The Effect of One Session of Exhaustive Swimming Training on Lactate Dehydrogenase, Creatine Kinase, and Lactate of Elite Male Swimmers

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Abstract
Introduction: Sports activities with increased mechanical pressure can lead to biochemical changes in the body. The purpose of this study was to investigate the effect of one session of exhaustive swimming training on the response of creatine kinase (CK), lactate dehydrogenase (LDH), and lactate in elite male swimmers.

Methods: In this experimental study, 28 elite male swimmers aging 15-20 years in Fars province who were performing their training every day at Enghelab International Pool of Shiraz were selected as the sample of study. At first, the health status form and informed consent questionnaire were completed by athletes and their parents. To eliminate the effect of previous training on levels of LDH, CK and lactate, a 48-hour rest before the implementation of the research protocol was given to swimmers. On the day of the research, blood samples were taken before training in the sitting position. The swimmers then warmed up for 10 minutes. Subsequently, all subjects performed exhaustive swimming training. Immediately after the end of the training, 15 and 30 minutes following the training, blood samples were again taken from all of the subjects similarly in the sitting position. To analyze the findings of the research, repeated measures ANOVA along with Bonferroni’s post hoc test were used at p≤0.05.

Results: One session of exhaustive swimming training did not have a significant effect on CK of elite male swimmers (P = 0.40); one session of exhaustive swimming training had a significant effect on LDH (P = 0.001) and lactate (P = 0.001) of elite male swimmers; LDH levels decreased significantly 15 minutes (P = 0.001) and 30 minutes (P = 0.009) after training, and lactate levels decreased significantly 30 minutes after training (P = 0.001).

Conclusion: It seems that one session of exhaustive swimming training in elite male swimmers leads to a significant increase in LDH and lactate. Also, LDH decreases significantly after 15 minutes and lactate after 30 minutes.

Keywords: Training, Swimmer, Creatine Kinase, Lactate Dehydrogenase, Lactate

Introduction
Different physiological, biomechanical, nutritional, and psychological factors have an effect on reaching an athlete to the peak of performance (1). Among the physiological factors, the type of energy systems for energy production, recovery of energy and disposal of metabolic substances are important for the growth and development of athletes (2). A lot of research has been done on the factors affecting the structure and application of energy production systems in athletes. Most studies have shown that energy systems are considered as essential factors in the development of sport skills (3). During physical activity, the pressure goes beyond the normal level of rest on the various physiological systems of the body. Changes
that occur in the body through exercise can make the systems more effective and efficient at the time of the match (2, 4). Tolerance of high intensity training in athletes depends on individual fitness against physical activity fatigue resulting from chemical interactions such as lactic acid production, lactic acid tolerance, buffering capacity, release and clearance of the metabolic substances produced from the muscle fibers (5, 6). Since swimming is a different sport, so to achieve principle of adaptation, swimmers have to go through different trainings in different energy systems (5, 6). The most effective factor in the decline of frequent performances of fast swimmers is the increase in fatigue and inefficiency of the glycolysis system. According to researchers, the decrease in lactate accumulation, along with the improvement of the psychological and technical conditions of swimmers, has been effective in preventing the decline of frequent and fast performances (6, 7). If the level of lactate production is sufficient to exceed the ability to oxidize mitochondria, the concentration of lactate increases. With increasing lactate, the pH of the muscle decreases. Although due to body buffering capacity changes in muscle pH are negligible, the same minor effect affects the production of energy and muscle contractions (8). By reducing muscle pH, intracellular fluid acidity stimulates pain receptors; as a result, the swimmer speed decreases. When the muscular pH reaches less than 7, it prevents phosphorylase and phosphofructokinase (PFK) enzymes activities (1). These enzymes regulate anaerobic metabolism (5). Creatine kinase (CK) is an intracellular protein that is leaked into the plasma after injury to the cell membrane due to oxidative pressure, and also by increasing the intensity of training and converting the activity from aerobic to anaerobic pathway, the amount of lactate accumulation followed by LDH accumulation is increased (11, 10). Most studies know the cause of the secretion of this enzyme to be changes in muscle tissue following high intensity activity (12). Considering the fact that there is no comprehensive information about the serum anaerobic system factors at different times after a session of exhaustive swimming training, the aim of this study was to investigate the effect of one session of exhaustive swimming training on the response of CK, LDH and serum lactate of elite male swimmers.

Methods
In this experimental study, 35 elite male swimmers aging 15 to 20 years from Fars province, who were performing their training every day at Enghelab International Pool of Shiraz were called for participation. After their declaration of preparation, they attended a briefing session, and were informed about the process of conducting the research (i.e., purpose and method of testing); then 28 qualified individuals were selected as the sample of study. At first health questionnaires and consent forms were filled and confirmed by athletes and their parents. The criteria for inclusion in this study comprised the ability to swim 4500 meters in 90 minutes and membership in the selected swimming team of production and are known as oxidative stress indices. Lactate dehydrogenase (LDH) and CK are serum cell damage indicator enzymes within the cell membrane; however, their release into the blood may increase due to cell membrane rupture, induction of enzyme synthesis, increased cell proliferation, and increased cellular degradation (10). Since cell membrane function is compromised by oxidative stress, this is evaluated by measuring the plasma CK, because CK is an intracellular protein that leaks into the plasma after membrane damage. Also, by increasing the intensity of training and converting the activity from aerobic to anaerobic pathway, the amount of lactate accumulation followed by LDH accumulation is increased (11, 10). Most studies know the cause of the secretion of this enzyme to be changes in muscle tissue following high intensity activity (12). Considering the fact that there is no comprehensive information about the serum anaerobic system factors at different times after a session of exhaustive swimming training, the aim of this study was to investigate the effect of one session of exhaustive swimming training on the response of CK, LDH and serum lactate of elite male swimmers.
Fars province. Exclusion criteria were related to being involved in specific diseases, the history of taking exercise supplements in the last month and smoking. In the present study, to eliminate the effect of previous trainings on LDH, CK and lactate levels, a 48-hour rest before the implementation of the research protocol was given to swimmers.

On the day of the research, blood samples were taken before training in the sitting position. The swimmers then warmed up for 10 minutes. Subsequently, all subjects performed exhaustive swimming training. Immediately after the end of the training, 15 and 30 minutes after training, blood samples were taken again from all of the subjects in the sitting position.

The exhaustive swimming training protocol was performed in three stages: warm-up, progressive, and lactate tolerance trainings. The warm-up included a 200-meter-long front (forward) crawl, 150 m backstroke, 100 m breaststroke, 50 m butterfly stroke, 50 m front crawl leg, and 50 m hand stretch swimming. The progressive training included front crawl (the first 50 m with 75% intensity and the next 50 m with 85% intensity) and lactate tolerance trainings including fast swimming with average intermittent rest periods including 8 repeats of 100 m and each 100 m with intensity of over the threshold (resting 1 minute in every 100 meters), so that these trainings caused severe acidosis among swimmers (2).

It should be noted that at each session of sampling in the sitting position, a dose of 5cc blood from the brachial vein was captured, of which 3cc was injected into the clot tube to prepare the serum for determination of CK and LDH, and 2 cc in an anticoagulant test tube to prepare the plasma.

Measurements of CK and LDH were performed by blood test using the DGKC photometric method (German Society for Biochemistry Standard), and plasma lactate was measured by enzyme and spectrophotometric procedure using the Cormay Randox 1530 H.W standard kit. 2-vial 25 ml cardboard box NAC Biochemistry Laboratory Kit supplied by Pars Co. was used to measure CK and LDH. Also, the Cormay Randox 1530 H.W standard kit, manufactured in Germany, was used to measure plasma lactate.

Results

The demographic characteristics of the subjects are presented in Table 1. Also, levels of LDH, CK and lactate before training, immediately after training, and 15 and 30 minutes after training are presented in Figures 1 to 3, respectively.

The results of repeated measure ANOVA test in Table 2 show that one session of exhaustive swimming training had a significant effect on LDH (F = 9.21, P = 0.001) and lactate (F = 64.45, P = 0.001) of elite male swimmers; however, it does not have a significant effect on CK (F = 0.98, P = 0.40).

The results of Bonferroni’s post hoc test in Table 3 show that LDH levels immediately after training increased significantly compared to before training (P = 0.001); however, 15 minutes after training (P = 0.001) and 30 minutes after training (P = 0.009) LDH levels decreased significantly after training.

Lactate levels immediately after training increased significantly compared to before training (P = 0.001). Also, 15 minutes (P = 0.001) and 30 minutes after training (P = 0.02), lactate levels were significantly higher than lactate levels before training; 30 minutes after training, lactate levels decreased significantly compared to immediately after training (P = 0.001).

Also, 30 minutes after training, the levels of lactate decreased significantly compared to 15 minutes after training (P = 0.001).
Table 1. Demographic characteristics of subjects

<table>
<thead>
<tr>
<th>Variable</th>
<th>Mean</th>
<th>Standard Deviation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (year)</td>
<td>17.5</td>
<td>17.5</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>74.33</td>
<td>5.96</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>179.50</td>
<td>4.01</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>23.07</td>
<td>1.72</td>
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</table>

Table 2. Results of repeated measures ANOVA to examine the effect of one session of exhaustive swimming training on LDH, CPK and lactate

<table>
<thead>
<tr>
<th>Variable</th>
<th>Source</th>
<th>Sum of Squares</th>
<th>Mean Square</th>
<th>F</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>LDH</td>
<td>Time</td>
<td>59844.32</td>
<td>19948.10</td>
<td>9.21</td>
<td>0.001</td>
</tr>
<tr>
<td></td>
<td>Error</td>
<td>175314.67</td>
<td>2164.37</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CK</td>
<td>Time</td>
<td>81797.45</td>
<td>27265.81</td>
<td>0.98</td>
<td>0.40</td>
</tr>
<tr>
<td></td>
<td>Error</td>
<td>2245252.29</td>
<td>27719.16</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lactate</td>
<td>Time</td>
<td>145985.37</td>
<td>48661.79</td>
<td>64.45</td>
<td>0.001</td>
</tr>
<tr>
<td></td>
<td>Error</td>
<td>61151.46</td>
<td>754.95</td>
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<td></td>
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</table>

Table 3. Results of Bonferroni’s post hoc to compare the changes of LDH and lactate before training, immediately after training, and 15 and 30 minutes after training

<table>
<thead>
<tr>
<th>Variable</th>
<th>Time</th>
<th>Mean Difference</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>LDH</td>
<td>Immediately after Training</td>
<td>-58.75</td>
<td>.001</td>
</tr>
<tr>
<td></td>
<td>15 Minutes after Training</td>
<td>-9.03</td>
<td>.99</td>
</tr>
<tr>
<td></td>
<td>Immediately after Training</td>
<td>49.71*</td>
<td>.001</td>
</tr>
<tr>
<td></td>
<td>15 Minutes after Training</td>
<td>49.60*</td>
<td>.009</td>
</tr>
<tr>
<td></td>
<td>30 Minutes after Training</td>
<td>-0.10</td>
<td>.99</td>
</tr>
<tr>
<td>Lactate</td>
<td>Immediately after Training</td>
<td>-89.16*</td>
<td>.001</td>
</tr>
<tr>
<td></td>
<td>15 Minutes after Training</td>
<td>-71.19*</td>
<td>.001</td>
</tr>
<tr>
<td></td>
<td>30 Minutes after Training</td>
<td>-21.48*</td>
<td>.02</td>
</tr>
<tr>
<td></td>
<td>Immediately after Training</td>
<td>17.97</td>
<td>.06</td>
</tr>
<tr>
<td></td>
<td>15 Minutes after Training</td>
<td>67.67*</td>
<td>.001</td>
</tr>
<tr>
<td></td>
<td>30 Minutes after Training</td>
<td>49.70</td>
<td>.001</td>
</tr>
</tbody>
</table>

Discussion
According to the findings of this study, one session of exhaustive swimming training has a significant effect on LDH and lactate in elite male swimmers; however, it has no significant effect on CPK. Also, the findings of the present study showed a significant increase in lactate levels immediately after training, 15 minutes after training and 30 minutes after training, compared to before training; also 30 minutes after training, the levels of lactate were significantly reduced compared to immediately after training and 15 minutes after training. Lactate is a by-product of glycolysis that can be produced and continuously implemented by a wide number of the body cells in rest and even when oxygen is available in sufficient quantities.
Figure 1. LDH changes before training, immediately after training, 15 minutes after training and 30 minutes after training

Figure 2. CK changes before training, immediately after training, 15 minutes after training and 30 minutes after training

This metabolite is a dynamic and important substrate during the ATP rebuilding period, which has a huge potential as an energy source and is effective in the reconstruction of energy sources (12). Increasing and exhaustive trainings, in addition to improving certain aspects of metabolism, can have serious and destructive effects on an individual’s performance. Acidosis caused by the practice of lactate tolerance is very severe. Structural injuries, with the passage of time, have the potential to be so prevalent that the athlete loses his strength and ability (13).
Performing such an exercise can undermine endocrine system and immunity. The need for energy in swimming competitions is far beyond what aerobic metabolism can provide (6). Thus, the amount of residual energy should be provided through an anaerobic metabolism and acid-lactic production. Any mechanism that can eliminate some of the lactic acid from its production site, that is, active muscle fibers, will delay the reduction of muscle pH in the course of the tournament (12). Consequently, improving lactate elimination by exercise will have a significant positive effect on athletic performance. To convert lactate to pyruvate and to generate energy, proteins such as LDH enzyme play a role (12, 14). LDH is an enzyme abundantly found in the cytoplasm of all tissues of the body with different concentrations, and in the glycolysis pathway, it can increase the rate of conversion of pyruvic acid to lactic acid or vice versa, and typically it gradually increases in the range of 24 to 48 hours after stimulation (12). Most studies know the cause of the secretion of this enzyme to be changes in muscle tissue following high intensity activity (15). During the practice of lactate tolerance, the use of glycogen stores will increase, and the amount of muscle glycogen decrease, especially in the fast-twitch muscle fibers, will be very high. In the long chain of lactate tolerance, some of the fast-twitch muscle fibers are released from the glycogen (13). Discharge of glycogen in various types of muscle fibers can be a factor in fatigue (2). High intensity trainings also lead to muscle damage, which can be explained by the mechanism of dilatation and shortening cycles (16). 24 hours after training, the immune system shows a dramatic increase, which reflects the body's defense against increased factors such as lactate dehydrogenase and creatine kinase. These factors justify increased lactate dehydrogenase enzyme. These changes may take a week to return to the base level (17). Also, the findings of this study showed a significant increase in this enzyme, as LDH levels immediately after training significantly increased compared to before training, however, 15 minutes after training and 30 minutes after training, LDH levels decreased significantly compared to immediately after training. Kabayashi et al. (2005) showed that aerobic exercise, such as running, may
increase LDH activity from 12 to 24 hours (17). Therefore, it has been suggested that immunological and hormonal changes are due to the increase in the volume and intensity of the trainings, which is related to stress in both types of training (i.e., aerobic and resistance) (12). The findings of this study are consistent with Mirzaei et al. (2007) (12). Mirzaei et al. reported that exhaustive training had no significant effect on CK changes. The results of this study are also consistent with the research by Close et al. (2004). They also did not observe a rise in CK levels in well-prepared athletes on ergometer bike after running 65 percent of maximum oxygen consumption (Vo2max) on a treadmill (18). In addition, Jamurtas et al. (2000), by studying the effect of introverted and extroverted activities on plasma CK levels, did not see any significant difference in CK enzyme in the experimental groups (19). CK is a key enzyme that promotes the metabolism of muscle cells and accelerates the conversion of creatine to creatine phosphate or vice versa. This enzyme is found in the healthy individuals in the cell membrane and is low in the blood. CK is an intracellular protein that leaks into the plasma after injury to the cell membrane due to oxidative stress and muscle damage. In the muscular injuries, CK enzyme indicates the most significant changes, and is the index for measuring muscle damage (20,21). It seems that the type of training, recovery time and training intensity affect the release of these enzymes. Meanwhile, factors such as age, gender, body fitness, season, and training are associated with increased fluctuations of these enzymes (22). Regarding the findings of this study, it can be concluded that the increase of LDH enzyme levels after exhaustive training activity has caused serious damage to skeletal muscle cells. Of course, it should be kept in mind that these enzymes are present in cells of other organs, such as the liver and the heart (13); therefore, a daily training session for athletes with this level of fitness, in addition to affecting their performance, may compromise cells of other organs, especially muscles. Therefore, coaches and athletes are recommended proper planning of trainings to prevent muscular injuries and reduce these injuries, as it can increase the life of the champions and maintain their health together with reducing the cost of treatment.

Conclusion
It seems that one session of exhaustive training in elite male swimmers leads to a significant increase in LDH and lactate; therefore, with a significant increase in LDH levels, which is one of the main markers of muscle damage, it can be concluded that one session of exhaustive swimming training can lead to muscular damage.

Ethical issues
Not applicable.

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

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References