Variations of serum creatine kinase and C-reactive protein levels in different recovery modalities following exhaustive exercise in soccer players

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Introduction:
The purpose of the present study was to explore changes in creatine-kinase (CK) and C-reactive protein (CRP) levels in a variety of recovery modalities following exhaustive exercise in elite soccer players.

Materials & Methods:
Thirty-male soccer players in Azadegan League (aged 22.4 ± 2 years, height 179.1±2.63 cm, weight 68.5±2 kg and BMI 21.5±1.1) completed one exhaustive exercise protocol of 6 bouts of 15 minutes specific football exercise and 20 m multistage shuttle run (Beep Test) level 7. They were randomly divided into active recovery, passive recovery and cold-water immersions for 15 minutes. To measure CK and CRP levels, a blood sample was obtained immediately, 24 and 48 hours after exercise protocol.

Results:
The results showed a significant reduction in blood CK and CRP levels after cold-water immersion compared with other recovery modalities. Moreover, CRP level in active recovery group was significantly lower than that in the passive recovery group (P<0.05).

Conclusion:
The current results reveal that cold-water immersion is superior to passive and active recovery modalities after exhaustive exercise protocols in elite soccer players.

Keywords: Creatine-Kinase, C-Reactive Protein, Recovery, Exercise

Introduction
Teams suffer a high level of physical and mental pressure during sports seasons. In weakly exercise plans, athletes must go through extensive exercise to be ready for the next practice on time (1). An important issue faced by professional athletes is the limited time for full recovery between exercise sessions and competitions (2, 3). Extensive exercise and successive competition impose pressure and stimulations on the body which, if not controlled, may cause nerve and muscle fatigue, disorders in energy systems and, therefore, debilitate physical performance (4, 5). Soccer is an intermittent sport with maximal and submaximal exercises done in about 90 minutes. Soccer players run for an average 9-12 km in each match. This sport requires a high level of energy for fast shuttle runs, dribbling, heading, and tackling (6). Continuous and prolonged effort in sports can induce muscle soreness, followed by muscle injury. These injuries can be diagnosed by measuring certain
blood markers, including creatine kinase (CK) and C-reactive protein (CRP) (7). The time of CK diffusion and excretion from plasma to the surface depends on the type, intensity, and duration of exercise. Peak serum level of CK occurs 8 hours after intense exercise (8). CK activity is significantly increased 24 hours after prolonged exercise. Intensive physical exercise, e.g. soccer, twice a day increases CK in the fourth day of exercise and its level remains elevated for 4-10 days (8). Also, soreness and inflammations caused by muscle injury occur in the process of physiological adaptation of a muscle to intensive exercise. One of the mechanical causes of muscle soreness can be the injury to sarcomeres in muscles, leading to z-disc fragmentation (9). The biochemical markers of delayed-onset muscle soreness include the increased level of CK which occurs when sarcomeres are torn. Muscle soreness results in discomfort, pain, and reduced physical performance. The pain caused by delayed-onset muscle soreness is usually manifested 12-24 hours after exercise and may continue 2-5 days (10). Another marker for the diagnosis of muscle injury and inflammation is CRP. This protein is a valid marker of inflammatory performance following exercise and is released from the liver in response to numerous injuries such as surgery, tissue damage, inflammation, and exercise (11). To treat these injuries, athletes usually have sessions of recovery similar to their regular exercise after every exercise or competition. These sessions resolve the pressure caused by exercise and matches. In general, the positive effects of recovery modalities allow athletes tolerate a heavier exercise load (higher intensity, volume, or frequency). Recovery modalities help excrete the products of metabolism faster and improve athletes’ physical and psychological state after effort. These modalities must be specifically defined for different sports so that coaches and athletes can have optimal conditions for competition considering the athletes’ physiological and psychological states (12). These are various recovery modalities, including stretching, massage, compression, anti-inflammatory drugs, antioxidants, active recovery, cold water immersion, contrast water immersion (alternating hot–cold water immersion), hot water immersion, electrical stimulation, and passive recovery (13). Cold water and contrast water immersion are effective recovery modalities which induce the excretion of lactic acid and CK after rugby matches and aerobic exercise (14, 15). On the other hand, some studies have reported no effect for cold water immersion. For instance, Pournot et al. (2010) examined the effect of various water immersion recovery modalities on maximal strength, power, and serum CK. Recovery modalities included temperate water immersion, cold water immersion, contrast water immersion, and passive recovery. Results showed no significant difference between the effects of cold water immersion and other recovery modalities on serum level of CK (3). Another recovery modality used after exercise is active recovery. According to research, active recovery is more effective than passive recovery. Moreover, the content of muscle glycogen remains almost constant during active recovery (16). However, the higher effect of cold water immersion than other strategies is still unclear and needs further research. The present study examined the effect of cold water immersion, active recovery, and passive recovery on the levels of CK and CRP in elite soccer players following simulated team exercise.
**Methods and Materials**

In this quasi-experimental study, 30 soccer players playing in the Azadegan League were selected as the sample. They had a mean age of 22.4±2.38 years, height of 179.1±2.63 cm, weight of 68.5±2.82 kg, and body mass index (BMI) of 21.5±1.10 kg/m² and were all healthy. Subjects were randomly assigned to three groups (n=10). Group 1 used active recovery modalities including jogging, walking, shuttle run, and stretching. Group 2 used passive recovery strategies including sitting and lying down. Group 3 used cold water immersion (immersion up to the neck in 10°C water).

To implement the exercise protocol, all subjects engaged in simulated soccer match exercise after an initial warm-up (7 minutes). This exercise comprised six 15-minute sessions of specialized soccer exercise, including walking, dribbling the ball between cones, backpedaling, and shuttle run along four straight lines for 50 m. After five minutes of rest, subjects took the Level-7 20m shuttle run test to definite exhaustion. Afterwards, they were divided into recovery groups (active recovery, passive recovery, and cold water immersion) based on BMI and performed recovery exercises for 15 minutes. Blood samples were taken from the right brachiocephalic vein right after the exercise, 24 hours later, and 48 hours later. At the time of sampling, the ambient temperature was about 22 °C and measurements were taken at similar time intervals. Serum levels of CK and CRP of subjects were measured with the ELISA method using a commercial kit (Pars Azmoun Co., Iran) in the noted time intervals (15 minutes after recovery). Data were expressed as mean and SD and the normality of distribution was determined using the Kolmogorov-Smirnov test.

Repeated-measures ANOVA with Tukey’s post-hoc test were used to compare variables in different time intervals. Statistical analyses were performed in SPSS 16 at the significance level of <0.05. Consent forms for participation in the study and medical tests were obtained from all subjects. In addition, all ethical requirements of research, including the confidentiality of information, were met.

**Results**

The physical markers, age, height, weight, and BMI of subjects are listed in Table 1. The three groups were similar in terms of the noted markers.

Based on Figures 1 and 2, serum levels of CK and CRP started to rise right after exercise and continued for 48 hours.

Based on Figure 1, mean serum CK level 24 hours after exercise was significantly higher in active recovery (680±26 U/L) than cold water immersion (612±18 U/L) (p=0.032) groups. However, active recovery (648±26 U/L) and passive recovery were similar in terms of serum level of CK. Moreover, mean serum CK level 48 hours after exercise was significantly lower in cold water immersion (764±56 U/L) than the other two modalities (883±48 U/L) (p<0.05). The difference between active and passive recovery modalities was not significant (p<0.05).

According to Figure 2, the level of CRP 24 hours after exercise was the same for active and passive recovery (5.8±0.27 mg/L). Nevertheless, mean CRP 24 hours after exercise was significantly higher in active and passive recovery than cold water immersion (5.28±0.1 mg/L) (p=0.023 and p=0.027, respectively). In addition, 48 hours after exercise, the level of CRP was significantly lower in cold water immersion (6.3±0.11 mg/L) than the other active...
(7.1±0.1 mg/L) and passive (7.4±0.3 mg/L) recovery modalities (p<0.05).

Table 1: General physical markers of subjects in three groups

<table>
<thead>
<tr>
<th>Groups</th>
<th>Age</th>
<th>Height</th>
<th>Weight</th>
<th>BMI</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Passive recovery</td>
<td>22.6±2.1</td>
<td>179.1±2.63</td>
<td>68.5±4.82</td>
<td>22.5±2.8</td>
<td>p&gt;0.05</td>
</tr>
<tr>
<td>Active recovery</td>
<td>21.9±1.38</td>
<td>180.1±2.1</td>
<td>67.9±4.2</td>
<td>21.5±2.10</td>
<td>p&gt;0.05</td>
</tr>
<tr>
<td>Cold water immersion</td>
<td>22.4±2.4</td>
<td>179.9±2</td>
<td>67.5±3.8</td>
<td>21.9±1.18</td>
<td>p&gt;0.05</td>
</tr>
</tbody>
</table>

*Age (year), height (cm), weight (kg), BMI (kg/m²)*

Figure 1: Variations in CK level in different recovery modalities.
★: significant difference between cold water immersion and other groups, p<0.05
Discussion

Results of the present study revealed that intensive exercise significantly increases the serum level of CK, but this elevation can be moderated using various recovery modalities. Mean serum level of CK 24 hours after exercise differed across the three modalities of active recovery, passive recovery, and cold water immersion. The difference between active and passive recovery modalities was not significant, but a significant difference was observed between cold water immersion and passive recovery. Moreover, based on results, the serum level of CK 48 hours after exercise differed across the three modalities (p<0.05). Serum level of CK level 48 hours after exercise was significantly lower in cold water immersion than the other two modalities (p<0.05). Based on these results, active recovery modalities can lead to fast recovery, which is consistent with the results of Gill et al. (2006), Ingram et al. (2009), and Elias et al. (2013). Gill et al. (2006) explored the effect of various recovery modalities on plasma CK activity in 23 elite rugby players after a match. Results showed that cold water immersion significantly reduces CK level compared to passive recovery (14). Cold water immersion prevents inflammation by vasoconstriction and, thus, reduces CK (3). Water immersion applies hydrostatic pressure on the body and blood flows from lower parts of the body to the thoracic region, thereby quickly excreting the products of metabolism (17). Moreover, cold water immersion reduces cell permeability through vasoconstriction, thereby decreasing inflammatory responses, muscle damage, edema, and pain (18). This method can relieve the inflammation resulting from intensive and damaging exercise as a treatment method. Therefore, it may be an effective method for reducing edema and inflammatory responses resulting from tissue damage (19).

Also, it is believed that active recovery is fast because it quickly restores glycogen reserves (12). If the intensity of effort is not controlled in active recovery, it may lead to further aggregation of metabolism products and thus have a negative effect on recovery (4). Demirhan et al. (2015) investigated the effect of cold water immersion and ice massage on levels of CK in wrestlers after

![Figure 2: Variations in CRP level in different recovery modalities](image-url)
exercise and reported no significant difference between the two modalities (7). The stimulation of CK depends on the duration of muscle contraction (9). Therefore, the similarity between the two modalities might be due to the special exercise protocol used. Furthermore, Vaile et al. (2008) examined the effects of cold water immersion on levels of CK in 28 male bodybuilders following strength training. They reported a significant decrease in CK level 24 and 27 hours after cold water immersion compared to passive recovery (20), consistent with the result of the present study.

Similar to previous studies, the level of CK in this study reached its peak 48 hours after exercise. This result is in line with results of previous studies which used cold water immersion to resolve neuromuscular and biomechanical symptoms of muscle damage in exercise models with similar needs and exercises (21). There is a general consensus regarding the ability of cold water immersion to reduce the perception of soreness and fatigue caused by exercise. However, its effectiveness compared to other methods is not still proven (1). Mechanisms responsible for reducing muscle damage and soreness following cold water immersion are not yet fully understood. One of the proposed mechanisms is that cold water immersion reduces the release of muscular enzymes (CK) in the lymphatic system. This reduction following cold water immersion may be the result of a reduction in vascular permeability (22).

Moreover, the findings of this study regarding variations in the level of CRP in three recovery modalities showed a significant difference among the three recovery modalities in terms of CRP level 24 hours after exercise. Serum level of CRP 24 hours after exercise was significantly lower in cold water immersion than the other two modalities. Ingram et al. (2009) compared the effects of contrast water immersion (10 °C and 40 °C), cold water immersion (10 °C), and passive recovery on the serum level of CRP after a simulated team sport exercise. Results indicated a significant decrease in CRP after cold water immersion compared to contrast water immersion after 1 and 24 hours (5), consistent with the present results. Also, Dabidi Rooshan et al. (2011) studied the effect of in-water versus out-of-water active recoveries on variations in the level of CRP following a sprint swimming bout. They observed no significant difference between the two modalities (23) which contrasts the results of the present study. This difference may be due to the type of exercise. The exercise used in Dabidi Rooshan et al.’s (2011) study was not intensive enough to change the level of CRP. Also, the levels of these variables in those who have regular light exercise are similar to those of individuals who are constantly active (24). Nevertheless, separate studies on various sports have shown that the level of CRP is higher in non-athletes than athletes. This may be due to exercise, adaptation to exercise, and the inhibitory effect of exercise on CRP (23).

Conclusion
Results of the present study showed that cold water immersion is an effective recovery method. The use of this method following exercise or matches can probably reduce inflammatory responses and muscle damage by decreasing the permeability of blood and lymphatic vessels which reduce inflammatory markers in muscles. Results also indicate that cold water immersion following simulated team exercise (here
soccer) quickly restores the physiological state of players. Therefore, recovery in general, and cold water immersion in particular, are recommended to soccer players after exercise and matches.

Conflict of Interest
None to declare

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References: