

Effects of whole-body cryotherapy on recovery after hamstring damaging exercise: A crossover study

B. Fonda¹, N. Sarabon^{1,2}

¹Laboratory for Motor Control and Motor Behaviour, S2P Ltd, Ljubljana, Slovenia, ²Science and Research Centre, Institute for Kinesiology Research, University of Primorska, Koper, Slovenia

Corresponding author: Nejc Sarabon, PhD, Science and Research Centre, Institute for Kinesiological Research, University of Primorska, Garibaldijeva 1, 6000 Koper, Slovenia. Tel: +386 51341573, Fax: +386 1520 7750, Cellular: +386 40 429 505, E-mail: nejc.sarabon@zrs.upr.si

Accepted for publication 4 March 2013

The purpose of this study was to examine the effects of whole-body cryotherapy (WBC) on biochemical, pain, and performance parameters during the 5-day recovery period after damaging exercise for hamstrings. Participants completed a bout of damaging exercise for the hamstring muscles on two separate occasions (control and experimental condition) separated by 10 weeks. During the control condition, subjects received no treatment after the damaging exercise. The experimental condition consisted of WBC everyday during the recovery period. WBC included single 3-min daily exposures to low temperatures (−140 to −195 °C) in the cryo-cabin. During the recovery period, subjects were tested for biochemical

markers, perceived pain sensation, and physical performance (squat jump, counter movement jump, maximal isometric torque production, and maximally explosive isometric torque production). Majority of the observed variables showed statistically significant time effects ($P < 0.05$) in control group, which indicates the presence of muscle damage. Significant interaction between the control and WBC condition was evident for the rate of torque development ($P < 0.05$). Pain measures substantially differed between the WBC and the control condition after the exercise. Results of this study are not completely supportive of the use of WBC for recovery enhancement after strenuous training.

After unaccustomed exercise, a sensation of discomfort within skeletal muscle, accompanied by a decrease in muscle force, range of motion, and physical performance, can appear in elite or novice athletes (Proske & Morgan, 2001). This sensation also referred to as delayed onset muscle soreness is one of the most common recurrent forms of sports injury and is associated with exercise-induced muscle damage (EIMD). Morphological markers of muscle damage are reported to be related to disruption of contractile and non-contractile proteins (Koh & Escobedo, 2004; Lovering & De Deyne, 2004) and the plasma membrane (Lovering & De Deyne, 2004). Additionally, increases in muscle proteins in the blood (Clarkson & Sayers, 1999), prolonged loss of muscle function (Clarkson & Hubal, 2002), swelling (Chleboun et al., 1998), and muscular microstructural abnormalities detected by imaging techniques (Yu et al., 2004) are observed after EIMD. The question of whether to use any recovery modality when a muscle has been damaged after strenuous exercise has been repeatedly addressed (Barnett, 2006; Bleakley et al., 2012), although the data on which modality to use for recovery are inconclusive.

Cryotherapy has gained increasing popularity as a means of improving recovery after strenuous training

(Barnett, 2006; Bleakley et al., 2012; Leeder et al., 2012). A decrease in tissue temperature stimulates cutaneous receptors to excite the sympathetic adrenergic fibers, causing the constriction of local arterioles and venules (Cheung et al., 2003). This results in a reduction in swelling and a decreased rate of metabolism, which, in turn, attenuate the inflammatory response, vascular permeability, and the formation of edema (Cheung et al., 2003). It has been shown that cryotherapy reduces cell necrosis and neutrophil migration, as well as slowing cell metabolism and nerve conduction velocity, which, in turn, reduce secondary tissue damage (Wilcock et al., 2006). It can be administered in a number of different ways (locally or more generally) and is frequently purported to reduce symptoms that are apparent following a damaging bout of exercise (Barnett, 2006). The most frequently used cryogenic recovery strategy is cold-water immersion as it is probably the best rationale in terms of cost (Bailey et al., 2007; Sellwood et al., 2007; Goodall & Howatson, 2008; Howatson et al., 2009; Rowsell et al., 2009; Ascensão et al., 2010; Bleakley & Davison, 2010; Leeder et al., 2012). Although some studies have shown no attenuation of the signs and symptoms of EIMD following cold-water immersion (Sellwood et al., 2007; Goodall & Howatson, 2008;

Howatson et al., 2009), some reviews demonstrated positive effects on recovery after strenuous exercise (Bleakley et al., 2012; Leeder et al., 2012).

Recently, a new form of cryotherapy, called whole-body cryotherapy (WBC), has been offered to athletes as an alternative to cold-water immersion or other cold exposure (Banfi et al., 2010). WBC involves exposing minimally dressed participants to very cold air (from -110 to -140 °C), either in a specially designed chamber for a short period of time or in a specially designed cabin, in which the head and hands are not exposed. In the cabin, the temperatures can drop even lower (-190 °C), while exposure usually lasts up to 3 min. In sports medicine, WBC has gained wider acceptance as a method to improve recovery from muscle injury. Recently, a review by Banfi et al. (2010) on the application of WBC in athletes was published. They concluded that WBC could be considered as a recovery modality to enhance athletes' recovery (Banfi et al., 2009; Banfi et al., 2010). Although, we should bear in mind that athletes usually have different creatine kinase (CK) response than non-athletes.

The enhancing effects of WBC are anecdotally widely used for recovering from trauma and for preventing overtraining symptoms, although very limited evidence-based knowledge can be found. To the authors' knowledge, only the studies by Costello et al. (2012) and Hausswirth et al. (2011) examined the effects of WBC on performance measures (isometric muscle strength) after EIMD, which has been caused by eccentric exercise and uphill/downhill running, respectively. These two studies reported inconsistent results of WBC as a recovery modality for muscle function after damaging exercise. Furthermore, Pournot et al. (2011) reported that a single session of WBC performed immediately after exercise can enhance muscle recovery by restricting the inflammatory process. Their data confirmed that WBC induces an anti-inflammatory protective effect and suggested that WBC could reduce the recovery time through positive effects on immunological parameters and the regeneration process (Pournot et al., 2011). Data by Pournot et al. (2011) and Hausswirth et al. (2011) have been collected using crossover design and could consequentially be polluted by repeated bout effect (McHugh, 2003; Howatson & van Someren, 2007; Starbuck & Eston, 2012).

Nevertheless, WBC lacks empirical data on sport performance parameters after damaging exercise. Therefore, the aim of the present study was to examine the effects of WBC on biochemical markers, pain, and performance parameters during a 5-day recovery period after a damaging plyometric exercise. It was hypothesized that WBC would have beneficial effects on muscle recovery after such an exercise bout.

Methods

Subjects

Eleven healthy young male adults [(mean \pm SD), age 26.9 ± 3.8 years, height 184.5 ± 7.7 cm, and weight 90.5 ± 3.8 kg] that are

regularly involved in moderate physical activity (agility and endurance) participated in this study. Inclusion criteria were that the subjects were familiar with plyometric exercise, but they did not perform this type of exercise for at least 3 months prior to the study, were not injured or receiving any medications in the last 9 months, were omnivore, and were within normal baseline levels for biochemical markers. Subjects were instructed to maintain their normal eating pattern during the experiment, but they were not permitted to drink alcohol or take any medications or dietary supplements. The interview, during which the details of the study were presented, was carried out prior to the start of the experiment. The study was approved by the Slovenian Medical Ethics Committee (Approval No. 108/01/12) and all subjects signed a statement of informed consent at enrollment.

Protocol

Subjects were randomly assigned into two groups and exposed to a crossover study design. Randomization has been carried out with a custom-written algorithm in LabVIEW (National Instruments, Austin, Texas, USA). The experiment was performed on two separate occasions whereby one group undertook the WBC condition (experimental condition) in the first session, while the other group did not use the recovery modality (control condition). After 10 weeks, on the next occasion, the groups were changed and the second group performed the WBC, while the first group underwent the control condition. On both occasions, the plyometric exercise to damage the muscles was the same. Between the sessions, the subjects were not allowed to participate in any kind of vigorous physical activity.

One day before the damaging exercise, subjects were tested for baseline values. The next day, following a 15-min warm-up (5-min easy running, 10 counter movement jumps and easy stretching for lower extremity) each subject performed a bout of damaging exercise consisting of drop jumps and leg curls (i.e., damaging protocol). Damaging protocol consisted of five sets of 10 drop jumps from a 0.6-m box with an emphasis on hip flexion-extension movement (range of motion $\sim 100^\circ$ from completely extended, i.e., 0°) were performed. Subjects were instructed to execute active amortization and maximally explosive push-off. Drop jumps were followed by five sets of 10 repetitions of bilateral leg curls (75% of concentric 1RM) in the prone-lying position (hips at 20° flexion). Leg curl range of motion was $\sim 90^\circ$, with fast eccentric-concentric coupling at $\sim 10^\circ$ knee flexion angle. Finally, an additional set of 10 repetitions of eccentric leg curls (130% of concentric 1RM; 3-s eccentric action with manually assisted lift) was performed in the same position. The series of drop jumps and leg curl exercises were separated by 1-min breaks. Similar damaging exercises have been used before to damage the muscle (Miyama & Nosaka, 2004). In the present study, we used this kind of exercise over eccentric exercise as it is more sport-specific and has been used in some other studies that examined effects of WBC on EIMD (Hausswirth et al., 2011; Pournot et al., 2011).

During the WBC condition, subjects performed the WBC approximately 1 h after the damaging exercise and at the same time of the day for the next 6 days. For each WBC, a subject was exposed for 3 min to low temperatures (from -140 to -195 °C) using a cryo-cabin (model Space Cabin; Criomed, Ltd, Kherson, Ukraine). It is noteworthy that the temperature is measured on the inner wall of the cabin and not next to the skin. The feet were protected with warm shoes, while the hands and head were not exposed. The subjects were instructed to turn around continuously in the cabin as recommended by the manufacturer.

Blood samples from the cubital vein were collected prior to, and 1, 24, 48, 72, 96 and 120 h after the plyometric exercise bout. After each blood withdrawal, subjects underwent a series of tests in a random order (pain sensation, squat jump, counter movement

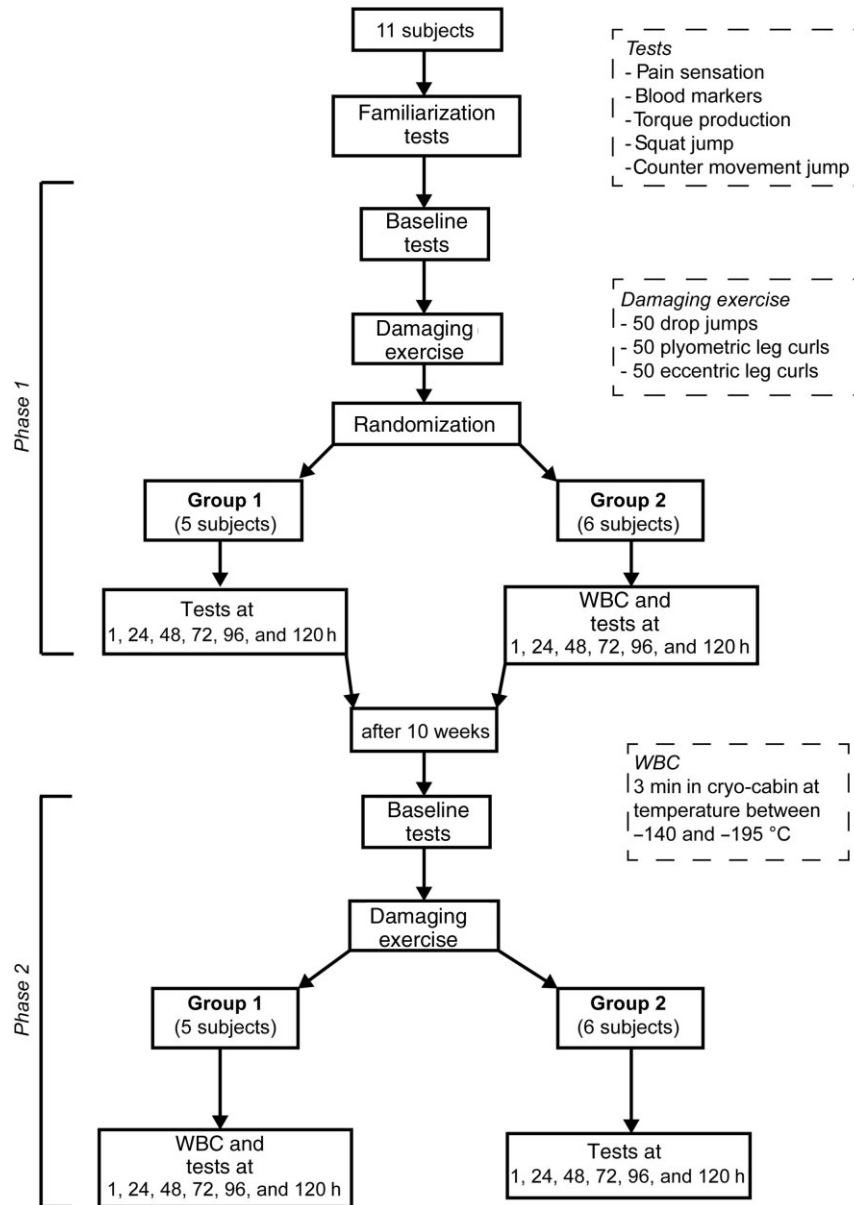


Fig. 1. Graphical presentation of the study protocol. WBC, whole-body cryotherapy.

jump, maximal isometric torque production, and maximally explosive isometric torque production). All dependent variables were taken after the WBC.

Prior to the experiment, the subjects made three visits to familiarize themselves with the testing procedures. During these three visits, they performed all the tests with the exception of taking of the blood samples and on the last familiarization day, they were tested for the concentric 1RM leg curl exercise by standard procedure (Baechle & Earle, 2008). Protocol is summarized in Fig. 1.

Material

Venous blood samples (8 mL) were collected directly into serum separator collection tubes. CK, aspartate aminotransferase, and lactate dehydrogenase were determined using an automated clinical chemistry analyzer (Olympus AU 680; Beckman Coulter, Nyon, Switzerland). Level of analytical sensitivity for CK, aspartate aminotransferase, and lactate dehydrogenase was 2.94, 0.94,

and 2.94 U/L, respectively [coefficients of variations (CVs) 11.4–22.1; intraclass correlation coefficients (ICCs) 0–0.9]. These blood markers were used as they were found to be the most commonly used biochemical markers to confirm the onset of muscle damage (Clarkson & Sayers, 1999). Perceived pain sensation was assessed using a 10-cm visual analog scale from 0 to 10, with 0 indicating no pain and 10 indicating severe pain. Precision of the scale was 1 cm (Mattacola et al., 1997). Squat jumps and counter movement jumps were performed on a force plate (model 9260AA6, Kistler, Winterthur, Switzerland). The signals were acquired at 1000- and 20-Hz low-pass filtered (second-order Butterworth) using commercially available software (MARS by S2P, Kistler) (Sarabon, 2011).

Maximal torque production and explosive contractions were performed on a static knee flexion measurement dynamometer bench (S2P Ltd, Ljubljana, Slovenia). Subjects were positioned prone on the bench with hip flexion of 45° and knees fixed at 60° of flexion. All tests were performed bilaterally. Custom-made software developed in LabVIEW 2010 (National Instruments) was

used for acquisition and analysis of the signals. The force signal was acquired at 1000- and 20-Hz low-pass filtered (second-order Butterworth).

Variables

For squat jump, the following parameters were analyzed: jump height, start power (first 50 ms), maximal force, maximal power, work, and push-off duration (CVs 3.6–25.7; ICCs 0.3–0.8). For counter movement jump, the following parameters were evaluated: jump height, maximal force, maximal power, work, push-off duration, and duration of the counter movement (CVs 3.7–5.9; ICCs 0.3–0.9). Out of three repetitions of squat jumps and three repetitions of counter movement jumps, the highest jump was used for analysis.

For the voluntary maximal torque production, the peak average torque on a 1-s time interval was calculated. Maximally explosive contractions of knee flexion were performed to evaluate the rate of torque development in the first 200 ms (start of torque rise at 3% of peak) (CV 11.6 and 11.7; ICC 0.7 and 0.8, respectively). Out of the three repetitions, the repetition with the highest value was used for later analysis.

Statistical analysis

For each of the measured parameters, means and standard deviations were calculated across subjects. Shapiro–Wilk test was used to test for normality of the distribution. All biochemical markers and squat jump push-off duration parameters were found to be significant. Hence, logarithmic transformation of these parameters has been carried out to achieve normal distribution. Descriptive statistics were used for variables of pain sensation. Differences in other measured variables between conditions and trials were analyzed with three-way repeated measure analysis of variance [Treatment (2) × Time (7) × Order (2)], using treatment and order as the inter-subject factor and time as the intra-subject factor. Bonferroni post-hoc tests were performed for each parameter to test for significant change for each day compared with baseline values and for significant change between groups for each day separately. Before that, Mauchly's test of sphericity was performed and appropriate corrections were used when significant ($P < 0.05$). The level of significance for all tests was set at $P < 0.05$. All statistical analyses were performed using the IBM SPSS statistics 19.0 software (Armonk, Westchester County, New York, USA).

Results

There were no statistically significant changes in baseline values between the two conditions for any of the measured parameters. Tables 1–4 show absolute data for blood parameters and perceived pain sensation, squat jump, counter movement jump, and torque production, respectively. Statistically significant time effects for the majority of the performance measures under the control condition were observed. The peak increase (eightfold) in CK was observed 24 h after the exercise. No significant Time × Condition ($P > 0.05$) interactions were observed for any of the biochemical parameters.

A statistically significant interaction (Time × Condition) was observed between the conditions for pain sensation during rest [$F(2.7, 24.3) = 5.120$; $P = 0.008$] and during squat [$F(2.5, 22.1) = 4.136$; $P = 0.018$].

Values of both pain sensation tests differed statistically significantly between the control and WBC condition 1–72 h after the exercise ($P = 0.038$ – 0.006 and $P = 0.050$ – 0.018 for pain during rest and squat, respectively). At all of these time points, pain values for the WBC condition were lower compared with the control condition, with the differences peaking at 48 h after the exercise ($P = 0.006$ and $P = 0.018$ for pain at rest and during squat, respectively). A statistically significant interaction (Time × Condition) between the conditions was observed for squat jump start power [$F(6, 60) = 3.558$; $P = 0.000$], with a statistically significant change between the control condition and the WBC condition 1 h after the exercise ($P = 0.010$). There was a statistically significant interaction (Time × Condition) between the conditions for maximal torque production [$F(6, 60) = 2.321$; $P = 0.048$] and rate of torque development [$F(6, 60) = 2.663$; $P = 0.036$]. Statistically significant differences in the rate of torque development between the control and WBC condition were evident 24 h after the exercise ($P = 0.012$).

Statistically significant interaction with order (Time × Order) was observed for pain during rest ($F = 6.649$; $P = 0.000$), pain during squat ($F = 6.649$; $P = 0.001$), squat jump start power ($F = 9.998$; $P = 0.000$), and squat jump work ($F = 2.329$; $P = 0.045$). All other interactions (Time × Order) were not statistically significant ($P > 0.05$). Furthermore, statistically significant three-way interactions (Time × Condition × Order) were found for maximal torque production ($F = 2.506$; $P = 0.033$), squat jump height ($F = 3.587$; $P = 0.005$), squat jump start power ($F = 9.998$; $P = 0.000$), squat jump push-off duration ($F = 4.482$; $P = 0.001$), and counter movement jump height ($F = 4.718$; $P = 0.001$). Other three-way interactions were not statistically significant.

Discussion

The present study examined the efficiency of repeated WBC to enhance recovery from EIMD. To the best of our knowledge, this is the first study to examine the effects of WBC on the recovery using combined biochemical, performance and pain measures after a sport-specific bout of damaging plyometric exercise. In line with the research hypothesis, the WBC protocol used in this study showed few enhancements in the recovery process following the damaging exercise as compared to the referent recovery in the control condition.

The plyometric exercise undertaken was successful in inducing EIMD, which was evident from the substantial increase in CK, pain sensation, and the majority of the performance measures in the control condition. Specifically, the largest, almost eightfold increase in CK compared with the baseline value, was evident 24 h after the exercise, while pain sensation was significantly increased throughout the recovery period, peaking 48 h after the exercise. Parameters of the vertical jumps

Table 1. Changes in biochemical markers and pain sensation, reported as mean (standard deviation)

	Control	WBC	P-value
CK (IU/L)	TE, <i>P</i> = 0.050	TE, <i>P</i> = 0.063	Time × Group = 0.578
Baseline	164 (107)	167 (83)	0.154
1 h	263 (167)	299 (137)	0.169
24 h	1265 (1707)	1660 (1693)	0.181
48 h	670 (608)	1021 (1040)	0.168
72 h	1078 (1032)	1265 (2055)	0.586
96 h	1110 (1888)	1073 (1695)	0.951
120 h	764 (1201)	810 (1269)	0.960
AST (IU/L)	TE, <i>P</i> = 0.120	TE, <i>P</i> = 0.000	Time × Group = 0.255
Baseline	24.5 (4.2)	26.3 (5.4)	0.154
1 h	29.4 (10.0)	31.7 (7.7)	0.169
24 h	43.7 (25.7)	69.5 (43.9)	0.181
48 h	39.9 (17.7)	56.3 (32.2)	0.168
72 h	45.0 (32.1)	53.3 (45.3)	0.586
96 h	47.1 (39.9)	48.2 (39.6)	0.951
120 h	42.5 (34.0)	43.5 (33.8)	0.960
LDH (IU/L)	TE, <i>P</i> = 0.325	TE, <i>P</i> = 0.249	Time × Group = 0.872
Baseline	175 (24.0)	189 (28.6)	0.410
1 h	184 (27.5)	194 (27.5)	0.319
24 h	187 (33.7)	210 (43.3)	0.173
48 h	195 (31.1)	213 (37.7)	0.142
72 h	210 (61.8)	214 (78.1)	0.890
96 h	195 (61.4)	197 (53.0)	0.887
120 h	187 (30.6)	195 (37.8)	0.314
Pain rest (cm)	TE, <i>P</i> = 0.000	TE, <i>P</i> = 0.000	Time × Group = 0.008
Baseline	0.0 (0.0)	0.0 (0.0)	0.341
1 h	0.8 (0.9)*	0.1 (0.3)	0.038
24 h	1.7 (1.2)*	0.5 (0.5)*	0.022
48 h	1.9 (1.2)*	0.8 (0.7)*	0.038
72 h	1.3 (0.7)*	0.2 (0.6)	0.006
96 h	0.2 (0.4)	0.0 (0.0)	0.167
120 h	0.1 (0.3)	0.0 (0.0)	0.341
Pain squat (cm)	TE, <i>P</i> = 0.000	TE, <i>P</i> = 0.000	Time × Group = 0.017
Baseline	0.0 (0.0)	0.0 (0.0)	0.341
1 h	2.3 (1.7)*	0.8 (1.1)	0.050
24 h	4.4 (1.6)*	2.6 (1.2)*	0.034
48 h	4.7 (2.1)*	2.8 (1.7)*	0.048
72 h	3.1 (1.7)*	1.5 (1.3)*	0.018
96 h	0.8 (1.0)*	0.4 (0.5)	0.138
120 h	0.4 (0.5)*	0.4 (0.5)	1.000

Time × Group interaction.

**P* ≤ 0.05 Bonferroni post-hoc paired *t*-test compared with baseline.

AST, aspartate aminotransferase; CK, creatine kinase; LDH, lactate dehydrogenase; TE, time effect within group.

showed a similar trend, with the peak drop in performance 24 or 48 h after the exercise. Our data for biochemical, pain, and performance measures concur well with previous literature that examined EIMD (Miyama & Nosaka, 2004; Goodall & Howatson, 2008; Howatson et al., 2009).

Contrary to our study, previous studies (Wozniak et al., 2007; Banfi et al., 2009) reported 30–40% lower CK values in athletes using WBC after training. The reason for this could be the training status of the subjects as athletes have different CK response compared with non-athletes. In line with the data of Hausswirth et al. (2011), the CK response after EIMD was not significantly affected by the WBC. Moreover, due to high inter-subject variation, the interpretation of CK activity should be made with caution. Therefore, the use of biochemical parameters and perceived pain sensation should be dis-

couraged for the purpose of quantifying EIMD and/or functional impairment (Warren et al., 1999).

The ability to develop high forces/torques over short time intervals (Wilson & Murphy, 1996; Ugarkovic et al., 2002) underlies sport-specific movement requirements such as speed, agility and quickness. It has been reported that a rapid increase in motor unit discharge rates and a high incidence of discharge doublets in the early phase of explosive contraction play a crucial role in the rate of torque development (Van Cutsem et al., 1998). In our study, the knee flexion rate of torque development recovered significantly faster in the WBC condition compared with the control condition.

It has been reported (Pournot et al., 2011) that multiple WBC exposures can enhance recovery by decreasing the acute phase inflammatory response after damaging exercise with release of anti-inflammatory

Table 2. Changes in squat jump parameters, reported as mean (standard deviation)

	Control	WBC	P-value
Jump height (m)	TE $P=0.002$	TE $P=0.000$	Time \times Group = 0.322
Baseline	0.265 (0.021)	0.264 (0.026)	0.944
1 h	0.254 (0.021)*	0.255 (0.017)*	0.841
24 h	0.247 (0.024)*	0.237 (0.016)	0.123
48 h	0.247 (0.024)*	0.254 (0.020)	0.422
72 h	0.257 (0.026)	0.258 (0.013)	0.961
96 h	0.260 (0.029)	0.265 (0.023)	0.474
120 h	0.265 (0.026)	0.265 (0.022)	0.926
Start power (W/kg)	TE $P=0.026$	TE $P=0.469$	Time \times Group = 0.000
Baseline	0.380 (0.118)	0.356 (0.111)	0.446
1 h	0.248 (0.055)*	0.330 (0.086)	0.011
24 h	0.247 (0.057)*	0.327 (0.165)	0.223
48 h	0.286 (0.081)	0.310 (0.111)	0.681
72 h	0.369 (0.109)	0.332 (0.124)	0.265
96 h	0.374 (0.111)	0.347 (0.131)	0.375
120 h	0.388 (0.122)	0.326 (0.120)	0.118
Max force (N/kg)	TE $P=0.279$	TE $P=0.038$	Time \times Group = 0.172
Baseline	22.3 (0.9)	22.1 (1.5)	0.650
1 h	21.8 (1.1)	21.3 (1.3)	0.131
24 h	21.4 (1.7)	21.5 (1.6)	0.737
48 h	21.9 (1.0)	21.8 (1.3)	0.797
72 h	21.9 (1.5)	22.0 (1.5)	0.808
96 h	22.2 (1.6)	21.4 (1.9)	0.230
120 h	22 (1.2)	21.0 (1.7)*	0.022
Max power (W/kg)	TE $P=0.000$	TE $P=0.148$	Time \times Group = 0.680
Baseline	47.1 (3.2)	46.1 (3.0)	0.228
1 h	45.3 (2.6)*	45.1 (3.3)	0.877
24 h	44.6 (2.8)*	44.1 (3.1)	0.566
48 h	44.3 (2.8)*	44.8 (2.9)	0.488
72 h	45.9 (2.3)	45.8 (2.7)	0.841
96 h	45.7 (2.7)*	45.7 (4.3)	0.735
120 h	46.2 (2.3)	45.1 (3.7)	0.226
Squat jump work (J)	TE $P=0.028$	TE $P=0.001$	Time \times Group = 0.807
Baseline	7.09 (0.60)	7.04 (0.60)	0.925
1 h	6.82 (0.67)	6.97 (0.44)	0.348
24 h	6.72 (0.75)*	6.62 (0.52)*	0.607
48 h	6.67 (0.62)*	6.99 (0.64)	0.964
72 h	6.72 (0.57)	6.84 (0.41)	0.756
96 h	6.91 (0.76)	7.11 (0.39)	0.682
120 h	7.03 (0.61)	7.30 (0.44)	0.950
Push-off duration(s)	TE $P=0.106$	TE $P=0.078$	Time \times Group = 0.819
Baseline	0.403 (0.044)	0.403 (0.041)	0.935
1 h	0.404 (0.041)	0.408 (0.040)	0.815
24 h	0.404 (0.033)	0.416 (0.065)	0.061
48 h	0.401 (0.026)	0.413 (0.049)	0.087
72 h	0.388 (0.021)	0.393 (0.044)	0.276
96 h	0.411 (0.063)	0.404 (0.039)	0.880
120 h	0.414 (0.024)	0.425 (0.031)	0.149

Time \times Group interaction.

* $P \leq 0.05$ Bonferroni post-hoc paired t -test compared with baseline.

TE, time effect within group.

cytokines and blocked pro-inflammatory cytokines, thus contributing to its beneficial role in muscle tissue protection from secondary muscle damage. Merged results of biochemical parameters, perceived pain sensation and performance measures from our study comprehensively reinforce the findings of previous studies (Wozniak et al., 2007; Banfi et al., 2009; Pournot et al., 2011) that examined WBC as a recovery modality in sport and exercise. In the present study, as well as in some previous studies that reported positive effects of WBC after EIMD (Hausswirth et al., 2011; Pournot et al., 2011), sport-

specific eccentric-concentric muscle contractions were used to elicit EIMD. On the contrary, Costello et al. (2012) used an eccentric exercise protocol (isokinetic leg extensions at an angular velocity of 1.57 rad/s) and reported no beneficial effects of WBC on muscle recovery. Eccentric exercise has been shown to cause more muscle damage than eccentric-concentric exercise (Jamurtas et al., 2000), which could partly explain the differences between the studies. Another noteworthy difference between our study and that of Costello et al. (2012) is that they used unilateral leg extension eccentric

Table 3. Changes in counter movement jump parameters, reported as mean (standard deviation)

	Control	WBC	P-value
Jump height (m)	TE <i>P</i> = 0.005	TE <i>P</i> = 0.675	Time × Group = 0.279
Baseline	0.312 (0.024)	0.304 (0.024)	0.297
1 h	0.298 (0.034)	0.303 (0.023)	0.618
24 h	0.289 (0.033)*	0.302 (0.027)	0.265
48 h	0.300 (0.032)	0.303 (0.022)	0.752
72 h	0.314 (0.036)	0.309 (0.021)	0.406
96 h	0.312 (0.046)	0.313 (0.030)	0.959
120 h	0.314 (0.038)	0.305 (0.030)	0.321
Max force (N/kg)	TE <i>P</i> = 0.000	TE <i>P</i> = 0.000	Time × Group = 0.857
Baseline	23.7 (1.5)	24.0 (1.5)	0.494
1 h	22.8 (1.5)*	23.0 (1.4)*	0.681
24 h	21.3 (1.2)*	21.6 (1.4)*	0.633
48 h	21.2 (1.1)*	21.7 (1.7)*	0.488
72 h	21.6 (1.3)*	22.1 (1.5)*	0.323
96 h	22.5 (1.6)	22.8 (1.5)*	0.403
120 h	22.7 (1.8)	22.5 (1.6)*	0.582
Max power (W/kg)	TE <i>P</i> = 0.001	TE <i>P</i> = 0.231	Time × Group = 0.259
Baseline	46.8 (2.4)	46.6 (3.0)	0.781
1 h	44.7 (3.1)*	45.0 (3.1)	0.511
24 h	44.2 (3.0)*	45.3 (2.6)	0.201
48 h	45.7 (2.9)*	45.2 (2.8)	0.310
72 h	46.2 (3.5)	45.3 (2.6)	0.082
96 h	46.5 (3.5)	45.6 (3.2)	0.339
120 h	46.3 (3.2)	45.5 (3.1)	0.435
Work (J)	TE <i>P</i> = 0.035	TE <i>P</i> = 0.026	Time × Group = 0.807
Baseline	8.52 (1.00)	8.55 (0.54)	0.925
1 h	8.27 (0.68)	8.52 (0.50)	0.348
24 h	8.03 (0.66)	8.19 (0.55)	0.607
48 h	8.25 (0.70)	8.26 (0.48)*	0.964
72 h	8.55 (0.94)	8.49 (0.51)	0.756
96 h	8.50 (0.91)	8.64 (0.66)	0.682
120 h	8.64 (0.79)	8.66 (0.68)	0.950
Push-off duration (s)	TE <i>P</i> = 0.001	TE <i>P</i> = 0.001	Time × Group = 0.188
Baseline	0.317 (0.025)	0.313 (0.020)	0.619
1 h	0.336 (0.022)*	0.339 (0.025)*	0.640
24 h	0.344 (0.027)*	0.334 (0.021)*	0.328
48 h	0.342 (0.026)*	0.334 (0.018)*	0.410
72 h	0.341 (0.029)*	0.323 (0.025)	0.066
96 h	0.339 (0.027)*	0.324 (0.024)	0.062
120 h	0.341 (0.024)*	0.324 (0.024)	0.051
CM duration (s)	TE <i>P</i> = 0.000	TE <i>P</i> = 0.362	Time × Group = 0.332
Baseline	0.492 (0.035)	0.486 (0.052)	0.847
1 h	0.522 (0.043)*	0.481 (0.077)	0.198
24 h	0.548 (0.036)*	0.499 (0.087)	0.152
48 h	0.532 (0.051)	0.499 (0.087)	0.285
72 h	0.523 (0.048)	0.489 (0.076)	0.288
96 h	0.517 (0.042)	0.471 (0.064)	0.050
120 h	0.509 (0.031)	0.477 (0.073)	0.234

Time × Group interaction.

**P* ≤ 0.05 Bonferroni post-hoc paired *t*-test compared with baseline.

CM, counter movement; TE, time effect within group.

exercise without damaging the contralateral leg, while we chose bilateral plyometric exercise as it is more common for sport-specific locomotion and reflects real-life situations. The main limitation of our study is the crossover design and possible influence of repeated bout effect (McHugh, 2003; Howatson & van Someren, 2007; Starbuck & Eston, 2012). We tried to limit that by taking order of treatment as one of the factors in statistical analysis. The influence of repeated bout effect was evident in pain measures, squat jump start power, and squat jump work. However, due to small sample size, it

is difficult to draw conclusions that in other parameters the repeated bout effect was not present. Future research should therefore consider other study designs.

To the authors' knowledge, the present study is also the first study performed using a cryo-cabin and not a cryo-chamber, as used in the majority of the previously published studies (Banfi et al., 2009, 2010; Hausswirth et al., 2011; Pournot et al., 2011; Costello et al., 2012). The main difference between the cabin and the chamber is in the type of exposure and the temperature. In the present study, we used temperatures between −140 and

Table 4. Changes in knee flexion isometric torque parameters, reported as mean (standard deviation)

	Control	WBC	P-value
Max torque (N·m)	TE $P = 0.000$	TE $P = 0.032$	Time \times Group = 0.048
Baseline	215 (41)	195 (35)	0.073
1 h	187 (48)*	178 (37)	0.499
24 h	180 (43)*	180 (44)	0.441
48 h	185 (45)*	194 (52)	0.424
72 h	198 (45)*	203 (45)	0.587
96 h	197 (37)*	200 (44)	0.734
120 h	203 (45)	208 (43)	0.560
RTD 200 ms (N·m/s)	TE $P = 0.000$	TE $P = 0.116$	Time \times Group = 0.036
Baseline	718 (114)	686 (129)	0.377
1 h	645 (132)*	604 (128)	0.350
24 h	586 (133)*	655 (143)	0.017
48 h	604 (145)*	659 (174)	0.030
72 h	635 (104)*	688 (169)	0.159
96 h	661 (121)*	695 (136)	0.216
120 h	676 (102)*	706 (150)	0.350

Time \times Group interaction.

* $P \leq 0.05$ Bonferroni post-hoc paired *t*-test compared with baseline.

RTD, rate of torque development; TE, time effect within group.

–195 °C, while in the cryo-chambers used in other studies (Wozniak et al., 2007; Banfi et al., 2009, 2010; Hausswirth et al., 2011; Pournot et al., 2011; Costello et al., 2012), the mean temperature used was approximately between –110 and –140 °C. However, the temperature provided by the manufacturers should be interpreted with caution as it is measured on the inner wall of the cabin and not next to the skin, where it matters the most.

Perspectives

The results of the present study do not provide conclusive support for the use of WBC as a technique to

enhance functional recovery after EIMD. WBC requires further research in order to be accepted as an effective recovery modality. Research should also be focused on between-subject design to limit the influence of repeated bout effect.

Key words: EIMD, DOMS, regeneration, performance.

Acknowledgements

The authors would like to thank Bostjan Benedetti (Krios Ltd, Slovenia) and Butan Plin Ltd for providing the cryo-cabin and the liquid nitrogen, respectively.

References

- Ascensão A, Leite M, Rebelo AN, Magalhães S, Magalhães J. Effects of cold water immersion on the recovery of physical performance and muscle damage following a one-off soccer match. *J Sports Sci* 2010; 29: 217–225.
- Baechle TR, Earle RW. *Essentials of strength training and conditioning*, 3rd edn. Champaign: Human Kinetics, 2008.
- Bailey DM, Erith SJ, Griffin PJ, Dowson A, Brewer DS, Gant N, Williams C. Influence of cold-water immersion on indices of muscle damage following prolonged intermittent shuttle running. *J Sports Sci* 2007; 25: 1163–1170.
- Banfi G, Lombardi G, Colombini A, Melegati G. Whole-body cryotherapy in athletes. *Sports Med* 2010; 40: 509–517.
- Banfi G, Melegati G, Barassi A, Dogliotti G, Melzi d'Eril G, Dugué B, Corsi MM. Effects of whole-body cryotherapy on serum mediators of inflammation and serum muscle enzymes in athletes. *J Therm Biol* 2009; 34: 55–59.
- Barnett A. Using recovery modalities between training sessions in elite athletes: does it help? *Sports Med* 2006; 36: 781–796.
- Bleakley C, McDonough S, Gardner E, Baxter GD, Hopkins JT, Davison GW. Cold-water immersion (cryotherapy) for preventing and treating muscle soreness after exercise. *Cochrane Database Syst Rev* 2012; (2): CD008262.
- Bleakley CM, Davison GW. What is the biochemical and physiological rationale for using cold-water immersion in sports recovery? A systematic review. *Br J Sports Med* 2010; 44: 179–187.
- Cheung K, Hume P, Maxwell L. Delayed onset muscle soreness: treatment strategies and performance factors. *Sports Med* 2003; 33: 145–164.
- Chleboun GS, Howell JN, Conatser RR, Giesey JJ. Relationship between muscle swelling and stiffness after eccentric exercise. *Med Sci Sports Exerc* 1998; 30: 529–535.
- Clarkson PM, Hubal MJ. Exercise-induced muscle damage in humans. *Am J Phys Med Rehabil* 2002; 81: 52–69.
- Clarkson PM, Sayers SP. Etiology of exercise-induced muscle damage. *Can J Appl Physiol* 1999; 24: 234–248.
- Costello JT, Algar LA, Donnelly AE. Effects of whole-body cryotherapy (–110 °C) on proprioception and indices of muscle damage. *Scand J Med Sci Sports* 2012; 22: 190–198.
- Goodall S, Howatson G. The effects of multiple cold water immersions on indices of muscle damage. *J Sports Sci Med* 2008; 7: 235–241.

Fonda & Sarabon

- Hauswirth C, Louis J, Bieuzen F, Pournot H, Fournier J, Filliard J-R, Brisswalter J. Effects of whole-body cryotherapy vs. far-infrared vs. passive modalities on recovery from exercise-induced muscle damage in highly-trained runners. *PLoS One* 2011; 6: e27749.
- Howatson G, Goodall S, van Someren KA. The influence of cold water immersions on adaptation following a single bout of damaging exercise. *Eur J Appl Physiol* 2009; 105: 615–621.
- Howatson G, van Someren KA. Evidence of a contralateral repeated bout effect after maximal eccentric contractions. *Eur J Appl Physiol* 2007; 101: 207–214.
- Jamurtas AZ, Fatouros IG, Buckenmeyer P, Kokkinidis E, Taxildaris K, Kambas A, Kyriazis G. Effects of plyometric exercise on muscle soreness and plasma creatine kinase levels and its comparison with eccentric and concentric exercise. *J Strength Cond Res* 2000; 14: 68–74.
- Koh TJ, Escobedo J. Cytoskeletal disruption and small heat shock protein translocation immediately after lengthening contractions. *Am J Physiol Cell Physiol* 2004; 286: C713–C722.
- Leeder J, Gissane C, van Someren K, Gregson W, Howatson G. Cold water immersion and recovery from strenuous exercise: a meta-analysis. *Br J Sports Med* 2012; 46: 233–240.
- Lovering RM, De Deyne PG. Contractile function, sarcolemma integrity, and the loss of dystrophin after skeletal muscle eccentric contraction-induced injury. *Am J Physiol Cell Physiol* 2004; 286: C230–C238.
- Mattacola CG, Perrin DH, Gansneder BM, Allen JD, Mickey CA. A comparison of visual analog and graphic rating scales for assessing pain following delayed onset muscle soreness. *J Sport Rehabil* 1997; 6: 38–46.
- McHugh MP. Recent advances in the understanding of the repeated bout effect: the protective effect against muscle damage from a single bout of eccentric exercise. *Scand J Med Sci Sports* 2003; 13: 88–97.
- Miyama M, Nosaka K. Muscle damage and soreness following repeated bouts of consecutive drop jumps. *Adv Exerc Sports Physiol* 2004; 10: 63–69.
- Pournot H, Bieuzen F, Louis J, Filliard J-R, Barbiche E, Hauswirth C. Time-course of changes in inflammatory response after whole-body cryotherapy multi exposures following severe exercise. *PLoS One* 2011; 6: e22748.
- Proske U, Morgan DL. Muscle damage from eccentric exercise: mechanism, mechanical signs, adaptation and clinical applications. *J Physiol* 2001; 537: 333–345.
- Rowell GJ, Coutts AJ, Reaburn P, Hill-Haas S. Effects of cold-water immersion on physical performance between successive matches in high-performance junior male soccer players. *J Sports Sci* 2009; 27: 565–573.
- Sarabon N. Development of software for comprehensive analyses of force plate measurements: technical note. *Kinesiology* 2011; 43: 204–212.
- Sellwood KL, Brukner P, Williams D, Nicol A, Hinman R. Ice-water immersion and delayed-onset muscle soreness: a randomised controlled trial. *Br J Sports Med* 2007; 41: 392–397.
- Starbuck C, Eston R. Exercise-induced muscle damage and the repeated bout effect: evidence for cross transfer. *Eur J Appl Physiol* 2012; 112: 1005–1013.
- Ugarkovic D, Matavulj D, Kukolj M, Jaric S. Standard anthropometric, body composition, and strength variables as predictors of jumping performance in elite junior athletes. *J Strength Cond Res* 2002; 16: 227–230.
- Van Cutsem M, Duchateau J, Hainaut K. Changes in single motor unit behaviour contribute to the increase in contraction speed after dynamic training in humans. *J Physiol* 1998; 513: 295–305.
- Warren GL, Lowe DA, Armstrong RB. Measurement tools used in the study of eccentric contraction-induced injury. *Sports Med* 1999; 27: 43–59.
- Wilcock IM, Cronin JB, Hing WA. Physiological response to water immersion: a method for sport recovery? *Sports Med* 2006; 36: 747–765.
- Wilson GJ, Murphy AJ. The use of isometric tests of muscular function in athletic assessment. *Sports Med* 1996; 22: 19–37.
- Wozniak A, Wozniak B, Drewa G, Mila-Kierzenkowska C, Rakowski A. The effect of whole-body cryostimulation on lysosomal enzyme activity in kayakers during training. *Eur J Appl Physiol* 2007; 100: 137–142.
- Yu J-G, Carlsson L, Thornell L-E. Evidence for myofibril remodeling as opposed to myofibril damage in human muscles with DOMS: an ultrastructural and immunoelectron microscopic study. *Histochem Cell Biol* 2004; 121: 219–227.