Effects of cold water immersion on the recovery of physical performance and muscle damage following a one-off soccer match

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Available online: 16 Dec 2010

To cite this article: António Ascensão, Marco Leite, António N. Rebelo, Sérgio Magalhães & José Magalhães (2011): Effects of cold water immersion on the recovery of physical performance and muscle damage following a one-off soccer match, Journal of Sports Sciences, 29:3, 217-225

To link to this article: http://dx.doi.org/10.1080/02640414.2010.526132
Effects of cold water immersion on the recovery of physical performance and muscle damage following a one-off soccer match

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(Accepted 20 September 2010)

Abstract
The aim of this study was to assess the effects of a single session of cold or thermoneutral water immersion after a one-off match on muscular dysfunction and damage in soccer players. Twenty-male soccer players completed one match and were randomly divided into cryotherapy (10 min cold water immersion, 10°C, n = 10) and thermoneutral (10 min thermoneutral water immersion, 35°C, n = 10) groups. Muscle damage (creatine kinase, myoglobin), inflammation (C-reactive protein), neuromuscular function (jump and sprint abilities and maximal isometric quadriceps strength), and delayed-onset muscle soreness were evaluated before, within 30 min of the end, and 24 and 48 h after the match. After the match, the players in both groups showed increased plasma creatine kinase activity (30 min, 24 h, 48 h), myoglobin (30 min) and C-reactive protein (30 min, 24 h, 48 h) concentrations. Peak jump ability and maximal strength were decreased and delayed-onset muscle soreness increased in both groups. However, differential alterations were observed between thermoneutral water and cold water immersion groups in creatine kinase (30 min, 24 h, 48 h), myoglobin (30 min), C-reactive protein (30 min, 24 h, 48 h), quadriceps strength (24 h), and quadriceps (24 h), calf (24 h) and adductor (30 min) delayed-onset muscle soreness. The results suggest that cold water immersion immediately after a one-off soccer match reduces muscle damage and discomfort, possibly contributing to a faster recovery of neuromuscular function.

Keywords: Soccer, intermittent exercise, cryotherapy, muscle damage, inflammation, soreness

Introduction
The activity of soccer players during a competitive season entails one-week cycles of training, taper, competition, and recovery, with top-level players having additional commitments such as national cups and other knockout matches, or representing their countries in international championships. These competitive demands may impose strains on various physiological systems, including the musculoskeletal system, to an extent where recovery strategies after exercise become influential in preparing for the next match so that performance can be restored to normal as soon as possible (Reilly & Ekblom, 2005).

As muscle damage is an important limiting factor for muscle performance during the days after intense exercise (Clarkson & Hubal, 2002), different methods of alleviating delayed-onset muscle damage, either singly or in combination, have been discussed, including stretching, massage, compression, anti-inflammatory drugs, antioxidants, exercise, and cold water immersion (Barnett, 2006; Cheung, Hume, & Maxwell, 2003).

Post-exercise cold water immersion (cryotherapy) is widely used to treat acute traumatic injury and may be appropriate as a recovery strategy after training and competition that cause some level of traumatic injury (Swenson, Sward, & Karlsson, 1996), although definitive support for its successful application against exercise-induced muscle damage is limited (see Barnett, 2006; Cheung et al., 2003). It has been suggested, however, that it might have some value as a recovery strategy in field settings where players experience acute soft-tissue injury as well as contraction-induced muscle disarrangements that typically result in delayed-onset muscle damage (Bailey et al., 2007; Eston & Peters, 1999; Yanagisawa et al., 2003a). In addition, the effectiveness of contrast and cold water immersions as recovery...
strategies by increasing creatine kinase washout as well as accelerating plasma lactate removal have been reported after rugby matches and intense anaerobic exercise (Banfi, Melegati, & Valentini, 2007; Gill, Beaven, & Cook, 2006; Morton, 2007).

Cryotherapy-induced reductions in cellular, lymphatic, and capillary permeability due to vasoconstriction are also thought to reduce the inflammatory response of damaged muscle, oedema, and pain perception (Wilcock, Cronin, & Hing, 2006). Cold immersion has been shown to reduce cell necrosis, neutrophil migration, as well as slow cell metabolism and nerve conduction velocity, which in turn reduce secondary damage (Wilcock et al., 2006). There are reasons to believe that cryotherapy may be somewhat advantageous against muscle damage symptoms and biochemical markers when more ecological whole-body exercise models are used (Bailey et al., 2007; Montgomery et al., 2008; Yanagisawa et al., 2003a, 2003b). Bailey et al. (2007) reported that a single session of cold water immersion after prolonged field exercise that simulated the activity pattern and the workload imposed by soccer (Nicholas, Nuttall, & Williams, 2000), reduced some indices of exercise-induced muscle damage in healthy active males. However, previous results suggest some differential alterations in physical performance indices and in markers of muscle damage and inflammation between soccer and the field exercise mentioned above (Magalhaes et al., 2010) and thus a more ecological approach is necessary. Recently, Rowsell and colleagues (Rowsell, Coutts, Reaburn, & Hill-Haas, 2009) assessed the effect of cold water immersion on physical test performance and perception of fatigue during a 4-day simulated soccer tournament in which the players played four games in 4 days, suggesting that cryotherapy only reduced the perception of general fatigue and muscle soreness, without any positive effects on muscular function, damage, and inflammation. However, to date no data on the effect of a single session of cryotherapy on neuromuscular, perceptual, and biochemical markers of muscle damage of soccer players during recovery from a one-off soccer match have been published, which is the aim of the present study.

Methods

Participants

Twenty male junior soccer players from two national league teams participated in the study (Table I) after being informed of the aims, experimental protocol, and procedures of the study and providing written informed consent. The protocol was approved by the local Institutional Review Board and adhered to the Declaration of Helsinki. Full backs, defenders, midfielders, and forwards were considered for analysis and only goalkeepers were excluded.

Experimental design and procedures

A schematic representation of the protocol is provided in Figure 1. Briefly, participants were randomly allocated to a thermoneutral water immersion or cold water immersion group before the match. Biochemical, neuromuscular, and perceptual markers of muscle damage were obtained at baseline, within 30 min of the end, and 24 and 48 h after a one-off friendly soccer match.

For 2 weeks before data collection and during the protocol, players were instructed not to change their normal eating habits and to refrain from additional dietary supplementation. One week before the experiments, the players performed the Yo-Yo intermittent endurance test (Bangsbo, 1994) and were familiarized with the functional tests performed. Participants were also instructed to abstain from exhaustive exercise for 48 h before and after the match.

Blood samples, perceived muscle soreness, and functional data (jump and 20-m sprint abilities, and muscle strength) were assessed before, within 30 min of the end, and 24 and 48 h after the friendly match. Environmental temperature during the match was 20°C. On the day of the game, players arrived at the club after an overnight fast of between 10 and 12 h. A resting blood sample was taken after participants had been standing for at least 15 min, after which they consumed a light standardized meal and drink and rested for 2 h. The meal consisted of 1.7 g white bread and 0.3 g of low-fat spread; both values are per kilogram of body mass (D. Thompson et al., 2003). Pre-match jump and sprint abilities and quadriceps strength were assessed during the 2 h between the pre-match meal and the start of the match. Players were required to ingest water in a bolus equal to 5 ml·kg⁻¹ immediately before the match and were allowed to ingest water ad libitum during the game when possible (match interruptions).

<table>
<thead>
<tr>
<th></th>
<th>Thermoneutral water immersion</th>
<th>Cold water immersion</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>18.3 ± 0.8</td>
<td>18.1 ± 1.8</td>
</tr>
<tr>
<td>Height (m)</td>
<td>180.2 ± 0.0</td>
<td>181.6 ± 0.4</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>70.6 ± 5.2</td>
<td>68.4 ± 3.8</td>
</tr>
<tr>
<td>Fat mass (%)</td>
<td>9.6 ± 2.5</td>
<td>8.9 ± 1.8</td>
</tr>
<tr>
<td>Training sessions per week (n)</td>
<td>6.0</td>
<td>6.0</td>
</tr>
<tr>
<td>Yo-Yo test (m)</td>
<td>1217.1 ± 409.1</td>
<td>1321.3 ± 224.8</td>
</tr>
</tbody>
</table>
For 2 days after the match, participants returned to the club after an overnight fast and at approximately the same time of the morning (within 1 h). A blood sample was taken from the forearm vein after the participants had been at complete rest for at least 15 min. Subsequently, perceived muscle soreness was assessed and the players performed the physical performance tests as outlined below.

**Cold water and thermoneutral immersions**

Immediately after the match, players from the cryotherapy group fully submerged their lower limbs to the iliac crest in a stirred cold water bath for 10 min (Bailey et al., 2007). The water was maintained at a mean temperature of 10°C by the addition of crushed ice. During the time of cold water immersion, participants in the thermoneutral group remained in the same long-seated position as their cryotherapy counterparts, fully immersing their lower limbs in a water bath at a mean temperature of 35°C (Rowsell et al., 2009). Room temperature where the immersions were performed was 20°C. Core body temperature was followed at regular intervals throughout the cryotherapy period. Ratings of perceived coldness were assessed during treatment using a visual scale that ranged from 0 (“not cold”) to 10 (“very, very cold”).

**Delayed-onset muscle soreness**

Within 30 min of the end and 24 and 48 h after the match, each participant was asked to complete a muscle soreness questionnaire for quadriceps, hamstring, calf, and hip adductor muscles, in which they rated their perceived muscle soreness on a scale from 0 (“absence of soreness”) to 10 (“very intense soreness”).

**Blood sampling and preparations**

All venous blood samples were taken by conventional procedures using ethylenediaminetetraacetic acid (EDTA) as anticoagulant. The freshly withdrawn blood was immediately centrifuged at 3000 rev · min⁻¹ for 10 min for careful plasma removal. Plasma was separated into several aliquots and rapidly frozen at −80°C for later biochemical analysis of myoglobin, creatine kinase, and C-reactive protein.

**Biochemical assays**

Plasma creatine kinase activity and the concentrations of myoglobin and C-reactive protein were determined spectrophotometrically using commercial test kits (A11A01632, Horiba-ABX, Montpellier, France; myoglobin bioMerieux 30446 and Roche Diagnostics, Carnaxide, Portugal respectively) according to the instructions of the manufacturers. To avoid variations in assay conditions, each assay was performed in duplicate, on the same day, and within one month of blood collection. The inter- and intra-assay coefficients of variation were 4.1–7.3 and 3.6–8.1, respectively.

**Performance tests**

Conventional squat and countermovement jumps were evaluated on a Bosco’s mat (Ergojump, Globus, Italy). The depth of the countermovement was self-selected and represented each player’s optimal depth for maximal jump. Each athlete performed three
jumps and the best jump height was recorded for analysis.

Sprint ability measurements were carried out using telemetric photoelectric cells placed at 0 and 20 m (Brower Timing System, IRD-T175, Draper, UT, USA). The players stood 1 m behind the starting line and began running upon a verbal signal. Timing began when the players crossed the first pair of photocells, and they then ran as fast as they could to complete the 20-m distance. Players completed two runs interspersed by 1 min of recovery and the best time was registered.

Maximal voluntary isometric torque of the quadriceps with knees positioned at 90° of flexion was measured using an isometric loading cell (Tempo Technologies, Globus Ergometer). After a warm-up set of five sub-maximal repetitions of knee extension at the referred angle, players completed two maximal repetitions separated by 60 s of rest. Participants received verbal encouragement and the best performance of the two was recorded.

**Fluid loss and intake**

To determine sweat loss during the match, players were weighed wearing dry shorts immediately before and after the match using a digital scale (Tanita Scale InnerScan Model BC533). Participants’ water intake was recorded.

**Statistics**

Means, standard deviations, and standard errors of the mean were calculated. A repeated-measures analysis of variance (ANOVA) was used to establish differences between treatments over time. When significant F-values were observed, a Bonferroni stepwise adjustment was applied for post-hoc comparisons. For single comparisons only (i.e. anthropometric and physical performance characteristics of soccer players in the two groups; Table I), a paired sample t-test was used to determine whether differences between groups lay. SPSS version 17.0 was used for all analyses. Statistical significance was set at $P < 0.05$.

**Results**

The anthropometric and physical performance characteristics of soccer players from both groups are presented in Table I. No significant differences were observed between groups regarding any variables.

During immersion, perception of coldness was higher in the cold water immersion (mean 7) than thermoneutral water immersion (mean 0.5) group and remained elevated during the 30-min recovery ($P < 0.05$).

As shown in Figure 2, post-match there was a significant increase in creatine kinase activity at 30 min, 24 h, and 48 h for both groups ($P < 0.05$). However, the increases at 24 h and 48 h were higher with thermoneutral water immersion than cold water immersion (Figure 2A). Myoglobin was increased in both treatment groups at 30 min, but more so in the
thermoneutral water than in the cold water group (Figure 2B). C-reactive protein concentrations were also increased in both groups at 30 min and 24 h, but again more so in the thermoneutral water immersion than in the cold water immersion group ($P < 0.05$) (Figure 2C).

A significant decrease in squat jump was observed at 24 h (Figure 3A) and in countermovement jump at 24 h and 48 h (Figure 3B) in the thermoneutral water immersion group ($P < 0.05$). A decrease in countermovement jump was observed only at 24 h in the cold water immersion group ($P < 0.05$) (Figure 3B). No significant differences were observed between groups in countermovement jump performance at any time point. Furthermore, sprint ability was not affected during the recovery and no differences were observed between treatment groups (Figure 3C).

Significant decreases in peak quadriceps strength were observed in the thermoneutral water immersion group at 24 h and 48 h and in the cold water immersion group at 48 h (Figure 3D). However, quadriceps strength was significantly greater at 24 h in the cold water than in the thermoneutral water immersion group ($P < 0.05$).

As shown in Figure 4, delayed-onset muscle soreness peaked at 30 min and again at 24 h for quadriceps, hamstrings, and calf. Cryotherapy only reduced the ratings of perceived soreness at 24 h for quadriceps and calf, and at 30 min for the adductor muscles.

Fluid loss during the game was $0.89 \pm 0.18$ and $0.92 \pm 0.3$ litre (or $1.3 \pm 0.4\%$ and $1.3 \pm 0.3\%$ of body mass) for the thermoneutral and cold water immersion group, respectively. Fluid intake was $0.75 \pm 0.2$ and $0.67 \pm 0.3$ litre, respectively. Thus, total fluid loss was similar between groups: $1.64 \pm 0.3$ and $1.59 \pm 0.3$ litres (or $2.3 \pm 0.3\%$ and $2.3 \pm 0.4\%$ of body mass) for the thermoneutral and cold water immersion group, respectively.

**Discussion**

**Main findings**

The present study was designed to examine the effect of immediate post-exercise cold water immersion on biomarkers of muscle damage, neuromuscular performance, and on perceptual measures of muscle soreness during 48 h recovery after a one-off soccer match. The main findings were that the players who underwent cold water immersion immediately after the match reported lower perceived muscle soreness in the quadriceps and calf at 24 h and hip adductor

![Figure 3.](image-url)
at 30 min, demonstrated a temporary recovery of strength at 24 h, a lower increase in creatine kinase activity up to 48 h, myoglobin concentration at 30 min, and C-reactive protein up to 24 h than players who underwent thermoneutral water immersion. Despite the controversy regarding the efficacy of cryotherapy against exercise-induced neuromuscular disturbances, our results are in line with those of other studies using this method to attenuate the neuromuscular and biochemical signs of muscle damage in exercise models with demands that are similar to those specific to intermittent team sports (Bailey et al., 2007; Ingram, Dawson, Goodman, Wallman, & Beilby, 2009; Montgomery et al., 2008; Rowsell et al., 2009). There is agreement on the ability of immediate cooling to reduce perceived soreness and general fatigue throughout recovery (Bailey et al., 2007; Rowsell et al., 2009), which corroborates the present data.

**Biochemical markers**

Concentrations of both creatine kinase and myoglobin in plasma have been reported to characterize muscle membrane disruption (Clarkson & Hubal, 2002; Clarkson & Sayers, 1999). The effectiveness of cold water therapies on the appearance of intracellular proteins in plasma during recovery from exercise-induced muscle damage is a matter of controversy, with studies reporting beneficial or no effects when conventional severe single muscle groups or the whole body are used as exercise models (Bailey et al., 2007; Banfi et al., 2007; Eston & Peters, 1999; Halson et al., 2008; Howatson, Gaze, & van Someren, 2005; Ingram et al., 2009; Isabell, Durrant, Myrer, & Anderson, 1992; Montgomery et al., 2008; Rowsell et al., 2009; Sellwood, Brukner, Williams, Nicol, & Hinman, 2007; Vaile, Halson, Gill, & Dawson, 2008a, 2008b).

Although lower activity could likely be expected for trained players, the absolute values of creatine kinase observed in the present study are of the same magnitude as those reported elsewhere after a match and after specific field tests designed to simulate a soccer match in elite and non-elite trained players (Ascensao et al., 2008; Bailey et al., 2007; Ispirlidis et al., 2008; Magalhaes et al., 2010). However, it is important to point out that in the study of Ispirlidis et al. (2008) the creatine kinase recovery profile was distinct from that of the present and other studies, as it continued to increase up to 48 hours. Due probably to the different blood kinetics and to inter-individual variability of some of these proteins, it is suggested that their release in response to

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**Figure 4.** Perceived muscle soreness in quadriceps (A), hamstrings (B), calf (C), and hip adductor (D) muscle groups following a one-off soccer match for thermoneutral water immersion (TWI, solid bars) and cold water immersion (CWI, open bars) groups. Values are means and standard deviations. *Significant difference versus baseline for both groups *(P < 0.05). #Significant difference versus TWI group *(P < 0.05). †Significant difference versus 24 h *(P < 0.05);
exercise is protein-specific (Bailey et al., 2007; Lee & Clarkson, 2003). Moreover, due to inter-individual variation, caution in the interpretation of creatine kinase activity as a marker of muscle damage is advised.

Our results demonstrate that cold water immersion was able to significantly attenuate the increased creatine kinase activity and myoglobin concentration observed after the one-off match (Figure 2A and 2B). These results are in line with those of Bailey et al. (2007) for myoglobin but not for creatine kinase after a prolonged shuttle test designed to simulate a soccer game. On the other hand, Banfi et al. (2007) suggested that cold water immersion accompanied by active recovery stabilizes creatine kinase activity in top-level rugby players and can be effective for improving recovery. Recently, others have reported no effect of cold water immersion on muscle damage and inflammation throughout a simulated tournament of four soccer matches on four consecutive days or during the recovery following exhaustive simulated team sports exercise (Ingram et al., 2009; Rowsell et al., 2009).

The mechanism(s) responsible for the lower exercise-induced intracellular protein release to plasma following cold water immersion remains unclear. It has been suggested that cryotherapy might reduce the post-exercise protein efflux from the muscle into the lymphatic system or reduce the amount of post-exercise damage. It is likely that this indirect indication of lower muscle damage could be associated with decreased vessel permeability probably due to cryotherapy-induced attenuation of the inflammatory response (Eston & Peters, 1999). Accordingly, an attenuation in C-reactive protein after the match was observed in the cold water immersion group compared with the thermoneutral water immersion group (Figure 2C). In fact, one of the major characteristics of the inflammatory response resulting from exercise-induced muscle injury is an increase in the permeability of vessel walls. Given that creatine kinase diffuses into the lymph vessels, it is possible that a reduced permeability of these vessel walls induced by cold water immersion reduced the rate of creatine kinase efflux from the muscle. However, further analysis of direct histological markers of muscle damage resulting from this type of therapy would be necessary.

Neuromuscular function

Jump and sprint abilities and muscle strength are frequently used as reliable means of quantifying exercise-induced muscle damage (Warren, Lowe, & Armstrong, 1999). This is supported by the consistent decrease in neuromuscular performance in response to a soccer match, and is accordance with other previous studies (Andersson et al., 2008; Ascensao et al., 2008; Ispirlidis et al., 2008; Magalhaes et al., 2010; Rowsell et al., 2009).

Cold water immersion following a soccer match resulted in a transient attenuation at 24 h for strength, but not for sprint and jump abilities (Figure 3). These findings appear to support the specific sensitivity of a more contractile-dependent muscular performance test such as strength compared with sprint and jump (Bailey et al., 2007; Warren et al., 1999). Accordingly, Rowsell et al. (2009) did not observe any treatment effect of cold water immersion against decrements in repeated sprint and countermovement jump caused by successive soccer matches, which together with our data, support the increased sensitivity of maximal strength. However, this lack of treatment efficacy against sprint abilities is at odds with the facilitated return of repeated sprint performance to baseline of a cold water immersion group versus controls and hot/cold contrast water immersion recently reported during recovery from exhaustive simulated team sports exercise (Ingram et al., 2009).

Perceived soreness

In the present study, the cold water immersion group reported less delayed-onset muscle soreness in hip adductors at 30 min, and in calf and quadriceps at 24 h compared with the thermoneutral water immersion again at the same time points (Figure 4).

The increase in muscle soreness observed following exercise is known to have a biphasic pattern: (i) immediately after exercise due to tissue oedema and/or accumulation of metabolic by-products, and (ii) delayed soreness associated with the inflammatory response and muscle damage (Cheung et al., 2003). With the exception of the hip adductors, no treatment effect against acute-onset muscle soreness was observed.

The precise mechanisms of cooling effects on the reported perception of delayed-onset muscle soreness and pain are unclear. The most widely accepted mechanism associated with cooling-induced reduction of pain perception is its analgesic effect. Indeed, muscle tissue temperatures of 10–15°C reduced nerve conduction velocity, mechanoreceptor activity including muscle spindles with a consequent blunted stretch-reflex response and inhibition of the pain-spasm cycle (Meeusen & Lievens, 1986). However, as the duration of these analgesic-related neural mechanisms is limited to 1–3 h, it is likely that this might only account for the attenuation in delayed-onset muscle soreness observed within 30 min of the end of the match. Other potential benefits of muscle cooling in combination with immersion-related changes in hydrostatic pressure could be associated
with the decrease in tissue oedema (Wilcock et al., 2006).

In general, our results are in line with those of others showing beneficial effects of cold water immersion against increased delayed-onset muscle soreness and general perceptions of fatigue observed after several models of intermittent field exercise, including a soccer match (Bailey et al., 2007; Ingram et al., 2009; Montgomery et al., 2008; Rowsell et al., 2009). In fact, subjective reports of faster recovery are common following cold water immersion.

Methodological issues and limitations

The compressive effects of hydrostatic pressure exerted on the body during water immersion are thought to create a displacement of fluids from the periphery to the central cavity, resulting in multiple physiological changes (Wilcock et al., 2006). These include increased central blood and extracellular fluid volumes via intracellular-intravascular osmotic gradients, and decreased peripheral resistance, contributing to increased removal of metabolic by-products with the potential for enhancing recovery from exercise (Wilcock et al., 2006). However, the recovery benefits from cold observed in the present study are most likely due to the water temperature than hydrostatic pressure, given that none of these alterations was observed in the thermoneutral water immersion group.

It is important to stress that this type of study does not incorporate a placebo control for cooling conditions and thus a treatment effect cannot be dismissed, with athletes commonly reporting enhanced feelings of alertness following cold water immersion. Evidence exists that athletes perform better when they believe they are receiving beneficial treatments (Beedie & Foad, 2009), thus the enhanced perceptions of recovery from increased delayed-onset muscle soreness after a soccer match observed in the current study. Moreover, one possible limitation to be considered in the present study was the absence of a passive control group of players is.

From a practical point of view, some disadvantageous results regarding immediate post-exercise forearm and leg cooling against endurance and resistance training effects on muscle performance and circulatory adaptation were recently described (Yamane et al., 2006). The possible effects of cooling on the modulation of the immune response, hyperthermia and vasodilatation-induced capillary permeability, and variations in oxygen tension with consequent regulation of endothelial nitric oxide synthase expression and subsequent release of the endothelium-derived relaxing factor nitric oxide, cytokine release, vascular endothelial growth factor and heat-related heat shock protein over-expression (Harris, Blackstone, Ju, Venema, & Venema, 2003; Liu et al., 1999; Malm, 2001; Thompson, Maynard, Morales, & Scordilis, 2003) as a consequence of exercise should be analysed. Further research is needed to test advantages and disadvantages of cooling effects after exercise, targeting the molecular mechanisms associated with muscle repair. Further research with ecological value for intermittent-based team sports such as soccer is needed to better clarify the mechanisms associated with this possible benefit.

In conclusion, despite the conflicting research regarding the molecular mechanisms behind muscle adaptation and regeneration after exercise, the present results suggest that cryotherapy applied as an immediate single bout of cold water immersion after a soccer match is effective in reducing some biochemical, functional, and perceptual markers of muscle damage.

Acknowledgements

We would like to thank to soccer players involved in the study for their committed participation. The authors are thankful to the excellent technical and practical assistance and skilful involvement of Emanuel Alves and Luis Pinto. The authors are also grateful to Naval 1° de Maio for providing the pitch and facilities where soccer match and evaluations were carried out. António Ascensão and José Magalhães are supported by grants from the Portuguese Foundation for Science and Technology (SFRH/BPD/42525/2007 and SFRH/BPD/66935/2009, respectively).

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