The effect of an Exhaustive exercise and Glutamine Supplementation on LDH, CPK and CPR indexes in non-athlete women students

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ABSTRACT
Decrease in blood’s viscosity in stressful situations such as injuries, hunger or rigid physical exercises undermines body’s immune system dramatically, therefore the current study was conducted to examine the impact of an exhaustive exercise alongside with the short time consumption of glutamine on the HDL, CPK and CRPs indexes in non-athlete women students’ blood. In this research 30 non-athlete women (age 21± 1.59 years, weight 59.75±7.96 Kg, height 160.63±4.89 Cm and BMI 22.38±3.62 Kg/m²) were randomly divided into two experimental and control groups and were studied utilizing two-ways blind. Both groups participated in an exhaustive exercise (Bruce test). Subjects consumed glutamine supplement and placebo (5 gram glutamine or placebo, solved in 300 ml solution of 5% Sucrose) one hour before the activity. Blood samples were taken swiftly after the experiment and one hour later. Obtained data in a form of mean and Sd/deviation was analyzed utilizing Co-Variance (ANCOVA) in level of P<0.05. Results showed a significant increase in CPK and HDL indexes in control group (P=0.05) while the same indexes did not show any significant alteration in experimental group. CRP’s level in both groups indicated a significant difference (P=0.001), while in comparison with two groups, CRP in experimental group showed a great reduction (p=0.001). Findings of the presented study indicated that the consumption of glutamine supplement could prove useful in reducing the CPK and HDL plasmatic levels following useful athletic activities.

Key Words: Exhaustive exercise, Glutamine, CPK, HDL and CRP

INTRODUCTION
Research has indicated that rigid and heavy athletic activities lead to chronic exhaustion, muscular texture damage, weight reduction and decrease in plasma’s glutamine and skeleton muscles [1]. Glutamine is the most abundant free amino acid in body and accounts for 50 to 60 % of whole free amino acid storage in skeleton muscle and 20% of amino acid plasma storage. In addition to establishing and controlling of body’s hemostasis, (liquid balance, PH and balancing body temperature and heart beat) glutamine is also effective in healthy performance of certain body textures particularly immune system and intestines [2, 3]. Glutamine viscosity decreases dramatically in stressful conditions such as infrlicting injuries, hunger or rigid and difficult physical exercises which are accompanied with inflammatory factor [4]. Studies have indicated in final minutes of heavy physical exercises and simultaneously with reduction in carbohydrate, glutamine and other amino acid (Leucine, Isoleucine, Valine) perform as fuel precursor in order to create energy so that they provide about 10 to 20 % of cells’ required energy [5,6]. Many studies have shown the reduction in glutamine's levels following difficult [7,8] and semi-difficult exercises [9,10]. In some studies, it has been reported that 2 hours of stamina-oriented activity with 75% VO2max, reduces plasmatic glutamine [8]. In another reported study, it has been reported 3 minutes of athletic performance over ergometer with intensity of 70% VO2max dose not have any significant impact on viscosity of plasmatic glutamine [11]. With regard to this issue, Kargotich and colleagues [2005] concluded that plasmatic glutamine decreases following a phase of heavy physical activity [10]. In first phases of returning to preliminary stages, it seems that due to reduction in glutamine levels there is feebleness in certain practical aspects of immune system and heavy physical performance causes
reduction in immune system function [12]. On the other hand, many researchers believe that inflammation processes (even in systemic or general mode) are main factors for atherosclerosis and cardiovascular diseases to occur [13-15]. In last decades, researches have paid special attention to inflammation markers, which predict the risk of cardiovascular diseases with higher level of accuracy and sensitivity [16-18]. The C Reactive Protein (CRP) has been introduced as one of the most sensitive and strong independent factors in cardiovascular diseases prediction [19, 20]. Meyer and colleagues (2001) stated the CRP’s level increases following heavy exercises [21]. Other studies have indicated that following marathon race, a short time but considerable increase in the CRP’s levels is detectable [22]. One of the main actions which could be taken to reduce the inflammation process and the unpleasant consequences due to exhaustive exercise is to consume nutrition supplements particularly glutamine amino acid as a source of energy for immune system and as an immune cell’s boosting factor to prevent infections [12, 23]. In an investigation it has been reported that consumption of glutamine supplement foments significant reduction in the CRP’s index with high sensitivity after performance and burning [24]. In contrast with that, certain researches have stated that the consumption of glutamine supplement had no effect on the CRP and had no impact on inflammation prevention in cardiovascular patients who were undergoing Bypass operation [25]. Anyway, while the accurate mechanism of the glutamine’s impact on inflammation reduction has not still been discovered, certain experts believe that by deactivating the message route of Nuclear Factor Kappa-B (NF-KB) and Nitric Oxide Synthase (NOS), glutamine causes the reduction in Tumor Necrosis Factor-α (TNF-α) (one of the proinflammatory cytokines introductory inflammatory cascade) and active types of nitrogen specially Nitric oxide (NO). In other words this acid amine could prevent the unpleasant increase in the CRP (one of the indexes of the inflammation cascade endings) [26, 27]. Certain reports show that heavy physical exercises damages cell’s structure, particularly, they damages muscular textures [28-30]. There is probability that free radicals due to their oxidative features and through the activating of enzymes and damaging to nucleic acids, proteins and cells’ membrane have destructive impact on cells and textures [31]. One of the procedures to measure oxidative pressure due to cellular texture destruction is to assess the amount of anti-oxidative enzymes’ secretion [32]. Lactate dehydrogenase and Creatine kinase are among enzymes which are known as oxidative pressure indexes [32, 33]. Glycolysis’s route actions and reactions alongside with hydrogen ion increase causes in-cellular pH plunge, some lactate leaves muscular cell with hydrogen ion and this phenomenon impacts out-cellular buffer capacity which triggers movement in hydrogen’s ions, otherwise, it caused the reduction in in-cellular pH to the point that foments disruption in cellular performance [34]. On the other hand, oxidative pressure disturbs cell membrane function and this could be evaluated by measuring plasma CK (CK secretes into serum plasma following the membrane damage) [35]. Since LDH and CPK enzymes’ levels increase after a heavy physical activity and they return to previous levels in recycling stage (36, 37), but there is not much research regarding the impact of glutamine supplement on CPK and LDH enzymes levels after heavy physical exercise.

Based on what stated and since physical education students participate in various classes during day which exerts pressure on them, the purpose of this study is to respond to this question that whether short-period glutamine consumption could impact on Lactate dehydrogenase and Creatine kinase’s oxidative pressure indexes and the CRP’s inflammatory index or not.

METHODS

The present research is semi-experimental and included 30 student women (22±3.17 Years, 7.96±59.75 kg, 4.89±160.63 cm, 22.55±2.75 kg/m²). All participants presented a normal health level with no chronic disorders or other respiratory by a physician. All participants granted written permission and the experiment was approved by Ethics Committee of the Hamadan University of Medical Science in Iran. Participants were excluded if they had participated in a heavy physical activity program or had eaten each drug or supplementing the 48-h prior to the trial. The subjects were divided into two groups: Experimental group (Exp, N=15); and Control group (Con, N=15). Height was measured using a stadiometer (with accuracy of 0.1cm) with the participants barefoot and standing upright. Body weight was assessed by digital scale (with accuracy of 0.1 kg; Model: 7071314004; Made in Germany). Body mass index (BMI) was calculated by dividing body weight measured in kilograms by height in square meters (kg/m²). Other anthropometric values of subjects are presented in Table 1.

<table>
<thead>
<tr>
<th>Group</th>
<th>Exp (N=15)</th>
<th>Con (N=15)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (year)</td>
<td>21 ± 1.59</td>
<td>23 ± 4.75</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>59.58 ± 8.9</td>
<td>57.42 ± 7.53</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>163.75 ± 4.4</td>
<td>158.75 ± 6.56</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>22.38 ± 3.62</td>
<td>22.71 ± 1.87</td>
</tr>
</tbody>
</table>

Abbreviations: Exp = Experimental Group, Con = Control Group, BMI = Body mass index

| Table 1. Descriptive characteristics of participants (Mean ± SD). |
The last inclusion criterion was ascertained by asking subjects to complete the Physical Activity Readiness Questionnaire (PAR-Q). One hour before the beginning of exhaustion tests, the experimental group was taken 300 ml of 5% sucrose solution with 0.15% lemon juice with approximately 5 g of glutamine, and the control group consumed only placebo (lemon juice).

The exercise intervention was performed on a treadmill according to the Bruce Protocol, and required the subject to run for as long as possible on a treadmill whose speed and slope incremented at timed intervals. The treadmill was started at 2.74 km/h (1.7 mph) and at a gradient (or incline) of 10%. The speed and incline of the treadmill was increased every three minutes. The test was stopped when the subject was unable to continue (38). Before the intervention and after 2-min the trial was completed and following a 12-h overnight fast, blood samples (10 ml) were drawn from the antecubital vein for analysis of Creatine Phosphate Kinase (CPK), Lactate dehydrogenase (HDL) and C-Reactive Protein (CRP). All blood samples were measured using the Auto Analyzer, Bio Tecnicon BT3000 and Immunoturbidimetry’s method, according to the manufacturer’s instructions. Manufactured by: Beckman Coulter, Inc., 250 S. Kraemer Blvd. Brea, CA 92821, USA.

Data analysis
The data were initially treated by means of descriptive statistics, with mean values and variability. After checking the normality of the data, outcomes were analyzed using ANCOVA with the baseline measure of each variable used as the covariate (39). As for the comparison of variables before and after the exercise program, the paired t test was performed. Statistical significance was assumed for P-values ≤ 0.05. The data were treated in the Statistical Package for the Social Sciences (SPSS), version 18.

RESULT
The 30 participants were divided into two groups: 15 in the experimental group, and 15 in the control group. The experimental and control groups were homogeneous regarding BMI (Table 1).

Table 2. Characteristics of physiological variables: all groups at pre- and post-intervention.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Group</th>
<th>Pre-test (Means ± SD)</th>
<th>Post-test (Means ± SD)</th>
<th>Within group P-value</th>
<th>Between groups P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>CPK (U/L)</td>
<td>Exp</td>
<td>106.93±25.47</td>
<td>113.60±41.75</td>
<td>0.245</td>
<td>0.095</td>
</tr>
<tr>
<td></td>
<td>Con</td>
<td>92.20±29.09</td>
<td>116.47±30.62</td>
<td>0.006*</td>
<td></td>
</tr>
<tr>
<td>HDL (U/L)</td>
<td>Exp</td>
<td>315.80±50.02</td>
<td>315.53±51.94</td>
<td>0.972</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Con</td>
<td>304.07±83.06</td>
<td>347.33±48.14</td>
<td>0.050*</td>
<td></td>
</tr>
<tr>
<td>CRP (mg/L)</td>
<td>Exp</td>
<td>3.50±1.41</td>
<td>4.19±1.33</td>
<td>&lt;0.001***</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Con</td>
<td>4.30±1.42</td>
<td>5.63±1.27</td>
<td>&lt;0.001***</td>
<td></td>
</tr>
</tbody>
</table>

# Paired samples t-test was used to establish differences between pre and post-intervention measures for each group (pre-test and post-test).
$ ANCOVA$ was used to test differences between the groups at post-intervention with dependent variable at pre-test as covariate.
* Significant difference between the values obtained for the before-exercise and after-exercise conditions (P ≤ 0.05).
** Significant difference between the values obtained for the before-exercise and after-exercise conditions (P ≤ 0.01).
*** Significant difference between the values obtained for the before-exercise and after-exercise conditions (P ≤ 0.001).
† Significant differences between the groups at post-intervention (P ≤ 0.05).
†† Significant differences between the groups at post-intervention (P ≤ 0.001).

Table 2 displays the mean ± SD for physiological measures for all groups at pre and post-intervention as well as the differences between, and within, the groups post-intervention. The control group experienced a significant increase in CPK, HDL and CRP whilst experimental group does not displayed significant changes in this value (Table 2).

DISCUSSION
In this research, the impact of glutamine supplement’s consumption on LDH and CPK’s enzymes and the C reactive protein was investigated.

Regarding the CRP, the results indicated that Bruce test exhaustive exercise fomented a significant increase in the CRP’s index in serum. In line with these findings, Meyer and his colleagues (2001), Feng...
and colleagues (2000), Church and colleagues (2004) and Stauffer and colleagues (2004) reported the increase in the CRP following exhaustive exercise. With respect to this, Scharbag and colleagues (2004) in their research which was conducted on various athletes concluded that exhaustive exercise regardless of its type could cause increase in the CRP in both athletes and non-athletes which could lead into cardiovascular diseases in susceptible individuals [21,40,41,42,43]. On the other hand Collins and colleagues [44] stated that exhaustive exercise in healthy individuals could not cause increase in the CRP’s levels. One of the reasons for this alteration may lie in the variety in type and number of subjects as well as the intensity and type of exhaustive exercise. Regarding the intensity of training in must be declared that Collins measured the intensity of exhaustive exercise in healthy individuals by counting the average distance covered by patients over round track with five degree and consequently healthy individuals did not reach to exhaustive state, therefore, it could be stated that the type of activity is very significant which means that its intensity and period must trigger acute phase response. Based on the current study findings, there was a significant difference between the average and slope of the CRP’s variations in two groups after performing the training protocol. In other words it could be stated that extend of the CRP’s fluctuations in glutamine’s consumers in experimental group is significantly less than what has been observed in control group. In contrast to this result, Castell and colleagues [45] reported that the consumption of 5 gram glutamine supplement swiftly and one hour after the termination of marathon competition (exhaustive exercise) did not have any effect in alleviating the amount of increase in the CRP. Engel and colleagues [25] declared that the consumption of the glutamine supplement did not have any significant impact on the CRP and was not capable to prevent inflammation in cardiovascular patients who were undergoing by-pass surgery operation. In this research since the increase in the CRP in glutamine group is less than of increase in control group, it could be stated that the consumption of glutamine supplement could prevent the unpleasant increase in the CRP and relatively could avoid increasing inflammation. It must be mentioned that there is not sufficient research investigating the CRP and glutamine.

The other aim of current study was to investigate the impact of consumption of glutamine’s supplement on LDH and CPK’s enzymes following an exhaustive exercise. Studies have shown amount of glutamine synthesize in stresses or physiological pressures in less than required needed levels is to maintain hemostasis [46]. During physical exercise, the increase and decrease in glutamine’s plasmatic levels have been observed and this transformation is a reflection of type, length and intensity of physical activity. Glutamine is a precursor of Glutathione and Glutathione could reduce pre-oxidization lipids and free radicals through increasing the capacity of anti-oxidants plasma [47]. Glutamine as one of the vital available anti-oxidants could prevent cellular damage [48]. As it was already stated, one of the procedures to measure oxidative pressure due to cellular texture destruction, is to assess the secretion level of anti-oxidative enzymes (32). In normal condition, Creatine kinase does not penetrate to out-cell space unless sarcolemma has been damaged. Vicissitudes in Creatine kinase is various due to muscular mass, intensity, length and volume of exercise and to extend that subjects are familiar with extrovert activities (49).

With respect to LDH and CPK, results showed that the consumption of glutamine causes significant decrease in LDH and CPK’s level following an exhaustive exercise. In line with these results, Cruzat and colleagues (2009) [50] in a study on rats found that supplementing free glutamine with alanine glutamine has been effective in glutamine stocks which could reduce CK’s plasmatic levels and inflammatory responses that are created by long-period physical exercise. Also in another study on rats and concerned with the impact of glutamine on CK and LDH as well as blood’s lactate after burn injury, it was observed that CK and LDH’s levels in glutamine consumption group compared to placebo group was significantly lower [51]. In another study which investigated the effect of glutamine supplement on the fluctuations of Creatine kinase enzyme’s level following extrovert trainings in non-exercised men, no significant difference between two groups was observed and indicated that the consumption of glutamine supplement could not avert Creatine kinase enzyme’s secretion and its increase in blood’s plasma [52]. The probable reason for this paradox may lie in physiological differences between humane and animal samples as well as by considering the anabolic impact of glutamine, there is possibility the utilized dose of glutamine in investigations has not been adequate or due to absorption difficulties glutamine has not been able to impact positively (53,54). Generally speaking and based on what was found in current study and certain previous investigations, it could be declared that probably the consumption of glutamine supplement could be effective in preventing and alleviating of inflammatory conditions and the CRP as an anti-oxidative factor. It deserves to be mentioned that unless more studied are conducted, no doubtless conclusion may be obtained.
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