The Effect of One Session of Exhaustive Training on Some Biochemical Markers of Skeletal Muscles and Hepatic Metabolism in Men Handball Players

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Abstract
Introduction: Evidence has shown that an increase in the skeletal and hepatic biochemical markers is one of the main factors in the exhaustion of the individual. The aim of this study was to investigate the effect of one session of exhaustive training on some biochemical markers of skeletal muscles and hepatic metabolism in men handball players.

Methods: The present study was a quasi-experimental research method in which 12 handball players with at least 2 years' experience in a superior league (age= 21.42±1.56 years, height= 186 ± 5.85 cm and weight= 83.25 ± 10 kg, body mass index= 24.09 ± 2.93 kg. m⁻²) were randomly selected. Fasting blood samples were collected before and immediately after the maximum Bruce protocol test in order to measure the biochemical changes in skeletal muscles (LDH, CPK and lactate) and hepatic metabolism (ALT and AST). Paired t-test was used to analyze the data. The significance level was less than 0.05.

Results: The results showed that the lactate dehydrogenase (P = 0.004), lactate (P = 0.001) and alanine aminotransferase (P = 0.001) levels increased significantly immediately after the exhaustive exercise. The levels of creatine phosphokinase (P = 0.20), aspartate aminotransferase (P = 0.16) increased but the increase was not statistically significant.

Conclusion: According to the findings of this study, it can be concluded that progressive exhaustive exercise may increase in some of the biochemical markers of skeletal muscle and liver metabolism. Therefore, it is recommended to trainers and athletes to pay more attention to the training principles of practice.

Keywords: Training, ALT, AST, CPK, LDH

Introduction
Delayed onset muscle soreness (DOMS) is muscle pain and discomfort that lasts about one to three days after the termination of exercise. It is believed that delayed muscular tension is due to new muscular activity and exercise activities with extrinsic muscle contractions (1). Creatine phosphokinase (CPK) is a key enzyme that stimulates muscle cell metabolism and accelerates the conversion of creatine to phosphate or vice versa. This enzyme is located in cell membranes of healthy individuals and its amount is low in the blood (2). Increasing physical activity increases plasma CPK (2). Lactate dehydrogenase (LDH), an enzyme found abundantly in the cytoplasm of all tissues of the body with different concentrations, accelerates the conversion of the pyrolytic acid to the lactate acid in the anaerobic glycolic pathway (2). The cellular mechanism of secretion of this enzyme is still unknown, but it is often thought to be due to structural changes in muscle tissue following intense activity (2). Exercise, especially if performed with severity or prolonged duration affects the activity of enzymes. Researchers have confirmed the relationship between
intramuscular acid-accumulation with decreasing peak tension. This effect is associated with an increase in the acid lactate followed by a hydrogen ion concentration and a decrease in PH (3). Numerous research results have shown that aspartate aminotransferase (AST) and alanine aminotransferase (ALT) levels are the best markers for assessing liver condition (4-6). These enzymes are parts of the transaminases. AST converts the Aminite agent's amino acids to alpha-keto acids and catalyzes the transfer of an amine group of α-Ketoglutaric acid and vice versa. For this reason, they are also called aminotransferase. ALT is also involved in the two-way reaction of the transfer of the amino agent of glutamic acid to pyruvic acid and the production of alanine and α-Ketoglutaric acid. The levels of these enzymes are spread in many of the body tissues and have a higher concentration in the liver. They are often considered as liver transaminases. In a study that the effect of one hour of weight lifting on creatine kinase was investigated, it was found that lactate dehydrogenase and aminotransferase enzymes level was increased (7). In a study, the effect of a single session of marathon activity on creatine kinase and lactate dehydrogenase enzyme was investigated in 27 males and 7 females. The results indicated the enzymes level increased after the exercise (8), while in another study no significant change in these enzymes was found after a session of activity (9) while in another study that the effect of warmup on delayed onset muscle soreness (DOMS) of non-athlete male students was examined after extrinsic contractions, it was found that warm up did not cause a significant change in the DOMS (10). The result of a study in which the effect of 30 minutes of step walking activity on serum ALT, AST and LDH enzymes in non-athlete participant was examined and another study where the effect of 246 km of running on liver and muscle enzymes was measured showed that enzyme levels increased (11, 12). In addition, limited studies have examined the effects of increasing exhausting exercises in athletes, and there is little information about the effects of increasingly exhausting exercises on the biochemical markers of skeletal muscle and hepatic metabolism. Therefore, new research is needed to clarify the ambiguities. In order to address these issues, in the present study has been investigated the effect of a on session of exhausting activity on biochemical markers of skeletal muscle and hepatic metabolism in male handball players.

Methods
In a quasi-experimental (pre-test and post-test design in an experimental and control group) randomly, 12 young men qualify from the Hepco Arak Handball team, who have at least 2 years of history in Iran's first and best handball league. They were selected according to the physician's confirmation of complete physical health. The inclusion criterion was age range of 19 to 25 years, body mass index of 18.5 to 24.9 kg / m2, at least 2 years of history of the game in the handball league of the country and the exclusion criterion were BMI greater than 25 or less than 18.5, having a history of disease, cigarette smoking, less than 2 years of history of game participation, drug use during the last 6 months. The participants were informed of the purpose, benefits and potential risks of the experimental design (Bruce Protocol) and completed the consent form before the start of the work. The participants referred to a laboratory at 8:10 am in a fasting condition to measure their body composition and giving blood sample. They received detailed explanation about the purpose of the research and possible risks of participation completed a written consent letter prior to the blood sample collection. Body weight was measured using Persian Digital Scale (QF-2003B model in Iran with a precision of ± 0.1 kg) bare footed with minimum dress, height using wall height (44440 model manufactured by Kaveh Co., Iran with accuracy of 0.1 ± cm) in an erect
standing position beside the wall while the scapula was in normal condition. Then the percentage of body fat was measured by 7-point right sides of the body by a caliper (PHYSICAL BEST) three times and 20 seconds between every turn. The mean of three times were used in Jackson-Pollack formula (13) and Siri's equation (14) to calculate the fat percentage. In order to reduce error, all measurements were taken by one person. Subjects' dietary information was recorded by a 24-hour food recall questionnaire in three days (two days at the beginning of the week and one end of the week) by the participants in a special diet. To analyze the data at first, the food consumed was converted to grams and then using processor Dorosty Food software (NII, FP2) to determine the amount of macronutrients. On the day of activity, the subjects used a standard diet (Dietary Reference Intakes-DRI) (15). The exercise program included a general warmup of 10 minutes (Stretching and soft motion), Bruce's maximum protocol and a 5-minute cool down (including 2 minutes of slow walking at a speed of 3 km.h-1 on treadmill to reduce heart rate and then movements Stretching). The maximum Bruce protocol on the treadmill runs at maximum seven steps. The duration of each stage is three minutes. Increasing the intensity of activity from one stage to the next stage is associated with increasing slope and velocity. The first stage was started at 1.7 mph a speed of (2.74 km.h-1) and a slope of 10%, and then the velocity and gradient increased with a constant ratio (slope 2% and speed of approximately 0.8 mph) at each stage (16). Subjects were asked to continue to work to exhaustion. In a morning before the Bruce maximum protocol, 5 cc of venous blood was collected in a lab by syringe (made in Iran, Model 5ML-22G) from the forearm vein Medium (basilic vein) in a sitting position from every fasting participants to measure the variables. The evening of that day, Bruce's maximum protocol was run to exhaustion. All the participants were asked not to consume any food except water 4 hours before the protocol execution. The blood samples were drawn 6 minutes before and 6 minutes after the Bruce protocol. The collected blood samples were kept in sterile tubes and transferred to the laboratory for analysis. After blood samples collection, the participants performed cool down exercise for 10 minutes. The collected blood samples were centrifuged at 3500 rpm for 10 minutes to separate the plasma. Then, the level of CPK, LDH, AST and ALT were measured by the Autotold analyzer HITACHI 911 made in Japan and the kits of Pars Azmoon Co. Made in Iran by ELISA method. Kolmogorov-Smirnov test was used to check the normality of variable and parametric statistical including paired sample t-test was used to compare the pre-test and post-test means. Then, the dietary analysis was performed using repeated Measures and Bonferroni post hoc test. All the analysis was performed by SPSS version 18 with alpha level set to 0.05.

Results
The result of descriptive analysis including weight, height, age, and body fat and body mass index is presented in table 1. Analysis of variance repeated measure (ANOVA-RM) was employed to compare the calorie intake of the different days prior to Bruce protocol. No significant differences were found. The result of analysis is presented in (Table 2). The result of paired t-test is presented in table 3. The results show that the lactate dehydrogenase (P=0.004) lactate (P=0.001) and alanine aminotransferase (P=0.001) level increased significantly immediately after the exhaustive exercise. The level of creatine phosphokinase (P=0.20), aspartate aminotransferase (P=0.16) increased but the increase was not statistically significant.
Table 1: Mean and Standard Deviation weight, height, age, body fat and body mass index

<table>
<thead>
<tr>
<th>Variable</th>
<th>Mean ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>2.88±14.24</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>4.1±7.13</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>10.5±83.42</td>
</tr>
<tr>
<td>Fat percentage (percent)</td>
<td>5.67±185.83</td>
</tr>
<tr>
<td>Body mass index (kg.m⁻²)</td>
<td>2.88±14</td>
</tr>
</tbody>
</table>

Table 2. Diet analysis of 24-hour dietary recall questionnaires.

<table>
<thead>
<tr>
<th>Variables</th>
<th>Saturday</th>
<th>Sunday</th>
<th>Friday</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Energy absorption (kcal)</td>
<td>170.6±3100</td>
<td>160.9±2900</td>
<td>190.4±3000</td>
<td>0.44</td>
</tr>
<tr>
<td>Carbohydrate(%)</td>
<td>5.6±51.96</td>
<td>6.6±51.5</td>
<td>4.8±49.3</td>
<td>0.58</td>
</tr>
<tr>
<td>Fat percentage</td>
<td>3.6±36.3</td>
<td>4.2±35.5</td>
<td>2.9±37.2</td>
<td>0.64</td>
</tr>
<tr>
<td>Protein(%)</td>
<td>2.8±12.3</td>
<td>2.6±13.3</td>
<td>3.1±13.9</td>
<td>0.65</td>
</tr>
</tbody>
</table>

Table 3. Paired sample t-test comparing LDH, CK, Lactate, AST, ALT levels before and immediately after the exhaustive exercise

<table>
<thead>
<tr>
<th>Variable</th>
<th>Before the activity</th>
<th>After the activity</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lactate dehydrogenase (mg.dl⁻¹)</td>
<td>99.79±446.08</td>
<td>95.83±539.17</td>
<td>0.004*</td>
</tr>
<tr>
<td>Creatine Kinase (mg.dl⁻¹)</td>
<td>211.75±242.08</td>
<td>258.24±271.75</td>
<td>0.207</td>
</tr>
<tr>
<td>Lactate (mg.dl⁻¹)</td>
<td>1.76±4.00</td>
<td>3.38±14.42</td>
<td>0.000*</td>
</tr>
<tr>
<td>Aspartate aminotransferase (mg.dl⁻¹)</td>
<td>13.67±31.17</td>
<td>15.56±34.17</td>
<td>0.163</td>
</tr>
<tr>
<td>Alanine aminotransferase (mg.dl⁻¹)</td>
<td>19.05±21.67</td>
<td>18.97±25.17</td>
<td>0.001*</td>
</tr>
</tbody>
</table>

*Significant relationship at the level of P <0.05

Discussion
The aim of this study was to examine the changes in biochemical markers of skeletal muscles and hepatic metabolism response to an intensive session of physical activity. It seems like when the intensity of exercise or activity is increased to the extent that the muscle tissue can tolerate (the fracture point), the markers of muscle and liver damage of the intracellular proteins leak into the interstitial fluid. These materials are collected by the lymphatic system and released into the bloodstream. Therefore, the amount of these enzymes can be a markers for measuring muscle and liver damage. This study was designed to examine the acute effect of a progressively increasing exhausting activity on changes of biochemical markers of skeletal muscle and hepatic metabolism in male handball players. The result of present study showed that increasing the level of exhausting activity leads to an increases in muscular delayed soreness indices in handball men. In this study, the increasing exogenous activity increased the amount of lactate dehydrogenase, lactate (significant) and creatine kinase (increased with insignificant). The results of this study is in agreement with the findings other investigation that showed increase of these enzymes following the exercise (17), however, another study showed that after an unloaded exercise session with 10 repetitions and one minute of rest resulted in no significant increase in the amount of these enzymes (9). It seems that the type of exercise, recovery time and exercise intensity have a significant effect on the release of these enzymes. Numerous researchers have reported that muscle contractions damage muscle fibers, connective tissues and cell membranes and increase the levels of LDH and CPK. However, muscle damage and oxidative stress are not directly correlated (18). In a study of muscle injury during training and after
competition in Thai boxers, it was found that even during the normal exercise, the activity of CK and LDH was significantly higher in the boxers than in the control group (19). It seems that the use of proper training method and arranging the training time and recovery according to the athletes' fitness levels reduce the disorder of biochemical changes. Researches in recent years have shown that 5 to 8 weeks of accelerated-frequency training increased blood flow and its circulation in the arteries, increased lactate and \( + \text{H} \) transfer capacity from active muscle, ion regulation, and the function of the network of sarcoplasmic reticulum (20). Although the timing of these adaptations is unknown, other studies show similar adaptations after only 5 to 7 days of anaerobic exercise in the blood flow (21) of the secretion of lactate from the training muscle and the positive pump activity (22). In study the present. It was found that one session of increasing exhaustive exercise has a significant effect on liver enzymes of men's handball including the levels of AST and ALT, (P = 0.001), but the increase in AST (P = 0.163) was not significant. The result of studies shows that ALT, AST enzymes fluctuate 24 hours a day or even seasonally (23). However, since exercise program in this study was limited to the light cycle and the participants performed the activity in the same research environment at all stages of the program and a within subject design was employed, there is insufficient evidence to attribute the changes in ALT and AST enzymes to the daily or yearly cycles. Barzgarzadeh Zarandi and colleagues showed that 6 and 12 weeks of continuous and periodic exercises significantly increase the levels of ALT and AST in elderly rats (24). This result is similar with the findings of the present study. In addition, Clarkson reported a significant increase of AST in active girls in a study that used increasing exhausting exercises in performed daily for a week (25). Rezaei et al. conducted a research in which Sprague-Dawley male rats performed three sessions of exercise on a negative slope and showed a significant increase in AST and ALT levels (26). The results of these studies support the findings of the present study. However, Zar et al. Examined the effect of eight weeks of aquaculture on liver enzymes and fatty profile of middle-aged women and sowed a significant decrease in AST and ALT (27). In another study, it was concluded that short-term exercise reduces liver enzymes' circulation in obese individuals with non-alcoholic fatty liver (28). In the same study, it was shown that 3 weeks of aerobic training with omega-3 supplementation, AST was decreased significantly (29). The findings of this study are not in agreement with the findings of the present study. Therefore, it is likely that this increase in serum levels is the result of injury and entry of these enzymes from all the organs mentioned, in addition, exercise with eccentric contraction and lack of sufficient opportunity to restore to the primary state results in damage to some organs of the body (29). In various studies, researchers have used different training protocols, thus, it may be another reason for this discrepancy in findings. Other factors like exercise experience, type of participants, physical fitness, and types of sport may also be the reason for these differences.

**Conclusion**

Based on the findings of this study, it may be concluded that increased levels of AST, ALT, LDH and CPK enzymes after exhaustive exercise activity may lead to serious damage to liver and skeletal muscle. It needs to be noted that these enzymes are present in cells of other organs, such as the heart muscle. Therefore, a daily exercise session for athletes with this level of fitness has an effect on their performance and may be associated with risks to their other cells within the body, particularly the muscles. Thus, the researcher advise the coaches and trainers to design proper planning for their exercises in order to prevent muscular injuries. Decrease of these
injuries increase the life of the athletes in sport and health condition in addition to cost of treatment.

**Ethical issues**
Not applicable.

**Authors’ contributions**
All authors equally contributed to the writing and revision of this paper.

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**References**


