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Recovery following Rugby Union matches: effects of cold water immersion on markers of fatigue and damage

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Abstract

We investigated the effect of post-match cold-water immersion (CWI) on markers of muscle damage, neuromuscular fatigue and perceptual responses within 72 h after a Rugby match. Twenty-two professional male Rugby players were randomized into CWI ($10^{\circ}\text{C}/10\text{min}$; $n=11$) or Control (CON:30min seated; $n=11$) groups. Activity profile from Global Positioning Satellite systems and post-match rating of perceived exertion were measured to determine match load. Biochemical (tumor necrosis factor alpha [TNF- α], interleukin-6 [IL-6]), neuromuscular performance (squat and countermovement jump [SJ; CMJ], peak power output [PPO], rate of force development [RFD], stiffness, 10- and 30-m sprint time and perceptual markers (soreness, perceived recovery) were obtained at pre, post, 30 min, 24, 48 and 72 h post-match. Magnitude-based inference and Cohen's effect size (ES) were used to analyze change over time and between-groups. Changes were *unclear* for the match loads, sprint times and perceptual markers between-groups. Higher $\%\Delta\text{SJ}$ at 24 h (*very-likely*[ES=0.75]) and in $\%\Delta\text{PPO_SJ}$ at 48 h (*likely*[ES=0.51]) were observed in CWI than in CON. Values in $\%\Delta\text{RDF_CMJ}$ were higher at post (*likely*[ES=0.83]), 30 min (*very-likely*[ES=0.97]) and 24 h (*likely*[ES=0.93]) in CWI than in CON. Furthermore, $\%\Delta\text{LogTNF-}\alpha$ were lower in CWI than in CON group at post (*almost-certainly*[ES:-0.76]), 24 h (*very-likely*[ES:-1.09]) and 72 h (*likely*[ES:-0.51]) and in $\Delta\text{stiffness_SJ}$ at 30 min (*likely*[ES=-0.67]) and 48 h (*very-likely*[ES=-0.97]), as well as, different within-groups effects throughout post-match were reported. Implementing post-match CWI-based strategies improved the recovery of markers of inflammation and fatigue in Rugby players, despite no change in markers of speed or perceptual recovery.

Keywords: Team sports, performance analysis, regeneration strategies, neuromuscular, inflammation, muscle damage.

Introduction

Rugby is an intermittent, high-intensity, contact sport encompassing high-speed runs, sprints, acceleration/decelerations and collision-based activities such as tackling, scrums, rucks and mauls (Johnston et al. 2014; Jones et al. 2015). This combination of physical load induces post-match fatigue, soreness and muscle damage (Johnston et al. 2014; Tavares et al. 2017). Further, the timeline of post-match suppression of performance and existence of damage, perceptual fatigue, and delayed-onset muscle soreness (DOMS) can occur for up to 48-h (Pointon and Duffield 2012; Webb et al. 2013) and even ~4-5 days in Rugby players (McLellan and Lovell 2013; Johnston et al. 2014). Thus, the use of recovery strategies, particularly interventions such as cold-water immersion (CWI), are popular in an attempt to improve the recovery timeline and preparedness for ensuing training/competition.

One of the most common recovery strategies in professional sport is CWI, often used to reduce the symptoms of fatigue and allow faster recovery (Pointon and Duffield 2012; Webb et al. 2013; Tavares et al. 2017). The physiological responses to CWI include decreased skin, tissue, core and muscle temperatures, leading to vasoconstriction and a theoretical reduction of swelling, edema and acute inflammation from muscle damage (Cochrane 2004; Wilcock et al. 2006). Furthermore, the use of CWI can also contribute to a reduction in nerve conduction properties and decrease in muscle spasm and pain (Cochrane 2004; Wilcock et al. 2006). However, despite widespread acceptance in sport, equivocal findings remain, with studies demonstrating beneficial effects of cold therapies on performance, damage and perceptual markers (Gill et al. 2006; Pointon and Duffield 2012; Garcia et al. 2016); whilst others have failed to find benefits of CWI on recovery in Rugby players (Higgins et al. 2013; Lindsay et al. 2015). In part, these differing outcomes have

been related to the contrasting cooling methodological approaches used in the literature. In addition, the lack of sport-specific tests and sensitive markers of exercise-induced muscle damage (EIMD) and fatigue relevant to the sport limit some of the previous findings.

In this regard, Tavares et al. (2017) in a recent literature review study in Rugby players concluded that acutely (<48 h post-match), cold strategies have a beneficial effect on markers of muscle damage (i.e., creatine kinase [CK]), neuromuscular function (i.e., jump height and maximal voluntary contraction [MVC]) and DOMS. However, although different markers of EIMD and fatigue have been previously assessed after CWI (Pointon and Duffield 2012; Higgins et al. 2013; Webb et al. 2013; Lindsay et al. 2015; Tavares et al. 2017), to the best of our knowledge, no previous studies have examined the changes specifically in inflammatory (i.e., interleukin 6 [IL-6], tumor necrosis factor alpha [TNF- α]) and neuromuscular function measured from the vertical ground reaction force (GRF) (Ache-Dias et al. 2016) (i.e., rate of force development [RFD], peak power output [PPO] and stiffness) in Rugby Union players. Consequently, more specific markers can provide additional information about neuromuscular responses following sports-specific fatigue to further understand the effect of CWI on recovery (Tillin et al. 2013; Peñailillo et al. 2015; Maffiuletti et al. 2016; Kennedy and Drake 2017).

Rugby matches are played with rest intervals of 6-8 days, although teams frequently train within this period and prior to subsequent matches. Therefore, recovery is a priority to allow optimal performance in the next training session or match. Given this 96 h timeline of recovery (McLellan and Lovell 2013; Johnston et al. 2014), methods to improve recovery are critical. Thus, the aim of this study was to determine the effect of post-match CWI on markers of inflammation, neuromuscular fatigue, perceptual soreness and recovery within 72 h after a Rugby match. We hypothesized that the CWI after Rugby match will result in

reduction of the inflammatory markers, neuromuscular fatigue and perceptual responses after 72 h, due to the CWI's purported anti-inflammatory and/or analgesic properties.

Material and methods

Participants

Twenty-two male Rugby Union players (25.2 ± 3.6 y; 96.8 ± 16.8 kg; 182.2 ± 6.3 cm), consisting of 11 Backs (24.1 ± 1.6 y; 96.1 ± 17.2 kg; 181.5 ± 6.8 cm) and 11 Forwards (25.6 ± 2.6 y; 97.7 ± 17.1 kg; 182.0 ± 7.09 cm) from a Brazilian professional team volunteered to participate in the study. At the time, athletes were engaged in a training program of 1 session per day for 5 days/week and 1 match/week. Inclusion criteria included performing all testing measures, be free from chronic diseases, not to be taking any medication, nutritional supplements with intracellular buffers, illegal substances or undertaking recovery techniques during the duration of the study. All participants were briefed about the experimental design and signed an informed consent form. The study was approved by a local Institutional Ethics Committee and followed the ethical guidelines of the Declaration of Helsinki.

Experimental design

One week prior to the study, players were familiarized with the physical tests performed, rating scales and procedures and undertook anthropometric measurements. Briefly, in this balance crossover study, the players were separated by playing position (Backs and Forwards) and randomized into CWI ($n=11$), or Control (CON $n=11$) groups by random permute block (available at <http://www.randomization.com>). However, three athletes were excluded from the CON group due to injury during games, thus the CON

group included eight participants. The other players and opposition team were of the same competitive level and participated solely in the friendly match without taking part in any other experimental test. The athletes were separated and randomized in two matches due to the limited number availability of the GPS units. During the experimental approach, athletes refrained from any exercise 24 h before and within 72 h after the match. The consumption of water during the match and post-match recovery period was *ad libitum*; however, players abstained from energy or sports drinks during this period.

Biochemical markers (inflammatory), physical performance (jumps and sprints) and perceptual markers (DOMS and Perceived Recovery Scale [PRS]) were obtained at pre, immediately post (not PRS), as well as 30 min (excluding biochemical markers) 24, 48 and 72 h after a one-off friendly Rugby match. Internal and external loads were measured using the rating of perceived exertion (RPE) and activity profile by Global Positioning System (GPS) during the game. Environmental temperatures during the matches were similar (i.e., $22.5 \pm 0.8^{\circ}\text{C}$, $55.7 \pm 1.5\%$ humidity). On the match day, the players arrived at the University's facilities at 12 p.m. A blood sample was obtained after the participants had been resting for approximately 15 min. Subsequently, DOMS, PRS scale, jumps (squat jump [SJ] and countermovement jump [CMJ]) and sprint abilities (10 m and 30 m) were assessed before the beginning of the match (~3 h p.m), as reported in Figure 1.

****Figure 1****

Match performance

Game movement patterns were obtained from GPS devices sampling at 5 Hz (SPI Elite, GPSports Systems, Australia). Devices were fitted to the upper back of each player using an adjustable neoprene harness. Velocity ranges were selected based on a previous study (McLellan and Lovell 2013). Match activities were divided into the following

categories: total distance covered (m), walking (0-6.0 km.h⁻¹), jogging (6.1-12.0 km.h⁻¹), cruising (12.1-14.0 km.h⁻¹), striding (14.1-18.0 km.h⁻¹), high-intensity running (18.1- 20.0 km.h⁻¹) and sprinting (>20.1 km.h⁻¹). Furthermore, approximately 15-30 min after the end of the match, players were required to report the intensity using the session rating of perceived exertion (s-RPE method) (Foster et al. 2001).

Recovery strategies

After the post-match measures, players in the CWI group submerged their lower limbs up to the iliac crest in a stirred ~10°C cold-water bath for 10 min (Lindsay et al. 2015), following which they undertook testing at 30 min post. The CON group remained seated for 30 min post-match in a controlled environment (22-23°C, 50–60% humidity) and undertook no other external recovery method before undertaking testing.

Biochemical markers

Blood samples were collected from a superficial forearm vein using standard venipuncture techniques. All samples were allowed to clot at room temperature for 30 min collected directly into serum separator collection tubes (Greiner Bio-one; Frickenhausen, Germany) and serum separated by centrifugation at 3.000 ×g 4°C for 15 min. The resulting serum was aliquotted into 3 x 0.5 ml Eppendorf tubes and frozen at -80 °C until the time of assaying (Brüggemann et al. 2017).

Circulating concentrations for total serum of interleukin 6 (IL-6) and tumor necrosis factor alpha (TNF-α) were determined using commercially available DuoSet Sandwich Enzyme-Linked Immunosorbent Assay (ELISA) kits (DuoSet, R&D Systems, Minneapolis, MN, USA) according to the manufacturer's instructions. The concentrations of cytokines

were estimated by interpolation from a standard curve by colorimetric measurements in an ELISA plate reader (Perlong DNM-9602, Nanjing Perlove Medical Equipment Co, Nanjing, China). All results were expressed in $\text{pg}\cdot\text{mL}^{-1}$. Interassay coefficients of variance (CV) ranged from 4.8% and 5.7%, respectively (Brüggemann et al. 2017).

Neuromuscular fatigue assessments

Neuromuscular fatigue was assessed by (1) vertical jump variables, and (2) 10 m and 30 m linear speed. Vertical jumps were performed on a piezoelectric force platform (Kistler® Quattro Jump 9290AD, Winterthur, Switzerland), with a sampling frequency of 500 Hz. Each participant performed three jumps of SJ and CMJ, with a randomized order and a rest interval of 30s between jumps. Jump height, PPO, RFD (30 and 50 $\text{N}\cdot\text{m}\cdot\text{s}^{-1}$) were calculated from GRF of the highest jump, according to Ache-Dias et al. (2016) and vertical stiffness from Morin et al. (2005) equations. In the CMJ protocol, subjects started from a static standing position and were instructed to perform a descent phase, free knee flexion followed by a rapid and vigorous extension of the lower limb joints (ascent phase). Subjects were instructed to jump as high as possible with trunk as vertically as possible and hands remaining on hips. SJ adopted the same instruction, despite of the difference that during the beginning movement all subjects started with knee flexed (90°).

Five minutes after the jumps, the athletes performed a single-sprint performance test consisted of three maximal sprints of 30 m, with a 90s passive rest interval. Each sprint time was recorded using a three pairs photocell system (CEFISE®, Speed Test 4.0, São Paulo, Brazil), with timing gates placed at the 0 m, 10 m and 30 m marks. All sprinting tests were conducted outside on grass play field.

Delayed-onset muscle soreness (DOMS)

Each participant was asked to complete a muscle soreness questionnaire for the lower and upper limbs in which they were required to rank their perception of soreness on a scale from 0 (“absence of soreness”) to 10 (“very intense soreness”). Subjects performed a standardized half squat for lower-body DOMS and a rotation of the trunk for both sides to rate the intensity of soreness in general (Thompson and Nicholas 1999; Vaile et al. 2007).

Perceived recovery status scale (PRS)

The subjects were asked to draw a vertical line that intersected the horizontal descriptor by a visual analogue scale at the appropriate position that best described their perceived level of recovery from 0 (“very poorly recovered”) and 10 (“high-perceived recovery”) (Laurent et al. 2011).

Statistical analyses

Data were analyzed for practical significance using magnitude-based inferences. The smallest worthwhile change (i.e., 0.2 x between-subjects standard deviation SD) and 90% confidence intervals (CI) were determined for between-trials comparisons (Hopkins et al. 2009). The quantitative chances of higher/beneficial, similar/trivial or lower/harmful differences were evaluated qualitatively as follows: <1%, *almost certainly not*; 1% to 5%, *very unlikely*; 5% to 25%, *unlikely*; 25% to 75%, *possible*; 75% to 95%, *likely*; 95% to 99%, *very likely*; >99%, *almost certainly*. True difference was assessed as unclear when the chances of having positive and negative results were both >5%. Threshold values for Cohen’s effect size (ES) statistics were >0.2 (small), >0.5 (moderate), and >0.8 (large). However, just differences classified as equal/higher *likely* and *moderate* effect size were considered for describing changes between groups. Delta changes were used for analysis to

reduce bias arising from uniformity errors. Values are reported as mean values \pm standard deviation (SD).

Results

Internal and external load during the match showed no clear and substantial difference for the all variables between groups (Table 1).

****Table 1****

Within-group Comparisons

Quantitative changes for inflammation, neuromuscular fatigue and perceptual markers within-groups throughout 72 h post-match are showed in table 2 and 3. IL-6 was increased at post and 24 h compared to pre in CWI, and CON. TNF- α decreased at 24 h in CWI; and increased at post and 72 h above pre in the CON group.

CMJ was reduced at post, 30 min, 24, 48 and 72 h compared to pre in CWI, and CON. A decrease in SJ occurred at post, 30 min, 24, 48 and 72 h compared with pre in CWI, and CON.

PPO_CMJ decreased at post and 30 min compared with pre in CWI, but it was unclear for CON, whilst PPO_SJ was reduced at 24 h compared to pre in CWI, and in addition at 48 and 72 h in CON.

RDF_30 CMJ decreased at 30 min and 48 h than pre in CWI, and in addition to post and 24 h in CON. Compared with pre, RDF_30 SJ decreased at 30 min, 24, 48 and 72 h in CWI, and CON. A decrease in RDF_50 CMJ occurred at 30 min, 24 and 48 h compared to pre in CWI, and in addition at post in CON, while RDF_50 SJ was reduced in 30 min, 24, 48 and 72 h compared to pre in CWI, and CON.

Stiffness in CMJ decreased at post, but increased at 48 h compared to pre in CWI, but unclear for CON. A decrease in stiffness in SJ occurred at post, 30 min, 24, 48 and 72 h than pre in CWI, and CON at 72 h.

10 m sprints decreased at post, 30 min, 24, 48 and 72 h than pre in the CWI, and CON. Compared with pre, 30 m sprints decreased at post, 30 min, 24, 48 and 72 h in CWI, and CON, but unclear at 72 h.

Quadriceps DOMS increased at post, 30 min, 24 and 48 h than pre in CWI, and CON, except at 48 h. An increase for hamstrings DOMS occurred at post, 24 and 48 h compared to pre in CWI, and CON, except at 48 h. Trunk DOMS increased at post, 30 min and 24 h than pre in CWI, and CON, but unclear at 24 h. PRS decreased at post, 30 min, 24 and 48 h compared to pre in CWI, and CON.

****Table 2****

****Table 3****

Between-group Comparisons

Changes in $\% \Delta \text{TNF-}\alpha$ for CWI were *almost certainly* lower than in CON group in post ([0/0/100]; ES: -0.76), *very likely* at 24 h ([1/3/96]; ES:-1.09), and *likely* at 72 h ([0/7/93]; ES:-0.51) (Fig 2B), while no clear and substantial difference between groups in $\% \Delta \text{IL-6}$ was observed (Fig 2A).

****Figure 2****

Effects in $\% \Delta \text{SJ}$ were *very likely* higher in CWI at 24 h ([95/4/1]; ES=0.75) and *likely* higher in $\% \Delta \text{PPO_SJ}$ at 48 h ([86/13/1]; ES= 0.51) than in CON group (Fig 3B and 3D). However no clear and substantial differences were evident in $\% \Delta \text{CMJ}$ and $\% \Delta \text{PPO_CMJ}$ (Fig 3A and C).

****Figure 3****

Values reported for the CWI in % Δ RFD_30 CMJ were *likely* higher than in CON group at post ([92/6/2]; ES= 0.83) and 24 h ([93/5/2]; ES= 0.93) and *very likely* higher at 30 min ([95/3/2]; ES= 0.97) (Fig 4A), while no clear and substantial analysis were reported for other measures (Fig 4B, C and D).

****Figure 4****

Changes in Δ stiffness in SJ were *likely* lower in CWI than in CON group at 30 min ([4/12/84]; ES=-0.67) and *very likely* at 48 h ([1/4/95]; ES=-0.97) (Fig 5B). However, Magnitude-based inference analysis showed unclear for Δ stiffness in CMJ (Fig 5A), % Δ sprints (10 m and 30 m) (Fig 5C and D), DOMS (Fig 6A, B and C) and PRS (Fig 6D) between groups.

****Figure 5****

****Figure 6****

Discussion

The present study verified the effects of CWI on post-match recovery relating markers of inflammation, neuromuscular fatigue, and perceptual markers in Brazilian Rugby Union players. As expected, significant impairments were evident with increased inflammation, neuromuscular fatigue, soreness and reduced perceived recovery in both groups within 72-h post-match. Furthermore, CWI positively affected inflammation markers (i.e., TNF- α) and neuromuscular fatigue (i.e., JH, PPO and RFD from CMJ), though no effects were observed in sprint speed or perceptual recovery. Our results were in general agreement with our main hypothesis in which CWI would result in a decrease in inflammatory markers and improved neuromuscular fatigue responses during the 72 h post-match.

To date, the effects of CWI on physiological recovery in Rugby players remain debatable. CWI is suggested to ameliorate EIMD via several mechanisms associated with cooling, hydrostatic pressures and redistribution of blood flow (Cochrane 2004; Wilcock et al. 2006; Ihsan et al. 2016). Cooling therapy induced vasoconstriction is suggested to aid the maintenance of cellular integrity by decreasing circulatory and lymphatic permeability, facilitating interstitial fluid gain and blunting pro-inflammatory events (Wilcock et al. 2006). Although IL-6 concentration peaked 24 h post-match in both groups, no treatment effect was observed. Conversely, a reduced peak in TNF- α was evident 24 h-post following CWI. Previous studies reporting the effects of CWI after EIMD on inflammatory markers are equivocal. For example, Tseng et al. (2013) showed that ice treatment did not influence plasma cytokine concentrations 1 h after eccentric exercise, despite reductions in plasma IL-6 and TNF- α 24 h post-exercise. In addition, CWI has been shown to decrease total leukocyte count and attenuate pro-inflammatory cytokines following EIMD (Pournot et al. 2011). Previous results indicated that CWI induced greater increases in pro- and anti-inflammatory markers after high-volume bouts of resistance exercise (Jajtner et al. 2015). However, other studies show no difference from using CWI on TNF- α (or TNFR1 expression) (Townsend et al. 2013), IL-6 (Gonzalez et al. 2014; Roberts et al. 2014) in Rugby players (Takeda et al. 2014; Lindsay et al. 2015). Whilst speculative, within the scope of the current literature the decrease evident in TNF- α in this study may suggest a role for inflammatory recovery following CWI (Baumert et al. 2016).

Regarding neuromuscular fatigue, CWI showed an improvement in recovery at 48 h post-match in functional tests (SJ) and in more specific and sensitive indicators of muscle function (i.e., PPO and RFD). In addition, despite smaller delta stiffness from SJ were reported in CWI at 30 min and 48 h between groups, unchanged effects were showed

within CON across 48 h post-match. Minett et al. (2014) showed enhanced recovery of MVC following CWI was attributable due to the faster return of central activation achieved via acute reduction in core temperature, by maintenance of contractile force, decreased muscle soreness or/and greater blunted blood-based markers after intermittent-sprint exercise. Webb and colleagues (2013) reported greater effects of CWI on jump height performance measured at 1, 18, and 42 h after professional Rugby league game. In this regard, CWI may have accelerated the rate of neuromuscular recovery (i.e., PPO and RDF) to restore force indices (Cormie et al. 2009; McLellan et al. 2011; Kennedy and Drake 2017). Furthermore, our results are in agreement with Roberts et al. (2014), who demonstrated that CWI enhances the recovery in muscle function as demonstrated by the capability to perform more volitional work in the squat exercise. Thus, this study shows that functional tests that may be more specific indicators of muscle function are improved by post-match CWI. Nevertheless, others studies have failed to find benefits of the CWI using different neuromuscular function (e.g., JH, sprints, reaction time, maximal pedaling power and agility test) (Higgins et al. 2013; Takeda et al. 2014; Garcia et al. 2016). Although speed impairments were reported here, no improvement in recovery with CWI was noted, corroborating previous investigations (Pointon and Duffield 2012; Takeda et al. 2014). Thus, strength tests appear to be more sensitive to a contractile-dependent muscular performance than sprints after CWI therapy (Bailey et al. 2007). Therefore, it is suggested the use of a more specific and greater indirect marker of eccentric exercise-induced muscle damage (Peñailillo et al. 2015; Kennedy and Drake 2017) (e.g., RDF and PPO), especially from jumps, may present different outcomes as reported in the aforementioned studies.

Despite increased DOMS and reduced PRS, no change was reported between groups. The analgesic effects of cooling has been documented (Leeder et al. 2012; White

and Wells 2013), potentially reducing ratings of muscle soreness by mitigating acute tissue edema and ensuing inflammatory responses to muscle damage (Vaile et al. 2007; Bailey et al. 2007). However, no effects were found regarding perceptual markers, controversially noted in studies with Rugby players (Pointon and Duffield 2012; Higgins et al. 2013; Webb et al. 2013; Takeda et al. 2014; Garcia et al. 2016). In fact, a close examination of the literature suggests that the perceived recovery is positively influenced by CWI (Pointon and Duffield 2012). This is in line with the suggestion that most of the effects of CWI are mediated by the placebo effect (Broatch et al. 2014). Furthermore, reductions in soreness may depend on the type of exercise performed (Leeder et al. 2012); whereby endurance-type activity responds more favorably to these recovery modalities than heavy stretch-shortening cycle based activity that is often undertaken by Rugby players. Still, whether and/or how improved perceptual ratings of muscle soreness and recovery may influence subsequent neuromuscular performance is yet to be identified.

This disagreement in aforementioned research findings used to assess the effects of CWI likely relates to the different contraction, physical demands, blood-borne markers and/or psychophysiological stress, the short timeline post-exercise recovery (<48 h) and soreness measures (Pointon and Duffield 2012; Webb et al. 2013; Takeda et al. 2014; Lindsay et al. 2015; Garcia et al. 2016). Nevertheless, while the efficacy of post-exercise cooling in improving biochemical perturbations and neuromuscular functional incurred during exercise is contentious, the greater positive changes in the jump height, PPO and RDF in the CWI post-match in the current study may indicate recovery in Rugby athletes. Finally, although these findings add novel insight into the effects of post-exercise cooling, especially on neuromuscular recovery, it is prudent that some limitations are acknowledged. These include the lack of control over confounding factors as recall foods or

diets, sleep, rest and the measurements of collision during the match. Furthermore, the placebo effect was not controlled in this study.

In conclusion, a Rugby match caused impaired neuromuscular performance, altered blood-borne markers of inflammation and changed perceptual markers of pain/recovery over 72 h post-match in the athletes. However, implementing a single bout of CWI did accelerate recovery, specifically a marker of inflammation and neuromuscular function measured from vertical GRF. In addition, the results suggesting that it is important to use more sensitive and specific markers compared with those usually reported in other studies (e.g., CK, MVC and jump height) to measure the effects of CWI post-match or training. Actually, some recovery markers in the CWI group were greater than CON, supporting the use of either this therapy to accelerate post-match recovery of Rugby players.

Conflict of interest statement

The authors express that there are no conflicts of interest to report.

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Table 1. Internal and external load between CWI and CON group during a Rugby Union matches.

	CWI	CON	ES	CI (90%)	% of chance (rating)
TD (m)	4276.1 ± 1428.5	3783.1 ± 1799.3	0.24	(-0.48 to 0.96)	54/31/15 <i>Unclear</i>
Time match (%)	83.8 ± 19.1	80.6 ± 20.0	0.14	(-0.63 to 0.90)	44/33/22 <i>Unclear</i>
RPE (a.u)	557.4 ± 156.0	601.0 ± 235.3	-0.16	(-0.90 to 0.58)	20/35/46 <i>Unclear</i>
0-6 km.h ⁻¹ (m)	1886.6 ± 703.8	1872.7 ± 777.9	-0.02	(-0.88 to 0.84)	33/31/36 <i>Unclear</i>
6-12 km.h ⁻¹ (m)	1369.9 ± 346.8	1130.9 ± 639.6	-0.62	(-1.92 to 0.68)	14/14/72 <i>Unclear</i>
12-14 km.h ⁻¹ (m)	343.0 ± 121.7	276.0 ± 164.7	-0.50	(-1.50 to 0.50)	12/18/70 <i>Unclear</i>
14-18 km.h ⁻¹ (m)	361.7 ± 187.3	321.3 ± 205.5	-0.19	(-1.05 to 0.66)	21/29/50 <i>Unclear</i>
18-20 km.h ⁻¹ (m)	124.8 ± 105.4	101.6 ± 69.1	-0.20	(-0.86 to 0.47)	15/35/50 <i>Unclear</i>
>20 km.h ⁻¹ (m)	200.7 ± 200.9	180.0 ± 52.4	-0.09	(-0.71 to 0.52)	20/42/38 <i>Unclear</i>
N° <i>Sprints</i>	10.38 ± 9.8	8.4 ± 3.8	-0.18	(-0.82 to 0.46)	15/37/48 <i>Unclear</i>

Note – TD: total distance; RPE: rating of perceived exertion; a.u: arbitrary units; m: meter; N°: number; k.mh⁻¹: kilometer per hour; CI: confidence interval; ES: effect size; CWI: cold water immersion; CON: control.

Table 2. Effect size and quantitative chances for inflammation, neuromuscular fatigue and perceptual markers within-CWI group throughout 72 h post-match.

	Pre x Post		Pre x 30 min		Pre x 24 h		Pre x 48 h		Pre x 72 h	
	ES	% Chance	ES	% Chance	ES	% Chance	ES	% Chance	ES	% Chance
Log IL-6 (pg·ml ⁻¹)	6.72	100/0/0 <i>Almost certainly</i>	-----	-----	0.38	79/20/1 <i>Likely</i>	0.22	53/42/6 <i>Unclear</i>	0.21	52/42/6 <i>Unclear</i>
Log TNF-α (pg·ml ⁻¹)	-0.07	1/89/10 <i>Trivial</i>	-----	-----	0.21	0/49/51 <i>Possible</i>	0.10	21/77/11 <i>Trivial</i>	0.09	6/89/4 <i>Trivial</i>
CMJ (cm)	-0.38	1/13/87 <i>Likely</i>	-0.52	0/2/98 <i>Very likely</i>	-0.28	0/23/77 <i>Likely</i>	-0.20	0/55/45 <i>Possible</i>	-0.20	0/58/42 <i>Possible</i>
SJ (cm)	-0.47	0/6/94 <i>Likely</i>	-0.58	0/1/99 <i>Very likely</i>	-0.20	4/47/48 <i>Possible</i>	-0.55	0/3/97 <i>Very likely</i>	-0.33	0/19/81 <i>Likely</i>
PPO CMJ (w/Kg)	-0.20	2/54/44 <i>Possible</i>	-0.21	0/54/46 <i>Possible</i>	-0.22	0/43/57 <i>Unclear</i>	-0.13	0/76/24 <i>Trivial</i>	-0.12	3/69/29 <i>Unclear</i>
PPO SJ (w/Kg)	0.04	29/49/22 <i>Unclear</i>	0.00	15/70/15 <i>Unclear</i>	-0.20	0/61/39 <i>Possible</i>	-0.11	1/79/20 <i>Trivial</i>	-0.03	5/84/11 <i>Unclear</i>
RDF_30 CMJ (N·m·s ⁻¹)	-0.33	10/27/63 <i>Unclear</i>	-0.50	0/10/90 <i>Likely</i>	0.11	41/37/23 <i>Unclear</i>	-0.33	0/19/81 <i>Likely</i>	-0.17	7/49/44 <i>Unclear</i>
RDF_30 SJ (N·m·s ⁻¹)	-0.08	22/41/37 <i>Unclear</i>	-0.98	0/1/99 <i>Very likely</i>	-0.63	0/3/97 <i>Very likely</i>	-0.50	0/2/98 <i>Very likely</i>	-0.47	0/6/94 <i>Likely</i>
RDF_50 CMJ (N·m·s ⁻¹)	-0.17	20/33/47 <i>Unclear</i>	-0.42	0/14/86 <i>Likely</i>	-0.25	2/39/59 <i>Possible</i>	-0.36	1/20/79 <i>Likely</i>	0.22	56/42/5 <i>Unclear</i>
RDF_50 SJ (N·m·s ⁻¹)	0.10	17/82/1 <i>Trivial</i>	-0.20	5/53/42 <i>Possible</i>	-0.49	0/8/92 <i>Likely</i>	-0.50	0/3/97 <i>Very likely</i>	-0.34	0/15/85 <i>Likely</i>
Stiffness CMJ (kN/m)	-0.21	5/44/51 <i>Possible</i>	-0.23	11/35/54 <i>Unclear</i>	0.17	47/36/17 <i>Unclear</i>	0.26	60/37/3 <i>Possible</i>	0.20	49/39/11 <i>Unclear</i>
Stiffness SJ (kN/m)	-2.22	2/2/96 <i>Very likely</i>	-2.21	3/1/96 <i>Very likely</i>	-2.22	4/3/93 <i>Likely</i>	-5.67	1/0/98 <i>Very likely</i>	-3.63	2/1/97 <i>Very likely</i>
Vel 10 m (m/s ⁻¹)	-0.38	0/12/88 <i>Likely</i>	-0.56	0/2/98 <i>Very likely</i>	-0.39	0/17/82 <i>Likely</i>	-0.42	0/10/90 <i>Likely</i>	-0.20	0/58/42 <i>Possible</i>
Vel 30 m (m/s ⁻¹)	-0.61	0/3/97 <i>Very likely</i>	-0.75	0/1/99 <i>Very likely</i>	-0.54	0/2/98 <i>Very likely</i>	-0.25	0/25/75 <i>Likely</i>	-0.22	0/39/61 <i>Possible</i>
DOMS Qua (a.u)	1.42	98/2/0 <i>Very likely</i>	1.04	95/1/1 <i>Very likely</i>	0.94	96/3/1 <i>Very likely</i>	0.28	62/34/9 <i>Possible</i>	0.12	41/41/18 <i>Unclear</i>
DOMS Ham (a.u)	0.82	88/9/3 <i>Likely</i>	0.30	68/20/12 <i>Unclear</i>	0.79	89/8/2 <i>Likely</i>	0.41	78/20/2 <i>Likely</i>	-0.12	28/28/44 <i>Unclear</i>
DOMS Trunk (a.u)	1.83	98/1/1 <i>Very likely</i>	1.37	98/2/0 <i>Very likely</i>	0.87	91/7/2 <i>Likely</i>	0.00	23/54/23 <i>Unclear</i>	0.25	54/29/17 <i>Unclear</i>
PRS scale (a.u)	-7.22	0/0/100 <i>Almost certainly</i>	-6.09	0/0/100 <i>Almost certainly</i>	-3.32	0/0/100 <i>Almost certainly</i>	-0.50	0/2/98 <i>Very likely</i>	0.00	22/57/22 <i>Unclear</i>

1 Note - Log IL-6: log transformation interleukin 6; Log TNF-α: log transformation tumor necrosis factor alpha; CMJ: countermovement jump; SJ: squat jump; PPO: peak power output; RFD: rate
2 of force development in 30 and 50 m/s⁻¹; Vel: velocity 10 and 30 meters; DOMS: delayed-onset muscle soreness; Qua: quadriceps; Ham: hamstrings; PRS: perceived recovery status; ES: effect
3 size; a.u: arbitrary units; CWI: cold water immersion; CON: control.

Table 3. Effect size and quantitative chances for inflammation, neuromuscular fatigue and perceptual markers within-CON group throughout 72 h post-match.

	Pre x Post		Pre x 30 min		Pre x 24 h		Pre x 48 h		Pre x 72 h	
	ES	% Chance	ES	% Chance	ES	% Chance	ES	% Chance	ES	% Chance
Log IL-6 (pg·ml ⁻¹)	1.04	98/2/0 <i>Very likely</i>	-----	-----	0.20	57/42/1 <i>Possible</i>	-0.07	2/92/8 <i>Trivial</i>	-0.04	3/88/9 <i>Trivial</i>
Log TNF-α (pg·ml ⁻¹)	4.59	98/1/1 <i>Very likely</i>	-----	-----	0.48	63/7/30 <i>Unclear</i>	0.16	48/17/35 <i>Unclear</i>	3.19	97/1/2 <i>Very likely</i>
CMJ (cm)	-0.24	0/35/65 <i>Possible</i>	-0.21	0/52/48 <i>Possible</i>	-0.20	0/59/41 <i>Possible</i>	-0.25	0/26/74 <i>Possible</i>	-0.34	0/18/82 <i>Likely</i>
SJ (cm)	-0.25	0/34/66 <i>Possible</i>	-0.20	0/55/45 <i>Possible</i>	-0.38	0/11/89 <i>Likely</i>	-0.37	0/13/87 <i>Likely</i>	-0.22	0/40/60 <i>Possible</i>
PPO CMJ (w/Kg)	0.08	24/69/7 <i>Unclear</i>	-0.15	1/65/34 <i>Unclear</i>	-0.07	2/86/12 <i>Trivial</i>	-0.14	0/74/25 <i>Unclear</i>	-0.07	1/88/11 <i>Trivial</i>
PPO SJ (w/Kg)	0.06	28/57/14 <i>Unclear</i>	-0.21	0/46/54 <i>Unclear</i>	-0.20	0/55/45 <i>Possible</i>	-0.20	0/52/48 <i>Possible</i>	-0.21	0/59/41 <i>Possible</i>
RDF_30 CMJ (N·m·s ⁻¹)	-0.42	1/16/83 <i>Likely</i>	-0.59	0/3/97 <i>Very likely</i>	-0.39	0/12/88 <i>Likely</i>	-0.56	0/6/94 <i>Likely</i>	-0.20	19/31/50 <i>Unclear</i>
RDF_30 SJ (N·m·s ⁻¹)	-0.16	16/38/46 <i>Unclear</i>	-0.27	1/34/65 <i>Possible</i>	-0.33	0/23/77 <i>Likely</i>	-0.30	0/21/76 <i>Likely</i>	-0.32	0/17/83 <i>Likely</i>
RDF_50 CMJ (N·m·s ⁻¹)	-0.20	0/49/51 <i>Possible</i>	-0.41	0/8/92 <i>Likely</i>	-0.23	0/39/61 <i>Possible</i>	-0.20	1/49/50 <i>Possible</i>	0.14	38/57/5 <i>Unclear</i>
RDF_50 SJ (N·m·s ⁻¹)	0.12	15/82/3 <i>Trivial</i>	-0.33	0/13/78 <i>Likely</i>	-0.41	0/13/87 <i>Likely</i>	-0.41	0/11/87 <i>Likely</i>	-0.48	0/8/92 <i>Likely</i>
Stiffness CMJ (kN/m)	0.25	54/26/20 <i>Unclear</i>	-0.09	29/30/41 <i>Unclear</i>	0.30	60/28/12 <i>Unclear</i>	0.20	50/38/12 <i>Unclear</i>	-0.18	47/42/11 <i>Unclear</i>
Stiffness SJ (kN/m)	0.30	57/25/18 <i>Unclear</i>	-0.07	30/29/41 <i>Unclear</i>	0.19	49/23/28 <i>Unclear</i>	0.04	38/31/31 <i>Unclear</i>	-3.23	4/10/88 <i>Likely</i>
Vel 10 m (m/s ⁻¹)	-0.27	0/36/61 <i>Possible</i>	-0.97	0/1/99 <i>Very likely</i>	-0.98	0/2/98 <i>Very likely</i>	-0.77	0/2/98 <i>Very likely</i>	-0.29	3/33/64 <i>Possible</i>
Vel 30 m (m/s ⁻¹)	-0.29	0/2/73 <i>Possible</i>	-0.75	0/1/99 <i>Very likely</i>	-0.25	0/38/61 <i>Possible</i>	-0.22	0/43/57 <i>Possible</i>	-0.16	3/56/40 <i>Unclear</i>
DOMS Qua (a.u)	0.50	93/6/1 <i>Likely</i>	1.02	88/10/1 <i>Likely</i>	0.89	92/6/2 <i>Likely</i>	0.20	51/45/4 <i>Unclear</i>	0.50	67/17/16 <i>Unclear</i>
DOMS Ham (a.u)	0.32	69/29/2 <i>Possible</i>	0.67	82/12/5 <i>Unclear</i>	0.89	87/11/2 <i>Likely</i>	0.45	66/19/14 <i>Unclear</i>	0.00	27/46/27 <i>Unclear</i>
DOMS Trunk (a.u)	1.58	94/3/3 <i>Likely</i>	1.18	94/4/2 <i>Likely</i>	0.66	73/14/14 <i>Unclear</i>	-0.15	26/28/46 <i>Unclear</i>	0.00	37/26/37 <i>Unclear</i>
PRS scale (a.u)	-5.5	0/0/100 <i>Almost certainly</i>	-4.35	1/0/99 <i>Very likely</i>	-3.49	3/1/96 <i>Very likely</i>	-2.37	1/1/98 <i>Very likely</i>	0.02	24/56/20 <i>Unclear</i>

4 Note - Log IL-6: log transformation interleukin 6; Log TNF-α: log transformation tumor necrosis factor alpha; CMJ: countermovement jump; SJ: squat jump; PPO: peak power output; RFD: rate
5 of force development in 30 and 50 m/s⁻¹; Vel: velocity 10 and 30 meters; DOMS: delayed-onset muscle soreness; Qua: quadriceps; Ham: hamstrings; PRS: perceived recovery status; ES: effect
6 size; a.u: arbitrary units; CWI: cold water immersion; CON: control.

7 **Figures Captions**

8

9 **Figure 1** – Experimental protocol.

10 IL-6= interleukin 6; TNF- α = tumor necrosis factor alpha; CMJ= countermovement jump;
11 SJ= squat jump; PPO= peak power output; RFD= rate of force development; DOMS=
12 delayed-onset muscle soreness; PRS= perceived recovery status; RPE= rating of perceived
13 exertion.

14

15 **Figure 2** – Percentage delta change between interventions throughout the 72-h recovery
16 period for (A) interleukin 6 and (B) tumor necrosis factor alpha. ^a *Almost Certain* moderate
17 effect compared with control group. ^b *Very Likely* large effect compared with control group.
18 ^c *Likely* moderate effect compared with control group. [^] *Almost Certain* large effect
19 compared with pre. ⁺⁺ *Very likely* large effect compared with pre. ⁺ *Likely* small effect
20 compared with pre. ^{*} *Possible* small effect compared with pre. Solid line with circle
21 represents Cold-water immersion (CWI). Dashed line with solid circle represents Control
22 (CON).

23

24 **Figure 3** – Percentage delta change between interventions throughout the 72-h recovery
25 period for (A) height countermovement jump, (B) height squat jump, (C) peak power
26 output for countermovement jump, and (D) peak power output for squat jump. ^a *Very likely*
27 large effect compared with control group. ^b *Very likely* moderate effect compared with
28 control group. [#] *Very likely* moderate effect compared with pre. ⁺ *Likely* small effect
29 compared with pre. ^{*} *Possible* small effect compared with pre. Solid line with circle
30 represents Cold-water immersion (CWI). Dashed line with solid circle represents Control
31 (CON).

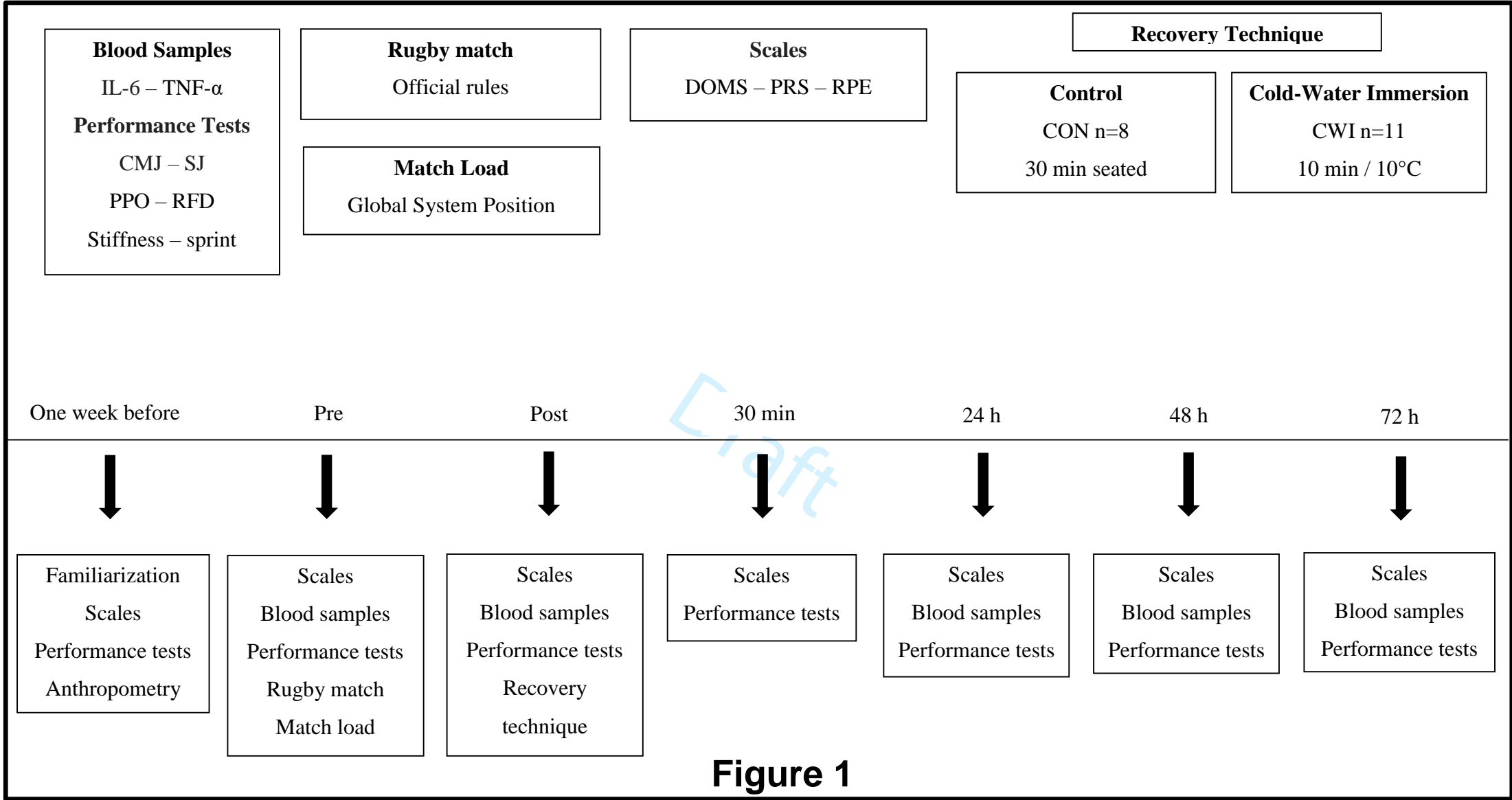
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33 **Figure 4** – Percentage delta change between interventions throughout the 72-h recovery
34 period for (A) rate of force development in 30 N·m·s⁻¹ for countermovement jump, (B) rate
35 of force development in 30 N·m·s⁻¹ for squat jump, (C) rate of force development in 50
36 N·m·s⁻¹ for countermovement jump, and (D) rate of force development in 50 N·m·s⁻¹ for
37 squat jump. ^a *Very likely* large effect compared with control group. ^b *Very likely* moderate

effect compared with control group. ⁺⁺ *Very likely* large effect compared with pre. [#] *Very likely* moderate effect compared with pre. [&] *Likely* moderate effect compared with pre. ⁺ *Likely* small effect compared with pre. ^{*} *Possible* small effect compared with pre. Solid line with circle represents Cold-water immersion (CWI). Dashed line with solid circle represents Control (CON).

Figure 5 – Delta change between interventions throughout the 72-h recovery period for (A) stiffness in countermovement jump, (B) stiffness in squat jump, percentage delta for (C) sprint 10-m, and (D) sprint 30-m. ^a *Very likely* large effect compared with control group. ^b *Very likely* moderate effect compared with control group. ⁺⁺ *Very likely* large effect compared with pre. [#] *Very likely* moderate effect compared with pre. ^{**} *Likely* large effect compared with pre. [&] *Likely* moderate effect compared with pre. ⁺ *Likely* small effect compared with pre. ^{*} *Possible* small effect compared with pre. Solid line with circle represents Cold-water immersion (CWI). Dashed line with solid circle represents Control (CON).

Figure 6 – Change between interventions throughout the 72-h recovery period for (A) for (A) DOMS quadriceps, (B) DOMS hamstrings, (C) DOMS trunk, (D) and perceived recovery scale. [^] *Almost Certain* large effect compared with pre. ⁺⁺ *Very likely* large effect compared with pre. [#] *Very likely* moderate effect compared with pre. ^{**} *Likely* large effect compared with pre. [&] *Likely* moderate effect compared with pre. ⁺ *Likely* small effect compared with pre. ^{*} *Possible* small effect compared with pre. Solid line with circle represents Cold-water immersion (CWI). Dashed line with solid circle represents Control (CON).



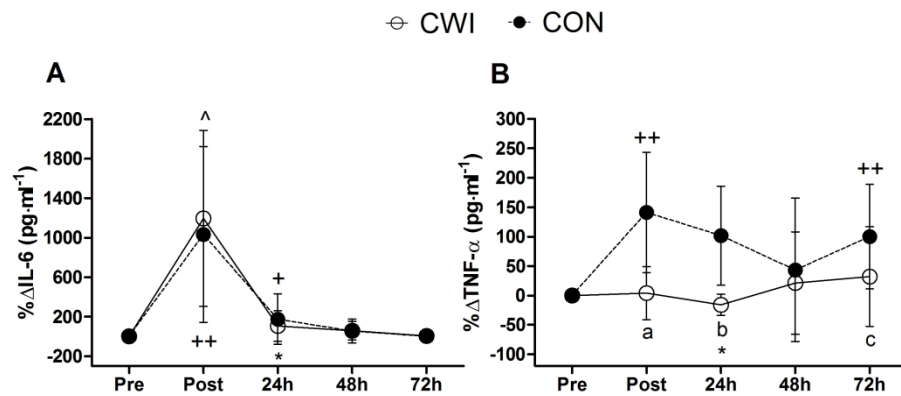


Figure 2

Percentage delta change between interventions throughout the 72-h recovery period for (A) interleukin 6 and (B) tumor necrosis factor alpha. a Almost Certain moderate effect compared with control group. b Very Likely large effect compared with control group. c Likely moderate effect compared with control group. ^ Almost Certain large effect compared with pre. ++ Very likely large effect compared with pre. + Likely small effect compared with pre. * Possible small effect compared with pre. Solid line with circle represents Cold-water immersion (CWI). Dashed line with solid circle represents Control (CON).

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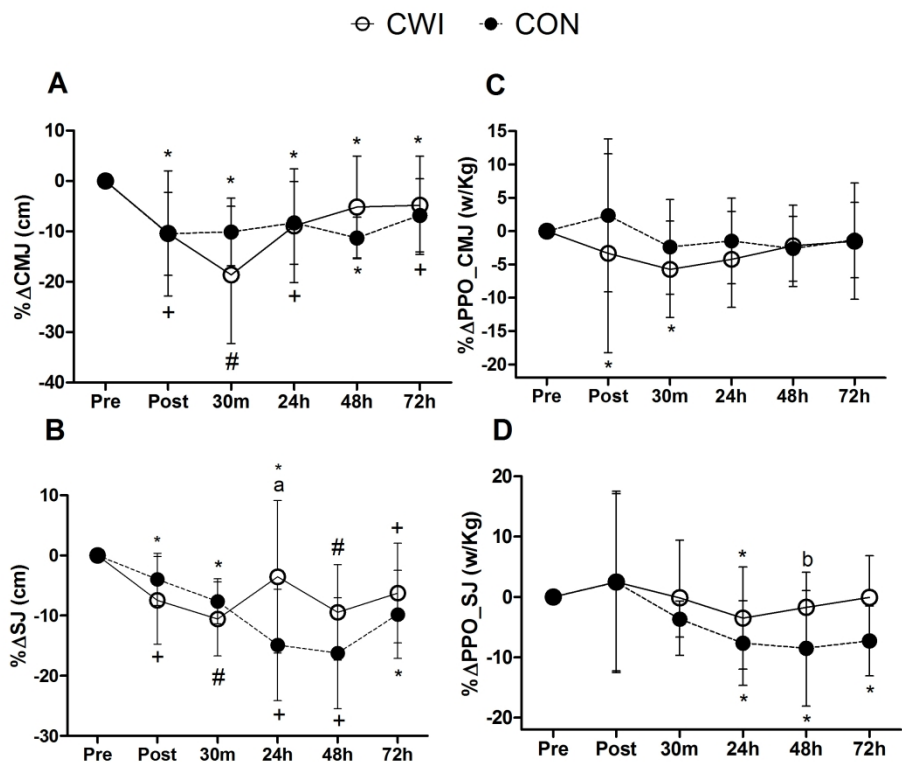


Figure 3

Percentage delta change between interventions throughout the 72-h recovery period for (A) height countermovement jump, (B) height squat jump, (C) peak power output for countermovement jump, and (D) peak power output for squat jump. a Very likely large effect compared with control group. b Very likely moderate effect compared with control group. # Very likely moderate effect compared with pre. + Likely small effect compared with pre. * Possible small effect compared with pre. Solid line with circle represents Cold-water immersion (CWI). Dashed line with solid circle represents Control (CON).

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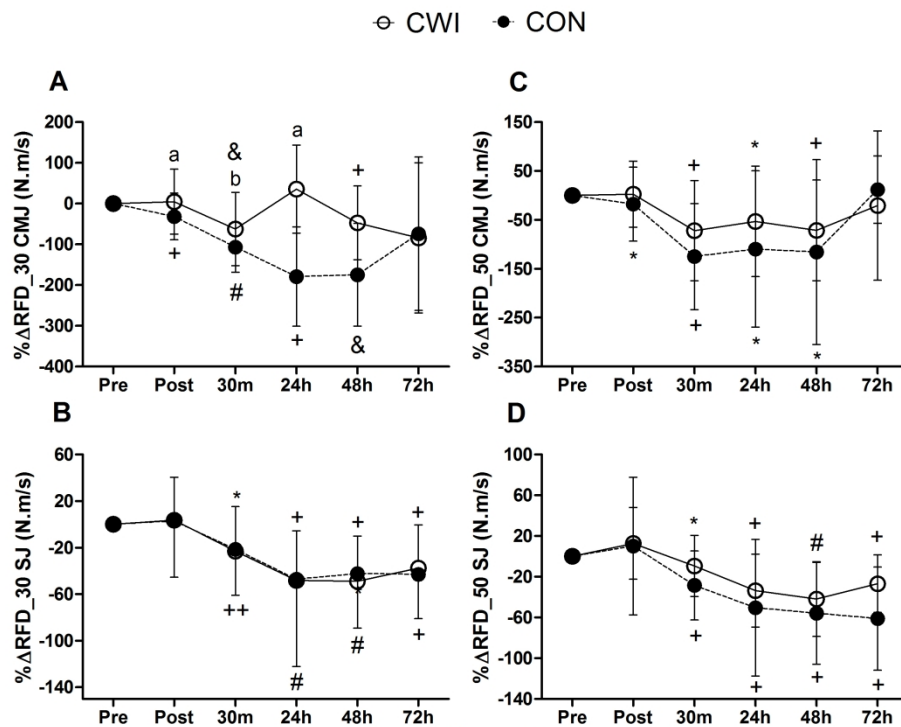


Figure 4

Percentage delta change between interventions throughout the 72-h recovery period for (A) rate of force development in 30 N·m·s⁻¹ for countermovement jump, (B) rate of force development in 30 N·m·s⁻¹ for squat jump, (C) rate of force development in 50 N·m·s⁻¹ for countermovement jump, and (D) rate of force development in 50 N·m·s⁻¹ for squat jump. a Very likely large effect compared with control group. b Very likely moderate effect compared with control group. ++ Very likely large effect compared with pre. # Very likely moderate effect compared with pre. & Likely moderate effect compared with pre. + Likely small effect compared with pre. * Possible small effect compared with pre. Solid line with circle represents Cold-water immersion (CWI). Dashed line with solid circle represents Control (CON).

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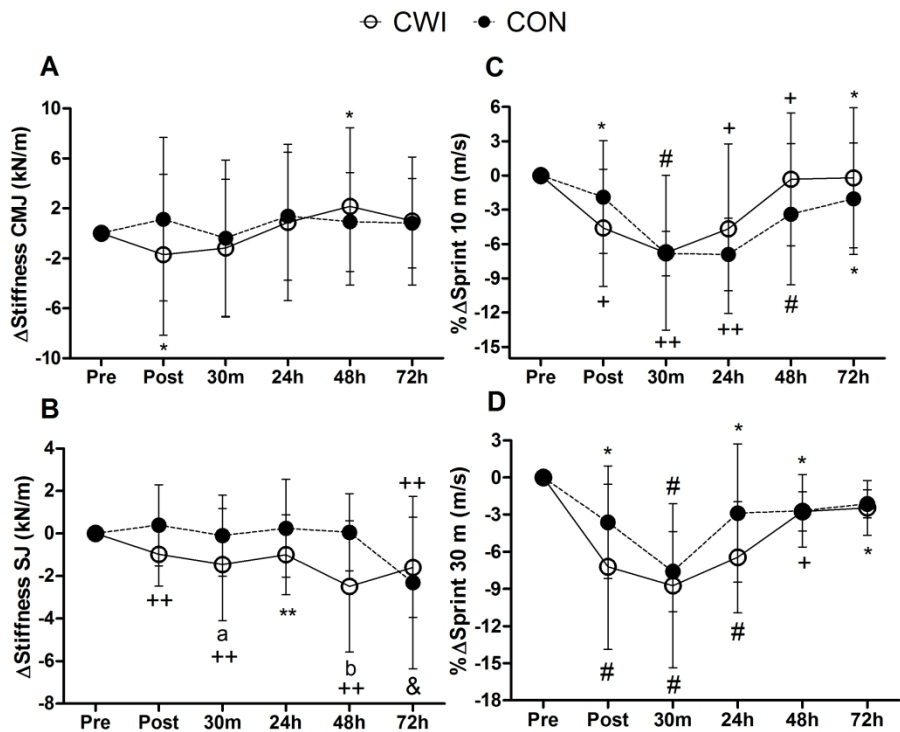


Figure 5

Delta change between interventions throughout the 72-h recovery period for (A) stiffness in countermovement jump, (B) stiffness in squat jump, percentage delta for (C) sprint 10-m, and (D) sprint 30-m. a Very likely large effect compared with control group. b Very likely moderate effect compared with pre. ++ Very likely large effect compared with pre. # Very likely moderate effect compared with pre. ** Likely large effect compared with pre. & Likely moderate effect compared with pre. + Likely small effect compared with pre. * Possible small effect compared with pre. Solid line with circle represents Cold-water immersion (CWI). Dashed line with solid circle represents Control (CON).

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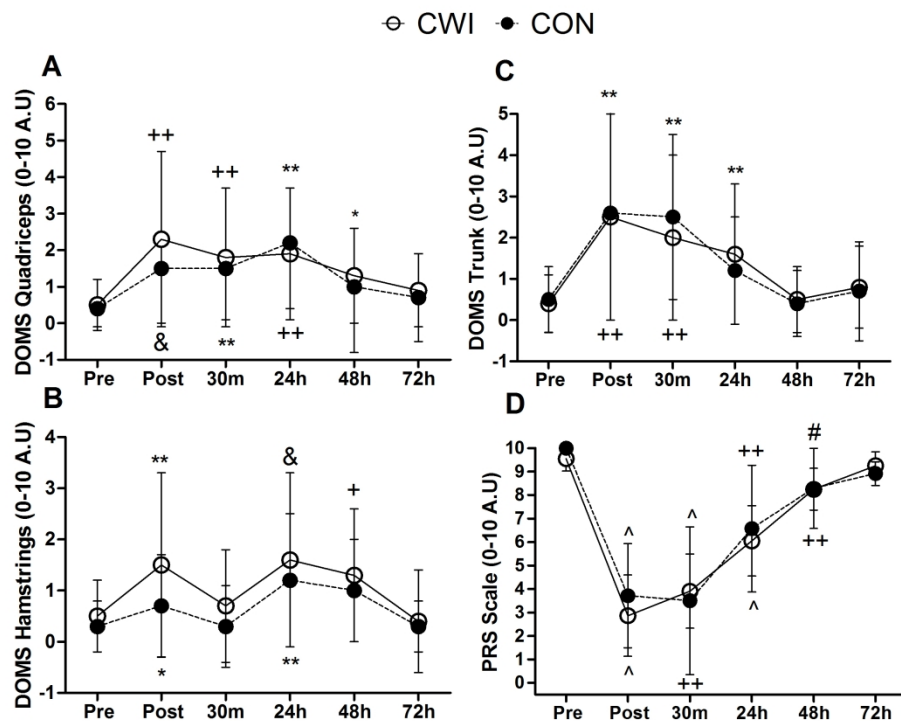


Figure 6

Change between interventions throughout the 72-h recovery period for (A) for (A) DOMS quadriceps, (B) DOMS hamstrings, (C) DOMS trunk, (D) and perceived recovery scale. ^ Almost Certain large effect compared with pre. ++ Very likely large effect compared with pre. # Very likely moderate effect compared with pre. ** Likely large effect compared with pre. & Likely moderate effect compared with pre. + Likely small effect compared with pre. * Possible small effect compared with pre. Solid line with circle represents Cold-water immersion (CWI). Dashed line with solid circle represents Control (CON).

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