COMPARISON BETWEEN TWO METHODS OF SUPPLEMENTATION WITH GLUTAMINE AND SODIUM BICARBONATE ON CPK, LDH AND CRP IN NON-ATHLETE WOMEN STUDENTS

M. SAMAVATISHARIF
A. HABIBINEGHAD
H. SEIAVOSHY

Abstract
The purpose of this research is to investigate the effect of an exhaustive exercise alongside with short-term consumption of sodium bicarbonate supplement and glutamine on CPK, LDH and CRP in non-athlete women students. Method: This research conducted on 45 non-athlete volunteer women (age 22.3±3.04 years, weight 59.78±8.14 Kg, height 161.66±5.18 Cm, BMI 22.87±2.88 kg/m²). Subjects were randomly divided into three control, sodium bicarbonate (15) and glutamine’s groups. All of them participated in an exhaustive exercise (Bruce test). One hour before experiment, experimental sodium bicarbonate group took 0.065 g of sodium bicarbonate for per Kg of body, glutamine group took 5 g glutamine or placebo in 300 ml solution of 5% Sucrose and control group took placebo (starch). Blood samples at pre-experiment level and 2 minutes after experiment to evaluate CPK, HDL and CRP’s serum level. Data was analyzed by utilizing Covariance (ANCOVA) in P<0.05 level. Result: 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 groups (Bic & Glu groups). CRP’s level in three groups indicated a significant difference (P=0.001). Conclusion: Findings of the presented study indicated that the consumption of sodium bicarbonate and glutamine supplement could prove useful in reducing the CPK and HDL plasmatic levels following useful athletic activities.

Keywords: Physical activity, Exhaustive exercise, Inflammation, Supplement

JEL classification: I23, O15, I29

Introduction
The purpose of this study is the comparison between the effect of short period consumption of sodium bicarbonate and glutamine following an exhaustive exercise on Creatine Phosphate Kinase (CPK), Lactate Dehydrogenase (LDH) and

1 M. Samavatisharif, Department of Physiology of exercise, Bu-Ali Sina University, Hamadan, Iran, m-samavati@basu.ac.ir
A. HabibiNeghad, Master of Physical Education and Sports Science, Chamran University, Ahvaz, Iran
H. Seiavoshy, Department of Physiology of exercise, Sport Sciences Research Institute of Iran (SSRI), Tehran, Iran
C-Reactive Protein (CRP). In pH physiologic values, Lactic acid almost completely divides into Lactic and Hydrogen ions. It seems that the congestion of Lactic and Hydrogen ions could -independently from each other- leads into exhaustion (1). When glycolysis process is conducted, cell’s access to NAD becomes restricted. If formed NADH through glycolysis process is not able to oxidize into NAD at the same amount, the glycolysis process will not endure. In most cells, not all of them-NADH oxidation and NAD reproduction could be conducted through two procedures. Resurrection of Pyruvate into Lactate by utilization of lactate dehydrogenase enzyme is a method to reach this end (1). However these actions and reactions cause increase in Hydrogen ion which leads into inner-cell pH reduction and this impacts out-of-cell buffer capacity that means certain Hydrogen ions are replaced, if not, inner-cell pH reduction exceeds so dramatically that triggers disruption in cellular function (1). Increase in Hydrogen ion viscosity disrupts the inductive connection through reducing calcium ions release from T-tubules sarcoplasmic matrix, which results in cell function’s deferment (2). Consumption of sodium bicarbonate (NaHCo3) as a robust Lactic acid tampon has become conventional to defer exhaustion among athletes who perform sports, which exert pressure on acid-base system (3, 4). Besides exhaustion consequences, studies have indicated that exhaustion due to intense athletic activities and heavy exercises causes muscular injuries, weight loss and reduction in plasmatic glutamine and skeleton muscles (5). Glutamine reduction due to rigid athletic performance or nutrition restrictions, increase its need (6-8). Considerable amount of research have shown that quantity of glutamine reduces after long (9, 10) or semi-rigid (11, 12) exercises. In some studied it has been mentioned that 2 hours of power-oriented endurance exercises with 75% VO2max reduces the viscosity of plasmatic glutamine (10). 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 (13). On the other hand, many researchers believe that inflammation processes (even in systematic or general type) are among main reasons, which prepares the ground for the onset of atherosclerosis and cardiovascular diseases (14-16). Therefore, since last decades physicians noticed Inflammation markers, which anticipate the risk of cardiovascular diseases with more accuracy and attention (17-19). In this regard, the C reactive protein (CRP) has been indicated as one of the most sensitive and strong independent predictors of cardiovascular diseases risk (20, 21). Concerned with this issue, Meyer and colleagues (2001) stated that the amount of the CRP increases after non-aerobic exercises (22). In another study, it was observed that swiftly after and 24 hours after marathon race, the CRP levels for a short time increases dramatically (23). One of the main measures which could be taken to alleviate inflammatory process and to reduce the negative implications of intense and acute exhaustive exercise is to consume nutrition supplements particularly amino acid glutamine supplement as a source of energy for immune system and as a factor to boost immune cells in order to prevent infections (11, 12). Based on
certain reports, rigorous and exhaustive exercise leads to the production of free radicals (24,25). There is a probability that free radicals with their specific features such as oxidative and through inactivating of enzymes and by damaging nucleic acids, proteins and acid’s membrane, have negative effects on cells and textures (26). One of the methods to measure oxidative pressure that is fomented by cellular texture destruction is through assessing of anti-oxidative enzyme’s secretion (27). Lactate dehydrogenase and Creatine kinase are enzymes, which show the amount of oxidative pressure (27, 28). During hard physical exercise, LDH and CPK enzymes increase and they reduce to the pre-physical exercise period during the recycling phase (29, 30). Another investigation showed that power-oriented exercises increase CPK and LDH plasma (31-33). Exhaustive stamina-oriented exercises increase the amounts of LDH and CPK’s plasma too (34, 35). In addition, reports show that by 48 hours after stamina-oriented activities, CK plasma levels increase (27). Clarkson and Thompson (2000) reported that regular exercise increases levels of anti-oxidative enzymes in muscles and decreases other oxidative pressure indexes (36). Of course by increase in exercise rigidity and transformation of activity from aerobic into non-aerobic, the amount of Lactate increases and following that the amount of LDH too (28). Regarding the impact of sodium bicarbonate consumption on blood Lactate’s response, research has shown that after consumption of this supplement and alongside with the improvement in performance, the amount of congestion of blood’s Lactate has significantly increased after an intermittent hard activity (37-40). Since there is an agreement that consumption of sodium bicarbonate increases the viscosity of bicarbonate plasma, buffering extra hydrogen ion and also by increasing blood’s pH reduces the clinical symptoms of acidosis, furthermore its consumption in little doses is safe (2), and since the amino acid glutamine supplement is source of energy for immune cells and works as a booster to block infections, many athletes are interested in consuming this supplement (11, 12). Regarding this fact that physical education students in some cases must participate in several practical classes without taking adequate rest, the researcher decided to examine and compare the effects of two types of glutamine and sodium bicarbonate supplements on certain indexes of oxidative pressure such as LDH, CPK and on the CRP inflammation marker.

Methods

The present research is semi-experimental and included 45 student women (22.33±3.04 Years, 59.78±8.14 kg, 161.66±5.18 cm, 22.87±2.88 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 supplements in the 48-h prior to the trial. The subjects were divided into three groups: Control group (Con,
N=15); Glutamine group (Glu, N=15); and Bicarbonate group (Bic, 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. 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 group Glu was taken 300 ml of 5% sucrose solution with 0.15% lemon juice with approximately 5 g of glutamine, and the group Bic also was taken 0.065 (g/kg) of sodium bicarbonate. The control group consumed only placebo (Starch).

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 (41).

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 (LDH) and C-Reactive Protein (CRP). All blood samples were measured using the Auto Analyzer, Bio Tecnicon BT3000 and Immunoturbidometry’s method, according to the manufacturer’s instructions (Manufactured by: Beckman Coulter, Inc., 250 S. Kraemer Blvd. Brea, CA 92821, USA).

Table 1. Descriptive characteristics of participants (Mean ± SD)

<table>
<thead>
<tr>
<th>Group</th>
<th>Glu (N=15)</th>
<th>Bic (N=15)</th>
<th>Con (N=15)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (year)</td>
<td>21 ± 1.59</td>
<td>23 ± 2.77</td>
<td>23 ± 4.75</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>59.58 ± 8.9</td>
<td>62.07 ± 8.38</td>
<td>57.42 ± 7.53</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>163.75 ± 4.4</td>
<td>162.5 ± 3.21</td>
<td>158.75 ± 6.56</td>
</tr>
<tr>
<td>BMI (kg/m2)</td>
<td>22.38 ± 3.62</td>
<td>23.52 ± 3.15</td>
<td>22.71 ± 1.87</td>
</tr>
</tbody>
</table>

Abbreviations: Glu=Glutamine Group, Bic=Bicarbonate Group, Con=Control Group, BMI=Body mass index

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.
(42). When significant differences were revealed, the LSD post hoc test was applied (43, 44). As for the comparison of variables before and after the exercise program, the paired t test was performed (45). 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

1. The 45 participants were divided into three groups: 15 in the Con, 15 in the Glu, and 15 in the Bic Groups. The groups Con, Glu and Bic were homogeneous regarding BMI (Table 1).

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 groups do not displayed significant changes in this value (Table 2).

Discussion

This research was conducted to examine and compare the effects of two types of sodium bicarbonate and glutamine supplements following an exhaustive exercise on LDH and CPK and the CRP in non-athlete women. How much lactic acid is produced depends on the intensity and duration of athletic performance and the numbers of engaged moving units. In time of blood’s acidosis and alkalosis,
sodium bicarbonate works as a tampon and by consuming sodium bicarbonate supplement, environment returns to normal (46). Studies indicate that mechanism of ergogenic buffers as a strong acid lactic tampon and hydrogen ion, helps return pH environment to natural state and consequently leads to function improvement and deferring of exhaustion (47). In literature of studies, there is no direct information regarding the effect of bicarbonate supplement on LDH and CPK’s vicissitudes but in many other similar studies, the effect of this supplement on blood’s lactate has been discussed. Results of this study showed that the consumption of sodium bicarbonate did not foment any significant changes in LDH and CPK’s levels following an exhaustive exercise. This result is in line with findings of McNaughton and Thompson (2001), Zajac (2009) and Tofighi (2013) (48, 49, 50). In the most recent research, Tofighi investigated the impact of bicarbonate sodium supplement on blood’s lactate following an exhaustive activity in young men. The findings of this result showed that the long consumption of sodium bicarbonate blocks blood’s lactate consumption (50). While investigating the response of lactate and levels of blood LDH after consuming sodium bicarbonate, some researchers found that consumption of sodium bicarbonate causes increase in blood’s lactate level (37-40) which is in contrast with results of present research. It may be commented that the possible difference between findings is due to the difference in subjects of two experiments; in presents study the subjects were non-athlete female whereas in other research the experiment was conducted on race horses (39). Other causes for this difference may lie in differences in the amount of supplement consumption, type of experiment, numbers of conducting the research, duration of experiment and other possible reasons.

Regarding the impact of glutamine supplement consumption on LDH and CPK indexes, results of this study shows that glutamine’ consumption did not cause any significant vicissitudes on LDH and CPK levels following an exhaustive exercise. The amount of glutamine’s syntheses in stresses or physiological pressures plunges under the essential levels to maintain hemostats (51). While doing sport, increase and decrease in glutamine plasmatic levels have been observed which this transformation is a reflection of type, span and intensity of athletic 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 (52). Glutamine as one of the vital available anti-oxidants could prevent cellular damage (53). 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 (27). 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 the subjects are familiar with extrovert activities (54). In a research that was in line with the current study and was conducted upon rats
researchers found that glutamine supplement was effective in reducing CPK and LDH’s plasmatic levels and inflammatory response caused by long period athletic activity (55, 56). 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 (57). 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 (58,59). Generally based on the current research results and some previous researches, it could be stated that probably glutamine could be effective in CPK and LDH anti-oxidative enzymes’ levels, although, there is not much research concerned with glutamine, CPK, and LDH anti-oxidative enzymes.

Statistically speaking and with regard to the impact of sodium bicarbonate and glutamine supplements on LDH and CPK’s indexes no significant alteration was observed and it could be stated that their effect on LDH and CPK’s indexes is the same.

Regarding the CRP, results of this study show that exhaustive exercise Bruce test causes significant increase of the CRP in serum. In line with what above-mentioned results, Meyer and colleagues (2001), Feng and colleagues (2000), Church and colleagues (2002) and, Stauffer and colleagues (2004) reported increase in the CRP levels following exhaustive exercise (22, 60, 61, 62). Concerned with same topic, Scharhag and colleagues (2004) (63) in a research on various groups of athletes conclude that exhaustive exercise regardless of its type could increase levels of the CRP in both athletes and non-athletes which may prepare the ground for atherosclerosis and cardiovascular diseases to develop in those who are susceptible. Collins and colleagues (2006) (64) stated exhaustive exercise in healthy people does not increase the CRP. One of the probable reasons for this result may lie in type and numbers of subjects and intensity and type of exhaustive exercise. About the intensity of exercise in Collins research, intensity exhaustive exercise was measured by counting the average of covered distance on running track with 5 degree steep by ill people which caused healthy people not to become exhausted; 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 presented research findings and regarding the glutamine supplement consumption, between the average and slope of the CRP, significant difference in both groups was observed after the performance of contracted exercise. It could be declared that the slope of the CRP’s fluctuations in experimental group which consumed glutamine is significantly less than control
group. In contrast to this result, Castell and colleagues (2008) (65) 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 (2008) (66) 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.

**Conclusions**

Based on presented research findings and regarding the consumption of sodium bicarbonate supplement, between the average and slope of the CRP’s fluctuations in two groups following the contacted exercise’s performance no significant difference was observed. Tofighi (2013) (50) investigation that examined the effect of sodium bicarbonate supplement in young men after exhaustive activity, consuming sodium bicarbonate for a long time did not have any significant effect on the amount of the CPR which is In contrast with results of presented research. Tofighi utilized balanced Bruce test to conduct the investigation. It must be stated that the type of activity has the most significant since the intensity and duration of exercise must reach such a level that causes acute phasic response. Based on the findings of this research, it could be stated that probably taking bicarbonate sodium supplement might be effective in preventing and alleviating inflammatory conditions and the CPR in non-athlete women’ students. Generally and based on presented research’s findings, between the CPR’s average and slope vicissitudes in both experimental sodium bicarbonate group and experimental glutamine group after the contracted exercise’s performance on significant difference was detected. According to these findings, in may be declared that probably the impact of both bicarbonate sodium and glutamine’s consumption on the CPR is similar, although, further research is required to confirm the obtained results.

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Comparison between Two Methods of Supplementation with Glutamine and Sodium Bicarbonate on CPK, LDH and CRP in Non-Athlete Women Students

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