Cold Water Immersion Improves Recovery of Sprint Speed Following a Simulated Tournament

Abstract

It is a common requirement in tournament scenarios for athletes to compete multiple times in a relatively short time period, with insufficient recovery time not allowing full restoration of physical performance. This study aimed to develop a greater understanding of the physiological stress experienced by athletes in a tournament scenario, and how a commonly used recovery strategy, cold water immersion (CWI), might influence these markers. Twenty one trained male games players (age 19 ± 2; body mass 78.0 ± 8.8 kg) were randomised into a CWI group (n = 11) or a control group (n = 10). To simulate a tournament, participants completed the Loughborough Intermittent Shuttle Test (LIST) on three occasions in five days. Recovery was assessed at specific time points using markers of sprint performance, muscle function, muscle soreness and biochemical markers of damage (creatine kinase, CK), inflammation (IL-6 and C-Reactive Protein) and oxidative stress (lipid hydroperoxides and activity of 6 lipid soluble antioxidants). The simulated tournament was associated with perturbations in some, but not all, markers of physiological stress and recovery. Cold water immersion was associated with improved recovery of sprint speed 24 h after the final LIST (ES = 0.83±0.59; P=0.034) and attenuated the efflux of CK pre- and post-LIST 3 (P<0.01). The tournament scenario resulted in an escalation of physiological stress that, in the main, cold water immersion was ineffective at managing. These data suggest that CWI is not harmful, and provides limited benefits in attenuating the deleterious effects of experienced during tournament scenarios.

Key words: muscle damage; recovery; strenuous exercise; athletes

Introduction

Elite athletes are frequently required to compete at national or international level in tournament scenarios, where multiple matches are completed in a short period of time. Insufficient recovery time between bouts of exercise does not allow full restoration of physical performance between matches, potentially reducing athlete performance levels. However, few studies have focussed on the specific demands of tournament scenarios. Spencer et al. (2005) has shown that movement patterns of players were negatively affected by residual fatigue in a tournament of 3 matches of hockey in 4 days. Montgomery et al. (2008) has also shown moderate impairments in physical performance tests in a simulated basketball tournament of 3 games in 3 days. Elevated inflammation, oxidative stress, creatine kinase (CK), delayed-onset muscle soreness (DOMS), which are indicative of exercise-induced muscle damage (EIMD) and strenuous exercise, have been shown to contribute to the reduction in muscle function in repeated sprint field sports (Bell, Stevenson, Davison, & Howatson, 2016; Thomas, Dent, Howatson, & Goodall, 2017). Research that has investigated these variables has only been following one or two exposures to repeated sprint exercise, therefore there is little understanding of the physiological demands of longer tournament scenarios.

To improve the rate of recovery during tournaments, athletes frequently use a variety of recovery strategies. One of the most popular interventions is cold water immersion (CWI), however evidence to support its use remains equivocal (Bleakley et al., 2012; Leeder, Gissane, van Someren, Gregson, & Howatson, 2012). Although the effectiveness of CWI following one-off bouts of repeated sprint exercise is not clear (Bailey et al., 2007; Corbett, Barwood, Lunt, Milner, & Tipton, 2012), CWI has been effective at reducing deleterious signs of EIMD during repeated exposures to intermittent sprint exercise designed to replicate competition demands (Montgomery et al., 2008; Rowsell, Coutts, Reaburn, & Hill-Haas, 2009, 2011; Vaile, Halson, Gill, & Dawson, 2008). Despite these positive findings, investigations did not include a valid control group by using a thermoneutral immersion (Rowsell et al., 2009, 2011), or did not incorporate any physiological markers of recovery (Montgomery et al., 2008; Vaile et al., 2008), therefore there is a limited understanding of how CWI might influence recovery in tournament scenarios.

To our knowledge, a comprehensive evaluation of CWI using markers of inflammation, oxidative stress, muscle damage, muscle soreness and performance, measured during a controlled simulated tournament is absent from the literature. Using a randomized controlled design, this study aimed to develop greater understanding of the physiological stress experienced by athletes in a tournament scenario, and how CWI might influence these indices. It was hypothesised that CWI in accordance with the majority of the literature to date, would be effective at reducing DOMS, but have no effect on other markers of recovery.

Methods

Participants

Twenty-one male games players, who frequently took part in collegiate repeat sprint field sports (rugby, hockey and football), volunteered for the study (Table 1). Participants completed informed consent and a health-screening questionnaire prior to testing. The study was approved by the University ethics committee for testing of human subjects, in accordance with the Helsinki Declaration.

Experimental Design

Participants visited the laboratory on 7 occasions over 2 weeks (visits 1-3 in week 1 and visits 4-7 on week 2). On visits 1 and 2, subjects were familiarised with tests of muscle strength (maximal voluntary contraction; MVC) and muscle power (countermovement jump; CMJ). On visit 3, subjects completed MVC and CMJ tests, followed by a multi stage fitness test (Ramsbottom, Brewer, & Williams, 1988) to ascertain the level required to complete the repeated sprint test designed to simulate competition demands (Loughborough Intermittent Shuttle Test; LIST; Nicholas, Nuttall, & Williams, 2000). Participants were briefly exposed to the LIST to familiarise themselves with the required running speeds. The LIST was conducted in an environmentally controlled indoor running facility with a temperature of 20°C and relative humidity of 60%. Finally, participants were then randomly assigned to a CWI group (n = 11) or a control group (n = 10). During week 2, participants completed the LIST on 3 occasions (visits 4-6), with 48 h recovery between each test. Venous blood was collected before and after each LIST test, as well as assessments of muscle soreness collected pre-, post- and 1 h post-exercise. On visit 7, 24 h following the final LIST, participants completed tests of sprint speed, muscle function, muscle soreness and had blood collected. Nutritional intake was

recorded for 2 d prior to testing and for the 6 d of data collection to ensure consistency between groups. Participants were required to refrain from exercise for 2 d prior to testing, during the test days and on recovery days between testing. Participants were requested to abstain from any therapeutic interventions, such as non-steroidal anti-inflammatory drugs or compression garments, throughout the experimental period.

Loughborough Intermittent Shuttle Test

The LIST is described in full elsewhere (Nicholas et al., 2000); briefly, the LIST comprised 5 sets of 15 min varying intensity exercise, ranging from walking, jogging (~55% V̇O2max), running (95% V̇O2max) and sprinting (11x15m sprints per set). Subjective ratings of perceived exertion (RPE) were recorded at the end of each 15 min set, as well as 15 m sprint times (Brower Timing System, Utah, USA). For each LIST, data used for analysis included the average RPE and average sprint times. Twenty-four hours following LIST 3, following the same standardised warm up as the previous 3 LISTs, participants completed 5 sprints in the first set of the LIST. These 5 sprints were then compared with the first 5 sprints of the three previous LIST tests.

Cold Water Immersion

Following completion of each LIST and measurement of the post-LIST venous blood sample and muscle soreness assessment, the control and CWI interventions were completed. The CWI group were immersed into a temperature controlled vessel (iCool Sport, Miami, Australia) in a seated position (n = 11; 14° for 14 min, hydrostatic pressure ~40 mmHg). The control group (n = 10) sat still in ambient conditions (20°C and 60% relative humidity) for the same time period.

Neuromuscular Function

Maximal voluntary contraction and CMJ were assessed before (Pre-LIST 1), and 24 h post-LIST 3. During the CMJ, participants stood on an electronic timing mat (KMS Switch Mat, Australia), placed hands on hips and dropped down to a self-selected level before jumping maximally; flight time was used to estimate jump height. The MVC of the knee extensors (at a joint angle of 90°, verified using a goniometer) using the dominant leg was assessed via a strain gauge (MIE Digital Myometer, Leeds, UK). One end of the load cell was attached to the ankle strap situated ~2 cm proximal to the malleoli, with the other end attached to a bespoke

steel chair. Assessment of both CMJ and MVC followed a standardised warm up and the average of the 3 measures were used for statistical analysis. Inter-day co-efficient of variation (CV) for CMJ and MVC were 3.7 and 3.5%, respectively.

Muscle Soreness

Delayed onset muscle soreness (DOMS) was measured via a 200 mm visual analogue scale with 0 mm representing 'no pain' and 200 mm 'extremely painful' (Leeder et al., 2014). Participants placed hands on hips and bent down in a squat position to a 90° knee angle prior to completing the visual analogue scale. Muscle soreness was assessed before, immediately after and 1 h after each LIST, and 24 h post-LIST 3.

Blood Markers

A venous blood sample (~30 mL) was taken from the antecubital fossa using standard venepuncture techniques to assess markers of muscle damage (CK), inflammation (Interleukin-6, IL-6; C-Reactive Protein, CRP) and oxidative stress (lipid hydroperoxides, LOOH; and activity of 6 lipid soluble antioxidants). Blood was immediately spun and the plasma was aspirated and stored at -80°C for later analysis. Blood was collected before, immediately after and 1 h after each LIST, and 24 h post-LIST 3. Plasma CK and CRP were derived using automated analysers (Advia 2400 Chemistry System, Siemens Healthcare Diagnostics, USA), with intra-sample CV of < 3% at low and high concentrations. Plasma IL-6 was determined in duplicate using the quantitative sandwich enzyme immunoassay ELISA technique (Quantikine, R&D Systems Europe Ltd., Abingdon, UK) with a spectrophotometric plate reader (Biochrom Anthos 2010, Cambridge, UK). Intra-sample CV was < 3% for IL-6. Lipid peroxidation was estimated using LOOH concentration using the ferrous iron/xylenol orange assay (Wolff, 1994). The inter-assay and intra-assay CV was less than 4% and 2%, respectively. The high-performance liquid chromatography method of Catignani & Bieri (1983) and Thurnham, Smith, & Flora (1988) was used simultaneously to determine the quantity of selected endogenous lipid soluble anti-oxidant activity (retinol, lycopene, α- and β-carotene and α- and γ-tocopherol). The inter-assay and intra-assay CV was less than 5%.

Statistical Analysis

To analyse changes across the three LISTs, a mixed model, repeated measures analysis of variance was used, analysing group (Control and CWI), bout (LIST 1, 2 and 3) and time (Pre, post- and 1 h post-exercise or Set 1-5; dependent upon the variable). This method enabled comparison of the potential different responses between the three LISTs, however when comparing information from LIST 1-3 with the 24 h post-exercise data, a two way repeated measures analysis of variance on time determined within subject effects: group (Control and CWI) and time (Pre-, post- and 1h-post LIST 1, 2 and 3, plus 24 h post-LIST 3). Where Mauchly's assumptions of sphericity were violated, Greenhouse–Geisser corrections were used. Significant interactions between means were identified using the least significant difference post-hoc analysis. Data were analysed using SPSS for Windows (v. 15.0 software package) and significance was set at $P = 0.05$.

Where appropriate, magnitude of effects of CWI on performance indices (sprint data, MVC and CMJ) were calculated as effect size (ES) statistics (Batterham & Hopkins, 2006); defined as 0.2 (small), 0.6 (moderate), 1.2 (large) and 2.0 (very large). Quantitative chances (%) of positive/trivial/negative effects were expressed qualitatively according to modified statistical spreadsheets (Hopkins, Marshall, Batterham, & Hanin, 2009). Data were presented in the text as ES ± 90% CL and likelihood the effect was positive/trivial/negative (%).

Results

There were no group differences in participant characteristics indicating that that groups were well matched (Table 1).

INSERT TABLE 1 NEAR HERE

Average sprint time during the 3 LISTs showed no main effects of bout, time or group, and no interaction effects ($P > 0.05$). Twenty-four hours post-LIST 3, despite no main effects of group or time, a group x time interaction ($F_{3,54} = 3.585$, $P = 0.019$) existed when comparing the average sprint time of the 5 sprints. Post-hoc analysis revealed that the control group average sprint time was moderately slower than the CWI group 24 h post-LIST 3 (Figure 1; $P = 0.034$.).

Magnitude-based inferences revealed this as a very likely positive moderate effect of CWI (ES = 0.83±0.59. 96/4/0). Expressed in raw units, this positive effect was on average -0.13 ± 0.09s over 15m.

INSERT FIGURE 1 NEAR HERE

Rate of perceived exertion showed main effects of bout ($F_{2,36}$ = 4.269, P = 0.022), time ($F_{1,24}$ = 70.841, P < 0.001), bout x time interactions ($F_{8,144}$ = 3.124, P = 0.003) and bout x time x group interactions ($F_{8,144}$ = 2.381, P = 0.019), but no differences between groups (P = 0.581). Post-hoc analysis revealed that RPE was lower in LIST 3 than LIST 1 (P = 0.009). RPE also increased from set 1 to set 5 in each LIST (P < 0.001).

At 24 hours post-LIST 3, CMJ reduced by 2.6±6.7% and 2.6±5.8% in the control and CWI groups, respectively. Although a trend existed towards main effects of time (P = 0.062), there were no main effects of group or group x time interactions (P > 0.05). Maximal voluntary contraction reduced by 2.9±13.3% and 3.2±7.3% in the control and CWI groups, respectively. There were no main effects of time, group or group x time interactions (P > 0.05). Magnitude-based inferences revealed CMJ (ES = -0.01±0.28; 11/77/12) and MVC (ES = 0.12±0.43; 37/52/10) showed only trivial differences between groups.

Creatine kinase (Figure 2) showed main effects of bout ($F_{1,21}$ = 72.454, P < 0.001), time ($F_{1,15}$ = 82.794, P < 0.001) and bout x group interactions ($F_{2,30}$ = 4.546, P = 0.019). Post-hoc analysis showed that CK increased pre- to post-exercise within each LIST (P < 0.001) and increased incrementally between each LIST (P < 0.001). Post-hoc analysis indicated that CK was lower in CWI than control at both pre-LIST 3 (P = 0.004) and post-LIST 3 (P = 0.001), yet there was no difference between groups 24 h post-LIST 3 (P > 0.05).

INSERT FIGURE 2 NEAR HERE

C-Reactive protein (Table 2) showed main effects of bout ($F_{1,14}$ = 9.909, P = 0.007), time ($F_{1,14}$ = 7.381, P = 0.017), and a time x group interaction ($F_{1,14}$ = 4.889, P = 0.044) but no group effects or interactions between other subject factors (P > 0.05). Post-hoc analysis revealed CRP increased pre- to post-exercise within each LIST (P = 0.017) and increased incrementally across the 3 LISTs (P < 0.05). There was no difference between groups 24 h post-LIST 3 (P > 0.05).

INSERT TABLE 2 NEAR HERE

IL-6 (Table 2) showed main effects of bout ($F_{1,23}$ = 9.659, P = 0.003), time ($F_{1,18}$ = 43.354, P < 0.001) and a bout x time interaction ($F_{1,22}$ = 9.640, P = 0.003) but no group effects or interactions (P > 0.05). Post-hoc analysis revealed IL-6 increased pre- to post-exercise within each LIST (P < 0.001), and the IL-6 response was lower in LIST 2 and 3 than LIST 1 (P < 0.05). The control group post-LIST IL-6 response in LIST 1 (P = 0.003) and LIST 3 (P = 0.038) was higher than CWI. There was no difference between groups 24 h post-LIST 3 (P > 0.05).

Lipid hydroperoxides (Table 2) data showed a main effect of time ($F_{1,20}$ = 17.049, P = 0.001), indicating that LOOH were elevated after each LIST. There were no bout or group differences and no interactions (P > 0.05). There was no difference between groups 24 h post-LIST 3 (P > 0.05). In regard to lipid soluble antioxidants (Table 2), there were effects of time (pre vs. post) were found for alpha tocopherol, gamma tocopherol, beta carotene, retinol and lycopene (P < 0.05). Main bout effects (LIST 1, 2 and 3) were found for alpha tocopherol, gamma tocopherol, alpha carotene and beta carotene (P < 0.05). Post-hoc analysis revealed that LIST 2 was lower to LIST 1 and 3 for gamma tocopherol and beta carotene (P < 0.05) and LIST 3 was different to LIST 1 and 2 for alpha tocopherol and alpha carotene (P < 0.05). Lycopene showed a main effect of group ($F_{1,20}$ = 11.669, P = 0.003). There were no group interactions indicating CWI had no effect after the simulated competition.

Perception of DOMS showed main effects of bout ($F_{2,34}$ = 16.521, P < 0.001), time ($F_{2,34}$ = 58.003, P < 0.001) and bout x time interactions ($F_{4,68}$ = 8.774, P < 0.001), but no group effects or other interactions between these subject factors (P > 0.05). Post-hoc analysis of bout data showed that DOMS increased from LIST 1 to LIST 2 (P < 0.001), then reduced in LIST 3 compared to LIST 2 (P = 0.046), however LIST 3 was still higher than LIST 1 (P = 0.001). Post-hoc analysis of time data showed that post- and 1 h post-LIST DOMS were higher than pre-LIST (P < 0.01). Post-hoc analysis also showed that at pre-LIST 3 time point, CWI was no longer elevated above baseline (P = 0.089) but the control group was still elevated (P = 0.010). Despite this, there were no between group differences for any muscle soreness measures (P > 0.05).

Discussion

This study investigated the effect of seated CWI during a simulated tournament scenario and aimed to determine if CWI had any effects on recovery of performance and indices of damage, inflammation and oxidative stress. The major findings of this study were: 1) the simulated tournament scenario was associated with perturbations in some, but not all, recovery markers, 2) cold water immersion attenuated the efflux of CK towards the latter part of the simulated tournament but had no effect on other recovery indices, and 3) cold water immersion was associated with faster sprint times 24 h following the tournament scenario.

Average sprint time did not change between the three LISTs, indicating 48 h between LISTs was sufficient for participants to recover sprinting ability. It was expected that participant RPE would rise across the 5 sets within each LIST (Nicholas et al., 2000), however, an unexpected finding was that participant RPE was lower in LIST 3 than LIST 1. Although not measured directly, it can be assumed the physiological load of each LIST was very similar because the protocol requirements do not change. Work from our laboratory (Leeder et al., 2014) has also shown a reduction in RPE to repeated LISTs and suggested it was likely due to a habituation to the exercise. The findings of the current study support this as the 3 d between LIST 1 and 3 is unlikely to bring about physiological adaption. IL-6 was lower in LIST 2 and 3 than LIST 1, potentially inferring habituation to the exercise, and DOMS was lower before LIST 3 than 2. The DOMS response is contrary to literature indicating athlete perception of leg soreness peaked after the 4th match in 4 days (Rowsell et al., 2009). The reason for this discrepancy is not clear, but conceptually the subsequent bouts do not cause further damage and hence do not further increase the magnitude of soreness. Future research is required to confirm this hypothesis.

Plasma CK increased incrementally across the 3 LIST tests, peaking after LIST 3, indicating an increased permeability of the membrane surrounding the muscle cells (Friden & Lieber, 2001). Previously, we have shown that CK peaks at 24 h and moves toward baseline by 72 h (Leeder et al., 2014), whereas in this investigation CK escalates across the week. Like-wise, C-reactive protein, an index of the non-specific response to inflammation, muscle damage and infection (Pepys & Hirschfield, 2003), also gradually increased across the 3 LISTs in a similar nature to CK. Collectively, this illustrates a cumulative stress effect of repeated simulated competition. These data provide good evidence that performing repeated bouts of high intensity exercise, in a similar fashion to a tournament scenario, can lead to an escalating

immune response and muscle damage. To the authors' knowledge, this is the first study to identify this incremental increase in these indices and further highlights the importance of effective recovery strategies to maintain a homeostatic state in situations of this nature.

The LIST was associated with elevations in five out of six lipid soluble anti-oxidants. It is speculated that rise in lipid anti-oxidants is a lipolysis dependent response, indicating the breakdown of lipids induced from exercise. This is supported by Davison et al. (2002) and Pincemail et al. (1988) who have observed elevations in lipid soluble anti-oxidants following intensive exercise. Lipid hydroperoxides were also elevated following the LIST, indicating the presence of free radical specific damage to cell membranes. As elevations in CK were also present, this provides strong evidence that sarcolemma integrity was compromised.

Twenty-four hours following LIST 3, the CWI group maintained average sprint speed but the control group experienced a decline. These data suggest CWI may be effective in protecting sprint running speed following repeated strenuous exercise. Counter-intuitively, CWI had positive effect on recovery of sprinting, but not tests of muscle power (CMJ) or strength (MVC) measured at the same time. The small decline in CMJ (-2.6%) and MVC (-2.9%) suggests that muscle function was not compromised and falls within the error range of the tests. Despite this, it is speculated two likely mechanisms could explain the differences in groups between recovery of sprint performance. Despite the limitations of CK as a marker of EIMD (Warren, Lowe, & Armstrong, 1999), the between-group difference in CK efflux pre- and post-LIST 3 could indicate a less damaged muscle cell membrane. Lipid hydroperoxides were also elevated following the LIST, although there was no group difference, this indicated the presence of free radical specific damage to cell membranes. Alongside the elevations in CK, this provides strong evidence that sarcolemma integrity was compromised. It is likely the magnitude of this cellular disruption was insufficient to cause a reduction in muscle function, given the absence in reductions of markers of muscle strength (MVC) and power (CMJ), but may have influenced running speed. A clear difference between isolated tests of muscle strength/power and 15m sprinting is the number of muscle contractions involved. Excitation-contraction uncoupling has been suggested as a mechanism of primary muscle damage (Warren, Ingalls, Lowe, & Armstrong, 2001), therefore it is speculated CWI may have limited the magnitude of this source of initial damage and thereby maintaining sprint speed, however future research is required to confirm such a hypothesis. A second, and simpler explanation, is related to participant motivation. Although there was no measured difference in muscle soreness between groups, a limitation of the current investigation was not utilising a more detailed profile of mood state.

Twenty-four hours after the LIST 3, it is possible participants in the control group could complete a one-off maximal physical effort, supported by the lack of group difference in MVC and CMJ measures. However, if control group participants felt more fatigued and lacked the motivation to complete 5 fast sprints, the average sprint time would be increased compared the CWI group, thereby explaining the difference in average sprint time 24 h post-LIST 3. There is evidence that athletes may regulate intensity of exercise based upon their sensation of fatigue (Noakes, St Clair Gibson, & Lambert, 2004; Rowsell et al., 2011).

The effect of CWI on plasma CK is equivocal. We have found no effect of seated or standing CWI following exposure to one LIST test (Leeder et al., 2015), which concurs other research investigating the efficacy of CWI following this exercise (Bailey et al., 2007; Ingram, Dawson, Goodman, Wallman, & Beilby, 2009; Rowsell et al., 2009). Our previous meta-analysis has shown a small reduction in efflux of plasma CK (Hedges' g = 0.221; P = 0.022) following analysis of 22 extracted data points, therefore this investigation adds to a body of evidence that CWI has small, but nonetheless significant effects in reducing CK efflux. The most likely explanation is a cold-induced reduction in the efflux of CK from the muscle to the extra-cellular space via a reduced permeability of vessel walls (Eston & Peters, 1999). A second potential explanation of reduced CK efflux could be CWI reducing the magnitude of secondary damage (Howatson & van Someren, 2008). However, this is unlikely given the lack of effect of CWI on markers of inflammation and oxidative stress in this investigation.

The most efficacious action of CWI is its analgesic effect (Leeder et al., 2012), with CWI exhibiting moderate effects in alleviating DOMS post-exercise. Whilst physiological mechanisms have been speculated to explain this finding, the perceptual nature of DOMS assessments makes it plausible that the placebo effect may have an impact on the efficacy of CWI (Beedie & Foad, 2009; Broatch, Petersen, & Bishop, 2014). Interestingly, there was no between-group difference in perception of soreness in the current investigation. This is likely explained by either a lack of participant belief in the efficacy of CWI, or an inability of CWI to reduce inflammatory responses and exudate formation. The latter is supported by the lack of group differences in CRP and IL-6. These data may provide useful insight to those interested in further understanding the influence of CWI on inflammatory markers in relation to training adaptation.

In conclusion, the improvement in sprint recovery time 24 h following the final LIST suggests potential beneficial effects of CWI on recovery following a simulated tournament. Cold water immersion was associated with attenuating the efflux of CK towards the latter end of the simulated tournament. Cold water immersion was ineffective in reducing markers of inflammation, oxidative stress or muscular soreness. These data suggest that during a tournament scenario, CWI is not harmful as suggested in some training scenarios, and may provide some benefits, albeit limited, in attenuating the negative effects of exercise stress and is therefore advocated in these specific situations.

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Tables

Table 1. Subject characteristics for control (n = 10) and seated CWI (n = 11).

Group	Age (years)	Mass (kg)	$\dot{V}O_{2max}$ (ml·kg·min^{-1})	MVC (N)	CMJ (cm)
Control	19±1	80.3±8.4	50.3±4.0	665±82	31.8±4.1
CWI	20±2	75.9±9.1	50.6±5.2	638±133	34.2±6.2
***P* Value**	0.228	0.263	0.521	0.586	0.314

Values are presented as mean ± SD. CMJ, countermovement jump; MVC, maximal voluntary contraction; CWI, cold water immersion group.

Table 2. Descriptive data for control and cold water immersion groups for CRP, IL-6, LOOH and 7 lipid soluble antioxidants across 7 time points.

Blood Marker	Statistical Significance		LIST 1		LIST 2		LIST 3		24h Post LIST 3
			Pre	Post	Pre	Post	Pre	Post	
CRP (mg·L-1)	T, B, TxG	Con	0.37±0.19	0.51±0.21	0.54±0.22	0.83±0.34	0.91±0.44	1.39±0.76	1.50±0.79
		CWI	0.49±0.22	0.45±0.22	0.90±0.70	1.04±0.76	1.43±1.29	1.44±1.41	1.51±0.62
IL-6 ($pg \cdot mL^{-1}$)	T, B, TxB	Con	1.12±2.09	15.17±12.30	0.51±1.62	8.40±5.45	0.52±1.16	8.55±6.17	0.84±1.14
		CWI	0.53±1.20	8.53±6.59	0.66±1.60	5.83±3.55	0.10±0.21	3.87±4.29	0.24±0.52
LOOH ($\mu Mol.L^{-1}$)	T	Con	1.75±0.32	1.88±0.60	1.54±0.27	1.83±0.37	1.49±0.13	1.53±0.31	1.49±0.18
		CWI	1.15±0.12	1.77±0.26	1.51±0.18	1.62±0.16	1.55±0.13	1.73±0.32	1.53±0.36
Gamma-Tocopherol ($umol.L^{-1}$)	T, B	Con	0.76±0.20	0.93±0.64	0.71±0.45	0.74±0.35	0.82±0.37	0.97±0.26	1.18±0.43
		CWI	0.69±0.59	0.95±0.54	0.62±0.29	0.51±0.50	0.58±0.57	1.02±0.75	0.93±0.60
Alpha-tocopherol ($umol.L^{-1}$)	T, B	Con	8.07±1.54	8.20±1.59	8.70±2.04	8.37±1.82	7.77±1.51	10.69±1.51	10.33±3.45
		CWI	9.22±2.13	9.13±2.02	9.04±2.31	8.42±2.54	8.26±2.00	11.42±3.12	10.10±3.04
Retinol ($umol.L^{-1}$)	T	Con	0.023±0.011	0.034±0.006	0.029±0.013	0.029±0.007	0.028±0.012	0.029±0.013	0.029±0.013
		CWI	0.022±0.012	0.028±0.008	0.023±0.010	0.023±0.013	0.023±0.021	0.030±0.010	0.024±0.010
Lycopene ($umol.L^{-1}$)	T, G	Con	0.005±0.003	0.009±0.009	0.006±0.003	0.006±0.003	0.007±0.005	0.014±0.015	0.017±0.010
		CWI	0.010±0.009	0.012±0.011	0.010±0.007	0.015±0.025	0.011±0.006	0.020±0.014	0.021±0.013
Alpha-carotene ($umol.L^{-1}$)	B	Con	0.028±0.023	0.046±0.036	0.031±0.031	0.032±0.031	0.025±0.024	0.005±0.010	0.006±0.008
		CWI	0.029±0.027	0.053±0.061	0.025±0.025	0.028±0.027	0.027±0.027	0.008±0.008	0.017±0.027

Beta-carotene ($umol.L^{-1}$)	T, B	Con	0.004±0.002	0.012±0.027	0.003±0.001	0.004±0.004	0.003±0.001	0.017±0.019	0.014±0.014
		CWI	0.008±0.014	0.008±0.007	0.004±0.003	0.005±0.005	0.005±0.005	0.021±0.020	0.022±0.020

Values are presented as mean ± SD. CRP, C-reactive protein; IL-6, interleukin-6; LOOH, lipid hydroperoxides; Con, control group; CWI, cold water immersion group. Significant differences ($P < 0.05$) are displayed for time (T; Pre and Post), group (G; Con and CWI), bout (B; L1, L2 and L3), time x bout interaction (TxB) and time x group interaction (TxG). Individual post hoc analysis data is included within the text of the results.

Figure Legends

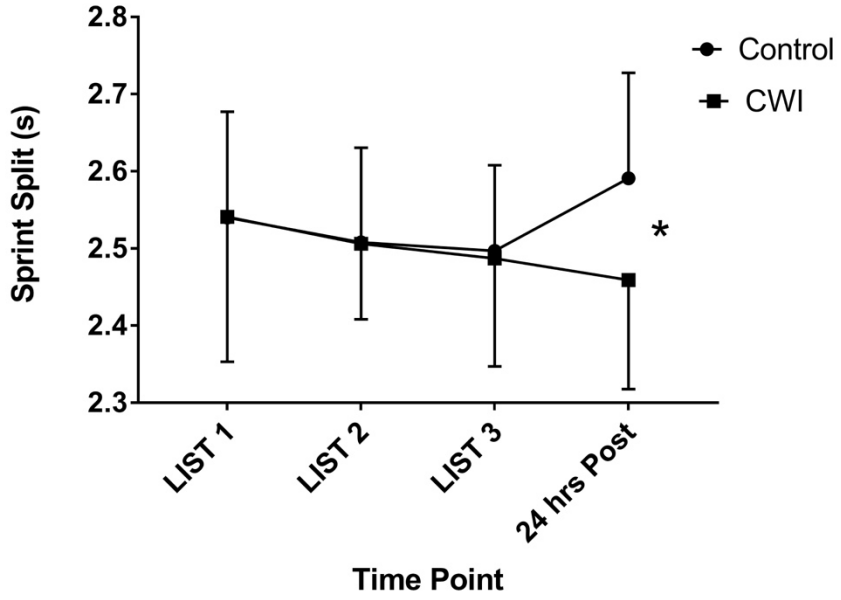

Figure. 1. Average sprint time of the first 5 sprints in each of the three LIST tests compared with 5 sprints completed 24 h following LIST 3. Values are presented as mean ± SD for each group. * denotes significant difference between groups (P<0.05).

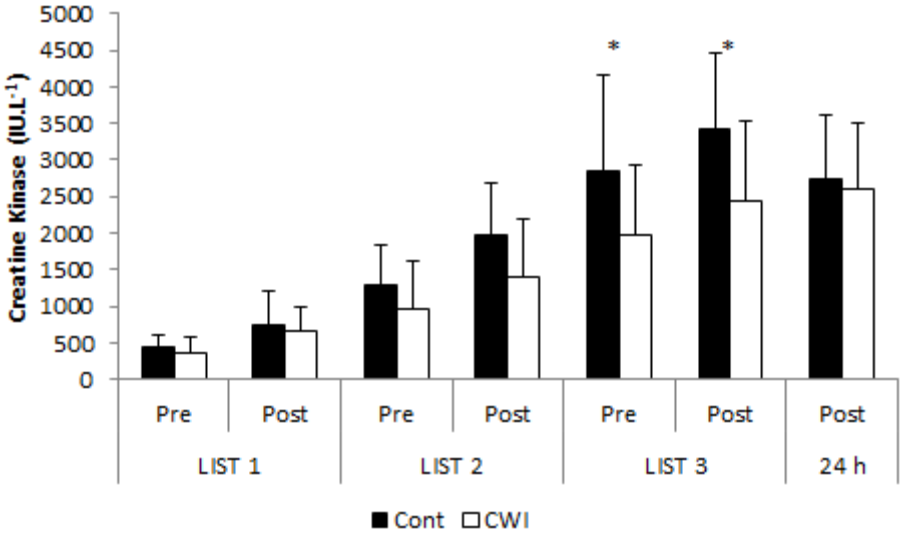

Figure. 2. Creatine kinase measured before and after each LIST, and 24 h following the third LIST. Values are mean ± SD for each group. * denotes between-group difference (P < 0.05).