1127 ± 259	1074 ± 244
	ACT		1066 ± 207	1156 ± 220	1117 ± 180
RFD 0 – 200 ms (N/s)	CWI	1349 ± 216	1285 ± 279	1378 ± 288	1263 ± 346
	ACT		1218 ± 270	1364 ± 296	1209 ± 418

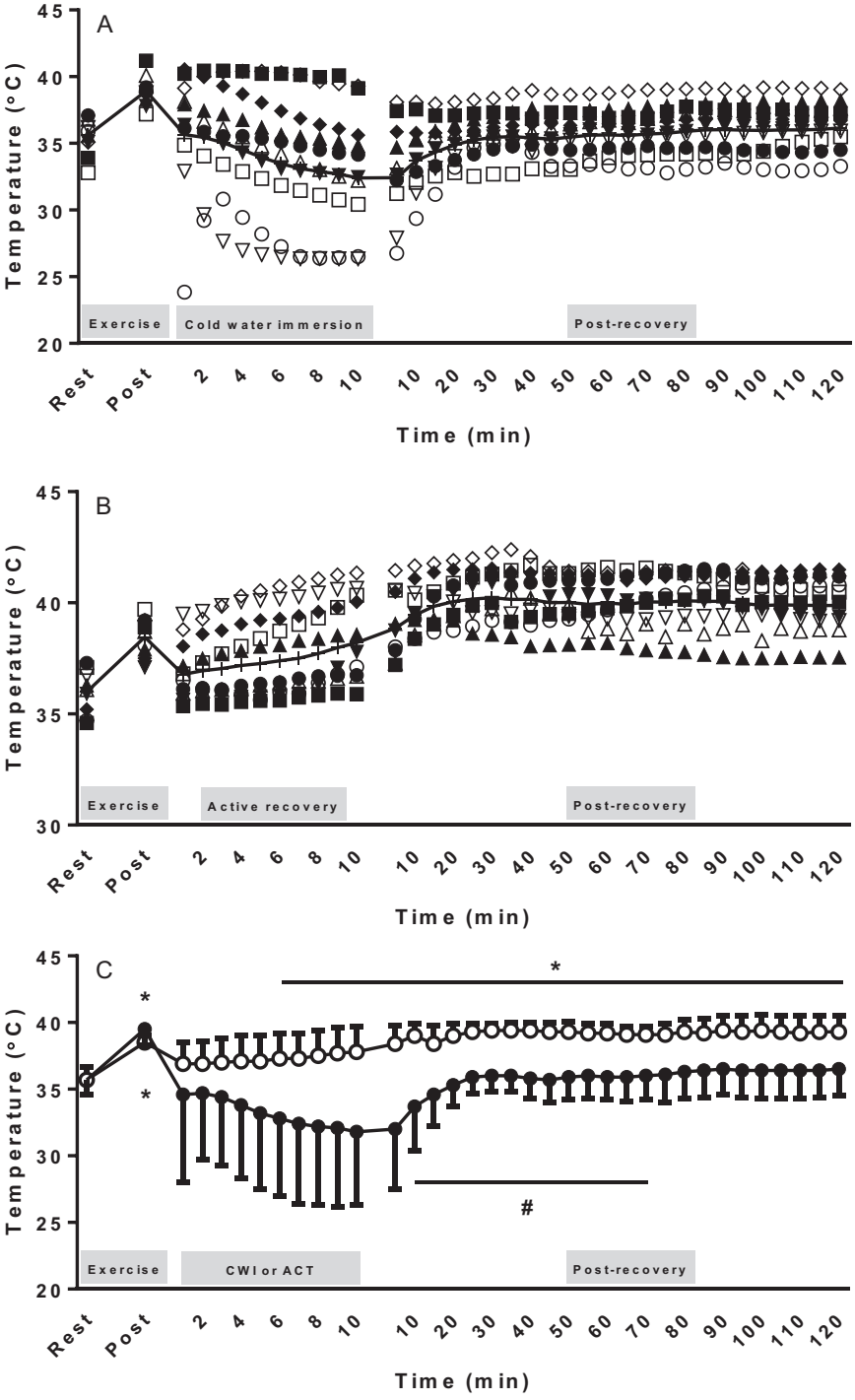
706

707

TABLE 3. Changes in lower and upper thigh volume, and muscle soreness while standing and squatting (mean □ SD) before (PRE), immediately after (POST) and 0.5 – 6 hours post-exercise for cold water immersion (CWI) and active recovery (ACT) conditions. *Significant difference from pre-exercise ($p < 0.05$). #Significant difference between trials ($p < 0.05$).

Column1	Column2	Time						
Variable	**Condition**	**PRE**	**POST**	**0.5 h**	**1 h**	**2 h**	**4 h**	**6 h**
Lower thigh volume (L)	CWI	3.1 ± 0.2	3.2 ± 0.3*	3.1 ± 0.3*	3.1 ± 0.3*	3.0 ± 0.2* #	3.0 ± 0.2* #	3.0 ± 0.3* #
	ACT	3.1 ± 0.2	3.2 ± 0.2*	3.1 ± 0.2*	3.1 ± 0.2*	3.1 ± 0.2	3.1 ± 0.2	3.1 ± 0.2
Upper thigh volume (L)	CWI	2.8 ± 0.5	2.9 ± 0.4*	2.9 ± 0.4* #	2.9 ± 0.5*	2.8 ± 0.4* #	2.8 ± 0.5* #	2.8 ± 0.4* #
	ACT	2.8 ± 0.5	2.9 ± 0.5*	3.0 ± 0.5*	2.9 ± 0.5	2.9 ± 0.5	2.9 ± 0.5	2.9 ± 0.5*
Standing soreness (mm)	CWI	9.6 ± 8.6	73.5 ± 17.6*	-	-	26.1 ± 28.1*	25.0 ± 26.7*	23.5 ± 30.0*
	ACT	10.0 ± 10.2*	68.1 ± 17.0*	-	-	30.8 ± 22.9*	30.8 ± 21.3*	30.4 ± 20.5*
Squatting soreness (mm)	CWI	3.8 ± 5.0	44.4 ± 20.4*	-	-	13.7 ± 16.0*	13.9 ± 16.5* #	12.2 ± 17.5* #
	ACT	4.0 ± 6.5*	41.8 ± 19.9*	-	-	20.3 ± 13.2*	18.7 ± 11.4*	19.4 ± 14.1*


1400
1200
1000
800
600
400
200
Mass (kg)
1
2
3
4
5
6
Sets
CWI
ACT
BAS
#
#
#
*
*
*
*†
*†


A
Temperature (°C)
45
40
35
30
25
20
Exercise
Cold water immersion
Post-recovery
Rest
Post
2
4
6
8
10
10
20
30
40
50
60
70
80
90
100
110
120
Time (min)
B
Temperature (°C)
45
40
35
30
Exercise
Active recovery
Post-recovery
Rest
Post
2
4
6
8
10
10
20
30
40
50
60
70
80
90
100
110
120
Time (min)
C
*
*
*
#
Temperature (°C)
45
40
35
30
25
20
Exercise
CWI or ACT
Post-recovery
Rest
Post
2
4
6
8
10
10
20
30
40
50
60
70
80
90
100
110
120
Time (min)

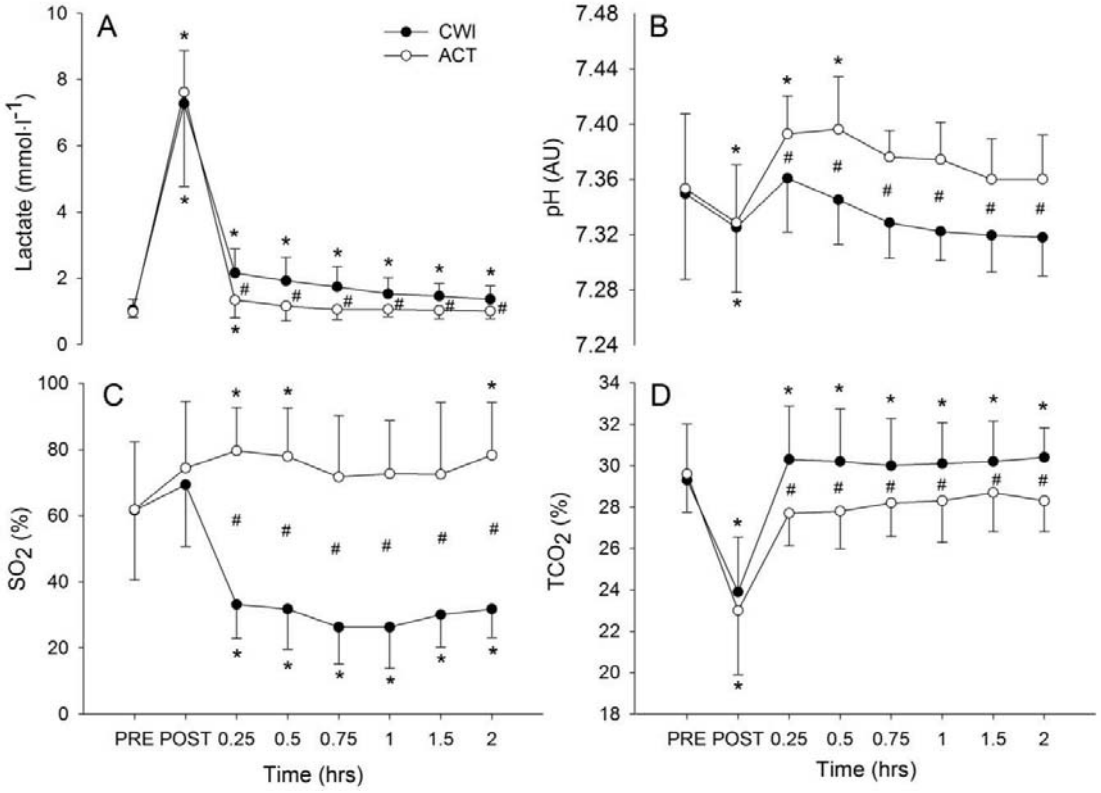
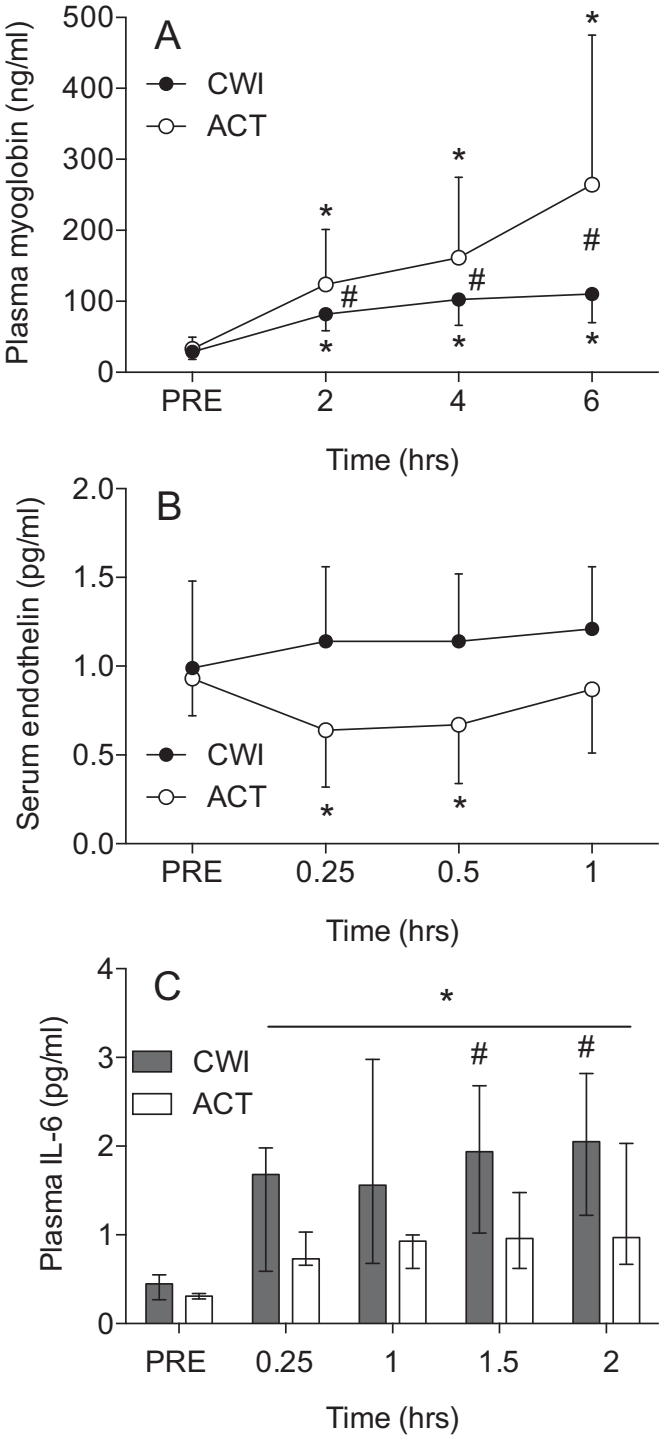

A
CWI
ACT
Lactate (mmol·l-1)
B
pH (AU)
C
SO2 (%)
D
TCO2 (%)
PRE POST 0.25 0.5 0.75 1 1.5 2
Time (hrs)

Figure 4.


A
Plasma myoglobin (ng/ml)
500
400
300
200
100
0
CWI
ACT
PRE
2
4
6
Time (hrs)
B
Serum endothelin (pg/ml)
2.0
1.5
1.0
0.5
0.0
CWI
ACT
PRE
0.25
0.5
1
Time (hrs)
C
Plasma IL-6 (pg/ml)
4
3
2
1
0
CWI
ACT
PRE
0.25
1
1.5
2
Time (hrs)