The Effects of Cold Water Immersion on the Recovery of Physical Performance and Biochemical Factors of Muscle Damage: A randomized Controlled Trial

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Abstract
This study has examined the effects of cold water immersion (CWI) on the Recovery of Physical Performance and Muscular Damage factors following a one boat eccentric exercise. Twenty female healthy elite athletes were voluntary participated and randomly divided into CWI (25 min, 13°C, n=10) and control (no treatment, n=10). Participants performed a delayed onset muscle soreness (DOMS) -inducing Curl Hamstrings protocol consisting of 3 × 15 eccentric contraction (60 seconds recovery between sets) at 80% of 1 repetition maximum (1RM). Muscle damage factors such as creatine kinase (CK) and C-reactive protein with high intensity (HS-CRP) and functional variables such as Pain, swelling, Range of motion of thigh flexors muscles (ROM), Sarjent’s vertical jump (SVJ) and maximal voluntary isometric strength (MVIS) were measured before, 24 and 48h after the exercise. Multiple ANOVA (treatment × time) with repeated measures was used with significant level p<0.05 and when the significant time and interaction effects were observed, a Sidak test and independent t-test were used respectively. No significant difference was observed between groups with regard to changes in Thigh circumference, SVJ. But between variables of pain, ROM, MVIS, CK and CRP were observed significant difference between groups as the changes in above variables were lower than control group (see table 2). The results suggest that cold water immersion immediately and after the eccentric exercise may reduce muscle damage and subsequent discomfort, possibly contributing to a faster recovery of muscle performance.

Keywords: cold water immersion, muscle damage, inflammation, DOMS, female elite athletes

Introduction:
Delayed Onset Muscle Soreness (DOMS) is a predictable painful condition which often occurs after unaccustomed eccentric exercise. The sore muscles are described as feeling stiff, tender, and aching especially after palpation or movement but these common symptoms rarely require medical attention (Elias et al, 2012; Webb et al, 2013). The intensity of soreness increase during the first 24 h, peaks 24-48 h, and subsides within 5-7 d post exercise. Recreational and elite athletes experience DOMS after unaccustomed exercise involving an eccentric muscle-loading component and this occur often introduction of a new phase or type of training. In the previous researches, it is well documented that eccentric contraction produces greater muscle damage and strength deficits than concentric or isometric contractions. This damage is evident as disruption of the normal banding patterns of skeletal muscle and broadening or complete disruption of sarcomere Z lines. Muscle cell damage allows release of enzymes including creatine kinase (CK), with serum CK consistently increased within 1-3 days of eccentric exercise and contributes to strength deficits seen in DOMS (Sellwood et al 2007). Within 8 h of the initial injury, chemo attractants released by the damaged muscle tissue attract neutrophils, which adhere to the endothelium of nearby blood vessels in a process called adhesion or margination. After adhesion, the neutrophils infiltrate the muscle tissue to phagocytosis damaged cells. If neutrophil function is not tightly controlled, healthy tissue is inadvertently destroyed and additional muscle damage occurs. Ultimately, the mechanical disruptions and inflammatory responses activate Type III and IV pain receptors, leading to the sensation of DOMS (Hilbert et al, 2003; Bleakly et al 2014).

Owing to a wide range of clinical features associated with DOMS, such as the lack of understanding of the underlying pathophysiology, many recovery strategies have been used by athletes, coaching staff and health professionals alike, in an attempt to minimize the symptoms and signs of this syndrome (Sellwood et al, 2007).
White et al., 2013). Some strategies proposed to alleviate DOMS include pre and post exercise stretching, light exercise, ultrasound, massage, topical analgesics, and pharmacological agents. None of these treatments, however, completely attenuate (Versey et al., 2012; Jajtner et al., 2014).

In the sports medicine setting, the use of cryotherapy is a widely accepted therapeutic modality (Leeder et al., 2014; Pournot et al., 2011). It has been long used to treat musculoskeletal soreness with the expectation that decreased tissue temperature will result in constriction of local blood vessels, thus diminishing inflammatory response and edema associated with muscle damage symptoms and biochemical markers when more ecological whole body exercise models are used (Darryl, 2004; Wilcock et al., 2006). Bailey et al. (2007) reported that a signal session of cold immersion after prolonged field exercise that simulated the activity pattern and the workload imposed by soccer reduced some indices of exercise induced muscle damage in healthy active males. Roswell and colleagues (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, because of results of previous researches are contradict and to date no data on the effect of cold water immersion on neuromuscular, inflammation and biochemical markers of muscle damage immediately and 24 h after a one boat eccentric exercise have been published, researchers decided to examine the effects of CWI on the recovery of physical performance and muscular damage factors.

**Methods**

**Participants:**

Twenty female elite athletes responded to poster advertisements distributed throughout department of physical education, Payame Noor university of Bonab, Tabriz, Iran. Adults aged > 19 years were eligible for inclusion (Table 1). Participants were healthy, with no history of lower extremity musculoskeletal pathology and who have potential vascular problems for which ice-water immersion is contraindicated (eg, diagnosis of diabetes or Reynaud’s disease) were excluded. They were had a regular menstruation period and none of them hadn’t participated in weight training in past month. Participants signed an informed consent as approved by the university of Tabriz medical science. We instructed participants not to use therapeutic modalities (such as ice or heat), massage, stretching or to ingest any medications during the course of the study. Also we asked participants to refrain from strenuous activity for the duration of their participation in this study.

**Design:** The design of this study consisted of pretest assessment, exercise protocol, posttest and treatment protocol immediately and 24 h after eccentric exercise. Participants reported to the lab one to two days prior for familiarization and baseline measurements. All participants had their height and weight determined on a scale (yagami) and calculated body mass index (BMI) for synchronization between groups. Approximately a week after the familiarization session, participants arrived at the Dr. Salehi laboratory in Bonab for taking blood sample. Venous blood was drawn from the median cubital vein of the right forearm in 8 ml serum separator collection tubes for the measurements of C-reactive protein (CRP) and Creatine Kinase (CK). Then participants went to gymnasium for other measurements and doing exercise. Upon arrival they complete the pain questionnaire mostly of them hadn’t any pain in their body. Pain ratings assessed with a visual analog scale were the dependent variables. After the questionnaire, range of motion (ROM), Sajent’s vertical jump test and maximum voluntary isometric strength with dynamometer were measured. The highest value was recorded as the baseline tests. After them, thigh circumference was measured that we will explain entirely all of measurements in next content.

**Eccentric exercise protocol**

The exercise protocol was carried out on a curl-hamstrings system. The 1RM weight lifted concentrically was determined for each subject. In all, 80% of 1RM was calculated and used as the
weight to be lowered eccentrically using the test leg. Each subject completed 3 sets of 15 repetitions in total, with a 1 min recovery between sets. The eccentric portion of each contraction was emphasized by allowing the participants to raise the weight with the aid of researcher. However, no aid was given during the eccentric phase.

**Measurements:**

Blood samples such as CK, HS-CRP and perceived muscle soreness (PMS), thigh circumference (swelling), range of motion of knee flexors muscle (ROM), Sarjent’s Vertical Jump (SVJ) and maximal voluntary isometric strength (MVIS) were measured and assessed before, 24 and 48 hours after exercise.

**Biochemical assays**

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 CK and HS-CRP. C-reactive protein is an acute-phase serum protein that plays a regulatory role in inflammation and is deposited at sites in the body where acute inflammation occurs. CRP was subsequently measured at a local pathology laboratory using an ELAISA and a particle enhanced immune turbid metric assay kit.

**Perceived Muscle Soreness (PMS)**

We used Graphic Pain Rating Scale (GPRS) of Talag, as developed by Denegar and Perrin, to assess the subject’s perceived level of pain. Graphic rating scales have been shown to be the best available method to measure pain and pain relief. This type of pain scale has been shown to be more sensitive than other scales and is easily used by participants, even if previous experience with the scale is lacking. The subject rated soreness on the scale while performing active knee flexion and active knee extension such as sit to stand. The scale was used in this investigation consisted of a 12-cm line with written descriptors placed both at the extremes and along the continuum. Beginning at the extreme left and proceeding to the right, the descriptors read “no pain, dull ache, slight pain, painful, unbearable pain”. We gave the participants standard instructions on how to complete the scale before each measurement. The participants placed an “X” at the point on the line that best described their pain. We measured the intersecting center point of the “X” to the nearest 0.5 cm, and, therefore, a total of 24 values could be obtained (Kuligowski et al 1998; Kianmarz 2011) (figure 1).

**Swelling**

Measures of thigh circumference were used to indicate swelling of the hamstrings muscle at each of the three reference points marked. Measurements were taken while the one leg of subject set on the seat and had stood by the other one leg. We measured distance of crest of iliac to epicondyle of patella by a flexible tape measure and divided two and then marked 5 cm to sides. The mean of three measurements was determined at each point. All of the measurements have performed by same researcher (Zinuddin et al, 2005; White et al, 2013).

**Range of motion**

Goniometry has been used to assess passive range of motion (ROM) before and after eccentric exercise. Goniometry has been shown to be both reliable and valid for determining ROM. When evaluating the effects of a treatment, other researchers have found that is necessary to use the same investigator when measuring ROM, so as to increase reliability. A pervious study indicated that intratester reliability for assessing knee ROM was r= 0.89. Bilateral knee-flexion passive ROM was measured using a 12-in (30.48 cm) clear plastic goniometer. The goniometer was marked in 1.0° increments. Bilateral knee flexion passive ROM was assessed using a standardized protocol. Participants were positioned on a plinth prone with the hips in 0° of abduction and flexion. A folded towel was placed...
under each distal thigh to stabilize the femur and reduce hip rotation, flexion, and extension. The fulcrum of goniometer was centered over the lateral femoral epicondyle, and the stationary arm of goniometer was aligned with the grater trochanter of the femur. The movable arm of the goniometer was aligned with the lateral midline of the fibula, using the lateral malleolus as a reference. The starting position was 0˚ of Knee extension (see figure 2). Bilateral knee-flexion passive ROM was assessed at the end range when participants either reported pain or soreness or no further movement occurred. Measurements were performed three times and average of them has recorded (Glasgow et al, 2014; Ernesto et al, 2011; Crystal et al, 2013). 

Reliability of the measurements
The same investigator took all the measurements. We used interclass correlation coefficient to analyze the reliability of the measurements with data from 10 participants for the 2 pre-exercise measurements taken during the familiarization session and before exercise. The formula for the interclass correlation coefficient was $R = (MS_s - MS_e)/MS_s$, where $MS_s$ was the mean square for the participants and $MS_e$ was the mean square for error, which is computed as follows: (sums of squares for trials + sums of squares for interaction)/(df for trials + df for interaction). The $R$ values for ROM and thigh circumference were 0.89 and 0.96, respectively.

Treatment protocol
Immediately and 24 h after the exercise, participants of CWI group fully submerged their lower limbs to iliac crest in a stirred cold water bath for 25 minutes. The cold whirlpool temperature was maintained at $12^0$Celsius. Temperature was closely monitored with a battery operated digital thermometer with validity $r= 0.94$ (Mizan Azma, co).

Statistical Analysis
Changes in variables over time were compared between the CWI and control conditions using a multiple ANOVA repeated- measure analysis of variance. When the analysis of variance showed the significant difference between conditions, we applied a Sidak test to find the location of significance. Independent T-test were also used to examine differences between groups. Data analysis was performed using a statistical software package (SPSS version 16.0). Statistical significance was set at $P< .05$ for all analyses. Data are presented as mean ± SEM unless otherwise stated.

Results

![Dynamomete](image)
Table 1 summary the characteristics of participants in the study. No significant difference was noted between the participants in the two treatment groups at baseline with regard to age, height, and weight or body mass index. Participants in both groups reported similar scores on all outcome measures at baseline and there were no significant differences between groups. The eccentric exercise protocol was successful in producing DOMS as indicated by the significant changes from baseline to 48 h in all of variables. The group means and standard deviations for dependent variable measurements of PMS, thigh circumference, ROM, SVJ, MVIS, CK and CRP are presented in tables 2.

Table 1. Characteristics of participants in this study

<table>
<thead>
<tr>
<th>CWI</th>
<th>Control</th>
<th>t</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>22.45±1.43</td>
<td>22.6±1.26</td>
<td>-0.24</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>161±3.9</td>
<td>163±4.96</td>
<td>-0.85</td>
</tr>
<tr>
<td>Weight(kg)</td>
<td>57±5.64</td>
<td>59±8.4</td>
<td>-0.59</td>
</tr>
<tr>
<td>BMI(kg/m²)</td>
<td>21.98±2.45</td>
<td>22.21±3.16</td>
<td>-0.19</td>
</tr>
</tbody>
</table>

As shown in table 2, Perceive Muscle soreness (PMS) developed after the eccentric exercise. The course of development of soreness differed, depending on the type of measurements. Peak soreness for palpation of the hamstrings was reported 1 to 2 days post exercise. Similarly, a significant decrease in ROM and MVIS was observed at 24, 48 h in control group than CWI group (p<0.05). In all sessions the vertical jump decreased after exercise, but without any statistically significant difference (P>0.05) between the groups.

Table 2. Means and Standard Deviations of physical performance variables.

<table>
<thead>
<tr>
<th></th>
<th>CWI</th>
<th>CON</th>
<th>T value</th>
<th>P</th>
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<tbody>
<tr>
<td>Perceive muscle soreness (PMS)</td>
<td></td>
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<tr>
<td>24 h</td>
<td>7.36 ± 1.68</td>
<td>11.6 ± 1.57</td>
<td>-5.92</td>
<td>0.001</td>
</tr>
<tr>
<td>48 h</td>
<td>9.8 ± 1.99</td>
<td>17.1 ± 1.72</td>
<td>-8.9</td>
<td>0.001</td>
</tr>
<tr>
<td>Thigh circumference (swelling)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre exercise</td>
<td>52.85 ± 3.63</td>
<td>54.15 ± 5.95</td>
<td>-0.61</td>
<td>0.54</td>
</tr>
<tr>
<td>24 h</td>
<td>53.93 ± 3.76</td>
<td>55.85 ± 5.85</td>
<td>-0.9</td>
<td>0.37</td>
</tr>
<tr>
<td>48 h</td>
<td>54.11 ± 4.08</td>
<td>54.11 ± 4.08</td>
<td>57 ± 5.28</td>
<td>0.2</td>
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<tr>
<td>Range of Motion (ROM)</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Pre exercise</td>
<td>145.9 ± 3.99</td>
<td>143.54 ± 7.26</td>
<td>-0.5</td>
<td>0.62</td>
</tr>
<tr>
<td>24 h</td>
<td>145.48 ± 4.83</td>
<td>139.38 ± 4.96</td>
<td>6.12</td>
<td>0.001</td>
</tr>
<tr>
<td>48 h</td>
<td>142.88 ± 3.2</td>
<td>131.8 ± 6.63</td>
<td>5.62</td>
<td>0.001</td>
</tr>
<tr>
<td>Sarjent’s Vertical Jump (SVJ)</td>
<td></td>
<td></td>
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<td></td>
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<tr>
<td>Pre exercise</td>
<td>27.5 ± 4.67</td>
<td>28.06 ± 3.79</td>
<td>-0.29</td>
<td>0.77</td>
</tr>
<tr>
<td>24 h</td>
<td>27.75 ± 5.07</td>
<td>28.95 ± 3.48</td>
<td>-0.62</td>
<td>0.54</td>
</tr>
<tr>
<td>48 h</td>
<td>29.32 ± 5.36</td>
<td>33.18 ± 7.05</td>
<td>-1.14</td>
<td>0.17</td>
</tr>
<tr>
<td>Maximal Voluntary Isometric Strength (MVIS)</td>
<td></td>
<td></td>
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<tr>
<td>Pre exercise</td>
<td>49.9 ± 7.04</td>
<td>52.9 ± 8.83</td>
<td>-0.86</td>
<td>0.4</td>
</tr>
<tr>
<td>24 h</td>
<td>45.45 ± 6.68</td>
<td>38.2 ± 8.36</td>
<td>2.2</td>
<td>0.04</td>
</tr>
<tr>
<td>48 h</td>
<td>43 ± 6.3</td>
<td>34.1 ± 6.27</td>
<td>3.27</td>
<td>0.004</td>
</tr>
<tr>
<td>Creatine kinase (CK)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre exercise</td>
<td>89.63±29.91</td>
<td>99.5 ± 52</td>
<td>-0.535</td>
<td>0.598</td>
</tr>
<tr>
<td>24 h</td>
<td>192.64±52.41</td>
<td>376.6±87.2</td>
<td>-5.922</td>
<td>0.001</td>
</tr>
<tr>
<td>48 h</td>
<td>257.27±56.3</td>
<td>511.4±125.3</td>
<td>-6.094</td>
<td>0.001</td>
</tr>
<tr>
<td>HS-CRP</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Pre exercise</td>
<td>0.7 ± 0.27</td>
<td>0.63±0.22</td>
<td>0.643</td>
<td>0.54</td>
</tr>
<tr>
<td>24 h</td>
<td>2.27±0.26</td>
<td>2.79±0.33</td>
<td>-3.99</td>
<td>0.001</td>
</tr>
<tr>
<td>48 h</td>
<td>1.33±0.35</td>
<td>2.51±0.45</td>
<td>-6.7</td>
<td>0.001</td>
</tr>
</tbody>
</table>
Similarly, no significant differences were found for thigh circumference between the three sessions and as shown in table 3, post exercise was a significant increase in CK activity and CRP at 24 and 48 h for both groups. However, the increases at 24 and 48 h in control group were significantly higher than CWI group.

**Discussion**

The purpose of this study was to investigate the effectiveness of cold water immersion (CWI) immediate and 24 h after eccentric exercise on physical performance, biochemical markers of muscle damage and inflammation, and perceptual measures of muscle soreness during 48 h recovery after exercise. The main findings of present study were as follows: (1) CWI alleviated symptoms of DOMS at 24, 48 h post exercise and was effective at 24 and 48 h following eccentric exercise. (2) CWI had a small but significant effect in reducing efflux of CK post exercise. (3) CWI was effect on recovery of muscle strength but had no effective in swelling and vertical jump.

In the present study, the cold water immersion group reported less delayed-onset muscle soreness in hamstrings muscles compared with the control group. Increase in pain and reductions in hamstrings strength at 24 h in both groups further attest to the success of the exercise protocol in inducing DOMS. One proposed mechanism of CWI is a cold-induced reduction in muscle blood flow and tissue temperature causing decreased inflammation induced via eccentric exercise. Gregson et al recently showed reduced femoral artery blood flow (>40%) and muscle temperature (2-4 °C) following 10 min of immersion in 8 °C and 22 °C water. The proposed reduction in inflammation is associated with a reduction of the sensation of pain caused by reducing the osmotic pressure of exudate, which pressurizes nociceptors, signaling via type IV afferent fibers. Cold-induced vasoconstriction is thought to reduce permeability of cellular, lymphatic and capillary vessels, which reduces fluid diffusion into the interstitial space, which is the suggested mechanism of reduced inflammation following muscle – damaging exercise. The reduction in muscle tissue temperature following CWI is another proposed mechanism of CWI and may reduce metabolic enzymatic activity and limit secondary hypoxic damage to uninjured cells (Duffield et al 2014).

**Biochemical markers**

Concentrations of both creatine kinase and C-reactive protein in plasma have been reported to characterize muscle membrane disruption (Williams et al., 2011; Ascensao et al., 2011; Glasgow et al., 2014). The effectiveness of cold water therapis on the appearance of intercellular 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 signal muscle mass or the whole body are used as exercise models (Bailey et al., 2007; Banfi et al., 2007; Ingram et al., 2009; Rowsell et al., 2009; Williams et al., 2011; Leal Junior et al., 2011; Peiffer et al., 2011; Bieuzen et al., 2013). In the current study, a peak median CK increase of 210% from baseline occurred at 48 h. this suggest that the eccentric exercise protocol used was successful in eliciting muscle damage, although peak increases up to 600% have been reported in other studies with more aggressive eccentric quadriceps loads. These results are in line with that of (Bieuzen et al., 2013, Rowsell et al., 2009). 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 research. As Glasgow et al (2014) showed that 10 min of CWI in 6 °C had minimal effect on decrease of CK. The mechanism responsible for the lower exercise-induced intracellular protein release to plasma following cold water immersion remains unclear (Bleakly et al 2014; Crystal et al., 2013; Duffield et al., 2014). 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 (Ascensao et al., 2011). Accordingly, attenuation in C-reactive protein after match was observed in the cold water immersion group compared with the control group. In fact one of the major characteristics of inflammatory response resulting from exercise-induced muscle injury is an increase in the permeability of vessel walls. Given that CK diffuses into the lymph vessels, it is possible that a reduced permeability of these vessels walls...
induced by CWI reduced the rate of CK efflux from the muscle (Higgins et al., 2013; Leeder et al., 2012). However, further analysis of direct histological markers of muscle damage resulting from this type of therapy would be necessary.

**Neuromuscular function**

Vertical jump and muscle strength are frequently used as reliable means of quantifying exercise-induced muscle damage (Vaile et al., 2009; Bieuzen et al., 2013; Rowsell et al. 2011). This supported by the consistent decrease in neuromuscular performance in response to eccentric exercise, and is accordance with other previous studies (AScensao et al., 2011; Rowsell et al., 2011; Elias et al, 2013).

CWI following an eccentric exercise resulted in a transient attenuation at 24 h for strength, but not for vertical jump ability (table 2). These findings appear to support the specific sensitivity of a more contractile-dependent muscular performance test such as maximal isometric voluntary strength (MVIS) compared with sprint and jump (Bailey et al., 2007; Warren et al., 1999; Ascensao et al., 2011). Accordingly, Rowsell et al (2009) did not observe any treatment effect of CWI against decrements in vertical jump caused by eccentric exercise, which together with our data, support the increased sensitivity of maximal strength and Vaile et al have consistently found positive effects form CWI on recovery of muscle power.

Future research may focus on specific groups of elite athletes who regularly use cold water immersion and develop reliable functional measures for each group that may demonstrate more subtle objective deficits resulting from muscle soreness. It would be valuable to assess athletes in their chosen sport to determine the specific muscle symptoms that result from an intense competition performance as it may be possible to identify a reliable model of sports-induced muscle damage, and reliable outcome measures that are affected by muscle soreness. From this, the effects of various interventions such as cold water immersion could be properly assessed in sports-induced muscle soreness.

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 and 24 h after an eccentric exercise is effective in reducing some biochemical, functional, and perceptual markers of muscle damage.

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**References**


