Investigation of the Effect of Creatine Monohydrate Supplementation on Muscle Damage in Male Climbers following Climbing to an Altitude of 4000 Meter

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Abstract

Purpose: Creatine is the most important and commonly used supplement in athletes. The purpose of this study was to investigate the simultaneous effect of moderate altitude and short-term Creatine supplementation on musculoskeletal damage indicators in male climbers.

Method: 16 male hikers with at least 2 years of hiking experience selected. Subjects (age 30.6±6.3 yrs.), (height 178.3±6/4 cm), (weight 77.6±11.25kg) were randomly divided into experimental (Creatine supplement) and control groups (placebo). The experimental group consumed 20 gr/day of the supplement for 5 days, while the control group received a placebo. The first blood samples were taken before the hiking session at the location of program. Hiking program consisted of ascending to Tochal peak at an altitude of 3961m. The second blood samples were taken after returning from the peak at an altitude of 3750 meters. The results were analyzed by SPSS software using independent t-test and K-S test with a significant coefficient of 0.05.

Results: The results showed that there was a significant difference between LDH and AST enzyme levels in post-test compared to pre-test in both AST (P = 0.13) and ALT (P = 0.001) levels Control group. Also, climbing to 4000 m altitude with Creatine supplementation had no significant effect on LDH (P = 0.78) and AST (P = 0.86) levels between groups.

Conclusions: The results showed that changes in muscle damage enzymes are independent of Creatine supplementation.

Keywords: Altitude, Aspartate Aminotransferase, Alanine Aminotransferase

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INTRODUCTION
Nowadays, dietary supplements are widely used in exercises so that most athletes consume at least one or more of them at some step of the own championship. These substances are used to improve the function and increase muscle mass by stimulating protein synthesis in the body or reducing body fat. Currently, there are many supplements that are used by athletes. Dietary supplements have important roles, such as energy production and increasing muscle mass. Creatine is one of the most widely used supplements, which has become increasingly popular among athletes (Rosene et al., 2009; De Fusco et al. 2019). Creatine increases phosphocreatine levels and muscular creatine by approximately 20% to 40% and improves skeletal muscle mass as well as functional strength and power (Zoeller, Stout, O’kroy, Torok & Mielke, 2007). Creatine (methyl guanidine-acetic acid) is normally found in meat and fish. 0.3 grams of this substance per kilogram of body weight is daily needed to replace the creatine used by muscle contractions. The main storage area of creatine in the human body is the skeletal muscles. In fact, about 95% of the body's creatine is stored in skeletal muscle. Creatine as a protein is produced endogenously in the human body and also can enter into the body from the diet. Creatine exists naturally in the daily meal of people; meat can generally provide one gram of this element for the body. The rest of the required creatine is synthesized by amino acids in the kidneys (Rad, 2010; Nebot et al., 2015).

Most of the studies indicate that creatine improves athletic performance in sports activities, especially during strenuous and short-term activities. This effect is more a result of increased muscle mass, which is a contributing factor in power and short-term activities and is obtained after using creatine (Cooper, Naclerio, Allgrove & Jimenez, 2012; Page, Reid, Speedy, Mulligan & Thompson, 2007). Researches performed to find the effects of creatine on athletic performance indicated that the use of creatine improves athletic performance, particularly in power activities (Kashif, Bonyan & Rad, 2012). These issues have been confirmed by Zoeller, Stout, O’kroy, Torok and Mielke (2007) and several other researchers. In this regard, Jones et al. examined the effects of creatine on quick movements of skiing in ice hockey players and concluded that creatine monohydrate improves anaerobic
performance and also has ergogenic effect in elite players (Zoeller, Stout, O’kroy, Torok & Mielke, 2007).

In contrast, the results of some studies have been showed no significant effect of creatine to enhance athletic performance. Snow et al. conducted a study to determine the effects of creatine supplementation on muscular function and metabolism, and came to the conclusion that this substrate cannot improve speed function or changes in muscular anaerobic metabolism. Sairotoic et al. in a study on 22 trained rowers investigated the effects of 5 days creatine loading, and 6 weeks maintenance on exercise performance; finally the researchers found that the use of creatine had no effect (Rawson, Conti & Miles, 2007). But most of researches so far corroborates the notion that creatine improves athletic performance during short intense exercise (Casey & Greenhaff, 2007; Piene, 2012). Conducted investigations on the effect of creatine on different damages have suggested that creatine supplements cannot be harmful. Michelle Green Hood et al. found that creatine not only does not damage, but also athletes who had used creatine in the study had less damages such as muscle cramps, dehydration and sprains and muscle strain than the control group (placebo). It is worth noting that the pathogenesis of creatine cannot be judged properly regardless of the unfavorable changes in cellular damage indices (Kashif, Bonyan & Rad, 2012; Scotney & Reid, 2015). Therefore, the present study aims to investigate the concurrent effect of climbing up to the average heights and short-term use of creatine on the muscle damage indices in male mountain climbers.

METHOD
This applied research was conducted as quasi-experimental with pretest-posttest design. First, different climbing groups were recalled. Finally, 20 male mountain climbers responded to this call. Among them, 16 subjects were randomly selected (Table 1), who were with 25-40 years of age having at least 2 years of climbing experiences and average age of 30.6±6.3 years, average height of 178.3±6.4 cm, average weight of 77.6±11.25 kg, average body mass index of 24.38±2.8 kg/m² and average oxygen consumption of 41.06±5.70 ml/min/kg, and divided into two experimental (case, n = 8) and placebo (control, n = 8) groups. Subjects were asked to refrain from drinking any caffeinated drinks and fatty foods, for up to three days prior to the implementation of the program.
A climb up session to the Mount Tochal with the height of 3961 meters was started from the Velenjak valley (Tochal Telecabin). Initially, the subjects sat on a chair in resting position for 10 minutes before sampling, then fasting blood samples were taken. Participants were transferred from station 1 to station 5 by telecabin, due to weather conditions and time limitation of program. Climbing began from station 5 (at the height of 2935 meters) by supervision of researcher. Climbing from stations 5 to 7 of telecabin lasted about 4 hours; the route was covered in snow, and weather conditions were desirable. The route slope fluctuates from about 25 to 35 degrees; the slope is reduced 10 to 15 degrees close to station 7 with gentle slope up to summit. It took about 50 minutes up to summit from stations 7. The difficulty of the route in mountain was semi heavy. The group climbed harmoniously with a proper rhythm. The second blood samples were taken after returning from the summit at the height of 3750 meters (Station 7) in a suitable place where participants can sit comfortably. In the short-term use of creatine, the experimental group consumed 20 grams supplement per day for 5 days with fruit juice and the control group received placebo. For data analysis, descriptive statistics (including measurements of height, weight, age, etc.) and inferential statistics were used to compare the mean pre- and post-test (paired sample t-test) and independent t-test was related to compare the average post-tests in both groups.

RESULTS
The individual characteristics of the subjects have been listed in Table 1. As shown in the table, physical characteristics and fitness in both groups are relatively close together.

**Table 1:** The individual characteristics of the subjects (independent t-test)

<table>
<thead>
<tr>
<th>Variables</th>
<th>Groups</th>
<th>Number</th>
<th>Mean</th>
<th>Standard deviation</th>
<th>Degree of freedom</th>
<th>T-test</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (y)</td>
<td>case</td>
<td>8</td>
<td>31.25</td>
<td>4.43</td>
<td>14</td>
<td>0.731</td>
<td>0.476</td>
</tr>
<tr>
<td></td>
<td>control</td>
<td>8</td>
<td>30</td>
<td>1.93</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Height (cm)</td>
<td>case</td>
<td>8</td>
<td>178.63</td>
<td>7.37</td>
<td>14</td>
<td>0.153</td>
<td>0.88</td>
</tr>
<tr>
<td></td>
<td>control</td>
<td>8</td>
<td>178.13</td>
<td>5.51</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>case</td>
<td>8</td>
<td>75.13</td>
<td>1.62</td>
<td>14</td>
<td>-0.91</td>
<td>0.377</td>
</tr>
<tr>
<td></td>
<td>control</td>
<td>8</td>
<td>80.25</td>
<td>11.87</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>case</td>
<td>8</td>
<td>40.56</td>
<td>5.70</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
As can be seen in Table 2, paired sample t-test showed that creatine supplementation had no significant effect on LDH (Lactate Dehydrogenase) and AST (Aspartate Aminotransferase) enzymes in the experimental group, and thereby led to no change and the inhibition of enzymes elevations.

**Table 2:** Paired sample t-test for case and control groups, pre-test and post-test for LDH and AST levels

<table>
<thead>
<tr>
<th>Groups</th>
<th>Variables (U/dl)</th>
<th>Mean</th>
<th>Standard deviation</th>
<th>T-test</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Case</td>
<td>LDH pre-test</td>
<td>299.75</td>
<td>55.65</td>
<td>-1.5</td>
<td>0.177</td>
</tr>
<tr>
<td></td>
<td>LDH post-test</td>
<td>322.125</td>
<td>63.82</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>AST pre-test</td>
<td>27.25</td>
<td>13.43</td>
<td>-0.55</td>
<td>0.59</td>
</tr>
<tr>
<td></td>
<td>AST post-test</td>
<td>27.25</td>
<td>12.54</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>LDH pre-test</td>
<td>274.125</td>
<td>76.15</td>
<td>-3.28</td>
<td>0.013*</td>
</tr>
<tr>
<td></td>
<td>LDH post-test</td>
<td>332.25</td>
<td>82.64</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>AST pre-test</td>
<td>23.5</td>
<td>7.27</td>
<td>-5.12</td>
<td>0.001*</td>
</tr>
<tr>
<td></td>
<td>AST post-test</td>
<td>26.62</td>
<td>6.80</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* p<0.05

As Table 3, independent t-test to compare the average post-tests for LDH and AST levels in case and control groups showed that creatine supplementation had no effect on LDH and AST enzymes in mountain climbers and cannot make a difference.

**Table 3:** Independent t-test to compare the means post-tests for LDH and AST levels in case and control groups

<table>
<thead>
<tr>
<th>Groups</th>
<th>Variables (U/dl)</th>
<th>Mean</th>
<th>Standard deviation</th>
<th>T-test</th>
<th>P-value (p&lt;0.05)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Case</td>
<td>LDH post-test</td>
<td>322.125</td>
<td>63.82</td>
<td>-0.274</td>
<td>0.78</td>
</tr>
<tr>
<td>Control</td>
<td>LDH post-test</td>
<td>332.25</td>
<td>82.64</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Case</td>
<td>AST post-test</td>
<td>27.5</td>
<td>12.54</td>
<td>0.17</td>
<td>0.86</td>
</tr>
<tr>
<td>Control</td>
<td>AST post-test</td>
<td>26.62</td>
<td>6.80</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
DISCUSSION
Dietary supplements have several important roles, such as energy production and increasing muscle mass. Creatine is one of the most widely used supplements, which has become increasingly popular among athletes (Saunders, Luden & Herrick, 2007). However, there are still many concerns about the unknown and adverse effects of creatine supplementation on athletes in different majors and there are doubts

Fig 1: LDH and AST in both groups (p<0.05)
about the effects (positive or negative) of short and long-term consumption on health indices and different body systems (Bashiri, Hadi, Bashiri, Nikbakht & Gaeini, 2010). Physical activities are considered as a mechanical stressor, which can cause biochemical changes; therefore, muscular activities can alter the blood concentrations of some cellular enzymes such as creatine kinase and lactate dehydrogenase (Taheri Gandomani, Faramarzi, Banitalebi, Shirvani, Taheri Gandomani, 2010). LDH level is increased obviously with exercise which depends on intensity and duration of activity (Santos, Bassit, Caperuto & Rosa, 2004). AST enzyme activity increases to a large extent immediately after intense muscular activity, and remains in high levels up to 24 hours. This increase relates to the workout duration, and can appear even without clinical signs. AST increases during chronic muscle damages (Saunders, Luden & Herrick, 2007). Skeletal muscle damage is a phenomenon that occurs for several reasons, including: tearing of muscle fibers or cell necrosis; and in damages without cell necrosis is the main reason for muscle fiber destruction, especially cellular basal lamina shell. In general, muscle damages occur in cell membranes accompanied by loss of cellular proteins such as creatine kinase (CK), myoglobin (Mb), lactate dehydrogenase (LDH), aldolase and troponin; release of these proteins from cells leads to increased inflammatory cells population such as macrophages and neutrophils in muscle fiber, increased delayed onset muscle soreness (DOMS), loss of power and possible damage to some structures such as irregularities of the Z line of a sarcomere. Therefore, promoted serum levels of these proteins may be indirectly a reason for muscle cell damage (Saunders, Luden & Herrick, 2007). Individual responses to the muscle damage indices depend on the level of health, as well as the type and duration of exercise. These factors affect the ratio of response and duration of release followed by damage (Santos, Bassit, Caperuto & Rosa, 2004). Various methods in measuring the muscle damages, a variety of indices, measuring time, type of training programs, the kind of carbohydrates and proteins, supplement levels, the percentage of carbohydrates and proteins found in supplementation and time of supplementation use have all a great impacts on the contradictory results (Saunders, Luden & Herrick, 2007). Most of active individuals are aware of the importance of carbohydrates in body function. During the inadequacy of carbohydrates in the diet, the body uses different methods
to prepare required carbohydrates (Hargreaves & Spriet, 2006). During the exercise, one or a group of muscles are responsible for ATP hydrolysis. The muscles without the knowledge of the mechanism of this phenomenon respond to it with appropriate method and using three different energy systems (Tiidus, Tupling & Houston, 2012).

Muscle glycogen and glucose in the blood that are derived from the liver and gastrointestinal tract by taking foods containing glucose are the main substrates for the regeneration of ATP in active skeletal muscle during intense and long-term activities (Hargreaves & Spriet, 2006). Mountain climbing is a popular endurance sport with abundant and diverse subdirectories. Mountain climbing is said to go up or down on foot in the natural heights (Abbasi et al., 2012). In the mountain climbing, clean mountain air is effective in preventing and treating many diseases. Studies have shown that people who do regular physical activity such as climbing feel more pleasing, self-confidence and better self-concept. Hormones such as endorphins and serotonin rise in the blood during climbing and are able to create a refreshing and invigorating in person with analgesic effects. Mountaineering plays an important role in reducing anxiety, depression and stress, having comfortable sleep, losing weight, strengthening the respiratory system, regulating the blood pressure, controlling the diabetes, preventing the incidence of osteoporosis, strengthening the heart, improving of digestion, reducing blood fat to natural levels and increasing muscle strength. Gently and prolong climbing causes blood vessels dilation especially inside the muscles and under the skin, and at the same time reduces vascular resistance; thereby reducing the blood flowing pressure as well as it is effective in lowering blood pressure and natural healing. In general, regular exercises including climbing prevent or delay the onset of diabetes because the rate of metabolism increases, resulting in the use of fat, sugar and overall body reserves. In other words, climbing and sporting activities act similar to insulin for patients with diabetes and play a deterrent role (Salehi Moghadam, 2008). The most obvious effect of trekking is the increase in muscle strength. It is estimated that the legs go up and down about 6,000 times during the one hour of walking on flat ground, while the climbing rhythm is 4,000 times (Shimizu et al., 2012). In each footstep, all the muscles of the legs, thighs, waist, back, abdomen and chest are strongly contracted and expanded. These repeated
contractions make the muscles more trained and more resistant and adds to the power of the person, also moving in the blood vessels gets better and so more blood will reach to skin. Because, capillaries activity in normal and rest circumstances can be about 15 percent of normal capacity, while the 95% of capillaries are activated during the climbing and food substances reaches to body more quickly (Salehi Moghadam, 2008).

In general, the results of present study showed that creatine supplementation leads to no change in muscle damage enzymes and inhibits their elevations in experimental group by climbing to the height of 4000 meters. But in the control group who did not use supplements, muscle damage enzymes were increased after climbing. However, there was no significant difference between the two groups in the rate of change in lactate dehydrogenase and aspartate aminotransferase (Agathanelou, 2016) Although these enzymes were promoted in both groups, but their means were lower in the creatine group compared with placebo, suggesting that short-term use of creatine can reduce muscle damage in mountain climbers; however, this decrease is not significant (Ramin, Ali, Akbar & Behrouz, 2012). Energy reserves decrease in long-term climbing activities, thereby increasing muscle damages; and to reduce muscle damage, the use of creatine, proteins and carbohydrates supplements is necessary (Cooke, Rybalka, Williams, Cribb & Hayes, 2009). Muscle damage was lower in the group receiving creatine supplementation due to metabolic energy supply. By comparing the two discussed groups no changes were observed in the enzyme levels, but the levels of muscle damage enzymes were higher in the placebo group, indicating that this supplement is effective on muscle damage though this change is no significant.

CONCLUSIONS
Based on the results of this study, creatine supplementation is not required in large amounts for storing and loading of muscle Pcr (phosphocreatine). Taking creatine supplements can be considered as a way to make immediate progress in athletes involved in explosive activities. The same studies also indicate that use of creatine can delay exhaustion during prolonged exercise, so athletes can practice with more intensity without fatigue. For this reason, creatine supplementation due
to nutritional properties can be effective in the development and improvement of athletic performance.

**Conflict of Interests**
The authors announce no conflict of interest.

**REFERENCES**


Piene, S. (2012). The effects of a sport drink containing carbohydrates and electrolytes with or without caffeine on 20km cycling time trial performance in men and women.

Rad, M. (2010). *Comparison the effect of taking two types of creatine monohydrate and creatine-carbohydrate on the anaerobic power and cellular damage indices (CK, LDH) among 15 to 18-year-old boys students* (M.Sc. Thesis). Shahid Rajaee Teacher Training University, Tehran, Iran. [In Persian]


