

The effects of interval training and age on blood lactate (La) levels and lactate dehydrogenase (LDH) activity in male Wistar rats

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Abstract

Introduction:

Physical activity and age are among the factors affecting lactate levels and lactate dehydrogenase activity. Physical activity appears to be able to counterbalance the morphological and metabolic changes associated with aging that decrease physical ability and performance.

Objectives: The purpose of the present study was to assess the effects of interval training on blood lactate levels and lactate dehydrogenase activity in young and old rats.

Materials and Methods:

A total of 40 male rats were selected and then divided into two age groups -the old group (20 rats aged 27 months and weighing 389 ± 31 g) and the young group (20 rats aged 3 months and weighing 224 ± 13 g). Each group was itself randomly divided into an experimental group (n=10) and a control group (n=10). The training protocol involved 4 minutes of running on the treadmill with intervals of 2 minutes active resting in 10 training bouts of 60 minutes, for 6 sessions per week and for 8 weeks and gradually increasing in intensity. Twenty-four hours after the last training session, the rats were anesthetized with a mixture of ketamine and xylazine in order for their blood sample to be collected from their cardiac puncture. Their lactate levels and LDH activity were then measured by an enzymatic method. Data were analyzed using the one-way ANOVA and Tukey's post-hoc test.

Results:

The results showed no significant differences in blood lactate levels between the four groups; however, LDH activity was significantly higher in the young experimental group than in the young control group ($p < 0/05$).

Conclusion:

The results indicate that lactate is increasingly cleared by interval training. It also appears that the effect of training on lactate clearance is similar in both young age and old age. Monitoring blood lactate levels benefits muscle glycogen replenishment and intracellular pH (pHi) regulation.

Keywords: Lactate dehydrogenase(LDH), interval training ,Lactate (La)

Introduction

Lactate is a dynamic substrate that has a great potential as an energy source and is

effective in the restoration of adenosine triphosphate (ATP) (1). The normal level of blood lactate is 0.5 to 2.2 mmol per liter

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(2). It is believed that this amount is increased in the range of 20 to 25 mmol per liter in the complete exhaustion (3). Peak blood lactate concentration occurs approximately 5 minutes after the cessation of intense exercise (2,4).

Lactate dehydrogenase is an enzyme that catalyzes the pyruvate-lactate reaction. This enzyme is found abundantly in the cytoplasm of all body tissues and usually its amount is gradually increased 24 to 48 hours after stimulation (5). The enzyme is secreted due to structural changes in the muscle tissue after vigorous activity (6).

During exercise, lactate is released from the contracting muscles and consumed by heart and various oxidative muscles. The diffusion of monocarboxylates such as lactate and pyruvate across the plasma membrane of mammalian cells is facilitated by carrier membrane proteins known as monocarboxylate transporters (7). Aging causes morphological and metabolic changes in skeletal muscle as reduction in the transverse section of muscles followed by reduced ability and weakened athletic performance (8). The number of type I and II fibers reduced in muscle fibers with a selective atrophy of type II (8,9). This phenomenon, referred to as sarcopenia, can be the result of a reduction in fiber size, fiber number, or a combination of both (10). Furthermore, lactate accumulation is reduced with age and results in increased resistance to exhaustion in skeletal muscles in the elderly (11). However, some studies indicate that lactate clearance is reduced in the elderly during and after exercise compared to younger individuals (12). Korhonen et al. reported that lactate level in male and female track and field athletes (mean age 40-88 and 35-87, respectively) is reduced after the match (13). It seems that the reduction in blood lactate in the elderly is related to factors such as the muscle mass and plasma volume which are reduced with age (14). In addition, it seems that old age is also associated with a minimum efficiency of glycolytic enzymes

(14). Pastorisa et al. stated that the activity of lactate dehydrogenase and hexokinase, citrate and citrate synthase is reduced in muscle as we age (15). Although the results of Benelli Piero et al's study in 2007 on the old swimmers showed no significant effect of age in women's blood lactate in the range of 40-79 years, a significant reduction was observed in men's blood lactate with increasing age (16). Although pieces of experimental evidence are not consistent, they show that the activity of the glycolytic enzymes is influenced by age. On the other hand, heavy exercise will produce large amounts of lactic acid in activated skeletal muscle and disrupts acid-base balance in the body, which reduces exercise performance ability (17). In addition, in the highly acidic conditions, some pH-sensitive enzymes such as phosphofructokinase are inhibited leading to decreased production of adenosine triphosphate (18); therefore, it is necessary to monitor lactate during exercise training. Furthermore, following the diffusion of lactate from skeletal muscle into the plasma, lactate and hydrogen ion are transferred into red blood cells particularly by monocarboxylate transporters and carried in the body in this way (19). The oxidative fibers absorb some lactate in the plasma and combine it with oxygen and the rest of the lactate is used in the liver for gluconeogenesis (20). These routes lead to removal and clearance of lactate. It seems that exercise training with appropriate intensity contributes to lactate clearance through increased expression of monocarboxylate transporters, increased mitochondria density and accelerated gluconeogenesis (21). Despite the importance of this issue, there are few studies which have investigated the effects of physical activity on the rate of lactate and lactate dehydrogenase activity, particularly in the elderly. Due to the positive effects of exercise on physical fitness and increased lactate clearance after training in the elderly, such studies can provide practical solutions against

complications of aging. In line with this issue, Sari Dewi N et al. reported that interval training reduces blood lactate levels of rats (22). Also Clarkson et al. (23) reported that values of lactate dehydrogenase and phosphofructokinase are increased significantly after training and competition. According to these studies, increased concentration of these enzymes is related to the intensity, type and duration of training (23). Carnevali Jr et al. also reported increased activity of lactate dehydrogenase and decreased lactate concentration in muscles of rats after interval training (24).

In general, the age and physical activity can be considered as two factors influencing on the amount of lactate and lactate dehydrogenase, but influencing factors related to age and the interaction between aging and physical activity are not yet fully known. In addition, the results of these studies are inconsistent and ambiguous. Also, few studies have examined the effect of interval training and there is little information about the effects of interval training on lactate clearance and its relationship with age. Hence, new studies are needed to clarify ambiguities. So, the present study was conducted to investigate the effect of interval training on blood lactate and lactate dehydrogenase activity in old and young male Wistar rats.

Materials and methods

This is an experimental study on an animal model with test-retest method and a control group. Animal samples were randomly divided into experimental and control groups and training protocol was conducted on the experimental groups for eight weeks. The control group did not exercise at this time, but otherwise they were housed under identical conditions as the experimental group.

Animal samples

A total of 40 male Wistar rats were obtained from the Pasteur Institute of Iran

and assigned into an old group (n=20) with the mean age of 27 months and a young group (n=20) with the mean age of 3 months. The rats were transferred to the animal house in the School of Pharmacy and Pharmaceutical Sciences, Isfahan University of Medical Sciences and housed at 22 ± 1 °C, $55.6\% \pm 4$ humidity, 12 hr light/dark cycle and appropriate amounts of food and water. After a week of adaptation to the lab environment, the old rats with the mean weight of 389 ± 31 g and young rats with the mean weight of 224 ± 13 g were randomly divided into experimental (n=10) and control (n=10) groups. The control group was housed in transparent polycarbonate cages during the exercise and were weighed weekly to control their health. First, the rats became familiar with training by running on a treadmill for a week, 10 minutes per session, 3 days a week, at the speed of 8 meters per minute and 2 meters per minute was added to the speed every day. Then, the training protocol was conducted for 8 weeks on the experimental group. In the end, both groups were anesthetized and the biopsy was performed.

All experiments were performed according to the guidelines of laboratory animal protection laws. The executive protocol was also approved by the Ethics Committee of Clinical Center of Isfahan University of Medical Sciences.

Training protocol

Training protocol consisted of 4 min running and 2 min active rest in 10 training phases. Running speed during training period was increased increasingly from 18 to 30 meter per minute. Training program was performed on treadmill for 60 minutes, 6 sessions a week for 8 weeks. In each training session, first a 7-minute warm-up was performed in the form of running or walking on the treadmill with the speed of 10 to 12 meters per minute and cooling down was performed for 5 minutes in the end. In the first week, the training was started with the speed of 18

meters per minute and in the last two weeks was gradually increased to 30 meters per minute. These data were obtained on a pilot study of 4 rats. Also, the study of Hafstad et al. was used as a model for designing the training (25). All training variables were kept constant in the last two weeks so that adaptations become stable at the time of anatomy (24).

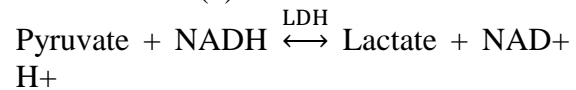
Sampling method

The rats were anesthetized with a combination of Ketamine (100 mg per kg of body weight) and Xylazine (10 mg per kg of body weight) 24 hours after the last training session, after an overnight fast (12 hours). To measure lactate, 3 cc blood were collected by a syringe with heparin directly from the left ventricle of the heart and immediately were centrifuged at 3500 rpm for 10 min to separate plasma from blood cells and then transferred to the laboratory. Another volume of blood – 3 cc – was also taken from the rats' heart to measure lactate dehydrogenase activity. Lactate dehydrogenase was measured in blood serum. For serum preparation, after blood was collected, it was poured into a test tube and allowed to stand at room temperature for 15 minutes to clot. Then the blood was centrifuged at a speed of 3000 rpm for 10 minutes to separate serum and the serum (supernatant) was kept at -20 C° to determine the lactate dehydrogenase.

Measurement of plasma lactate and serum lactate dehydrogenase

Plasma lactate and lactate dehydrogenase were measured in laboratory by spectrophotometric method with Hitachi autoanalyzer made in Japan. The method of measuring lactate is in such a way that the lactate in the sample is converted into pyruvate and hydrogen peroxide due to lactate oxidase, hydrogen peroxide generated in the presence of peroxidase and 4-amino-antipyrine and an exclusive 1-chrome is changed into a purple

material. An increase in optical absorbance which is read at the wavelength of 540-660 nanometers is proportional to the amount of lactate (5). The German Society of Clinical Chemistry and Biochemistry (DGKC) method was used to measure lactate dehydrogenase activity. In this method, the enzyme activity is determined according to the change in NADH concentration (5).



Lactate dehydrogenase is oxidized by NADH activity. In the process, the reduction of NAD to NADH is directly proportional which can be measured by photometric method.

Statistical analysis

Data from research are expressed as mean \pm SD. The Kolmogorov-Smirnov test was used to ensure the normality of sample distribution and the one-way ANOVA and Tukey's post-hoc test were used to compare differences between groups. Given the normal distribution of all variables, parametric statistical tests were used to analyze the data.

All statistical analyses were performed by SPSS 16 at the significance level of $P < 0.05$.

Results

According to the results, at baseline and after a 8-week interval training, no significant change was observed in rats' weight and the weight gain was normal in all groups (Figure 1).

The results of the blood lactate levels and lactate dehydrogenase activity are reported in Table 1.

The mean of blood lactate levels in the old experimental group and the old control group was 2.8 and 2.4 mmol per liter, respectively, and in the young experimental group and the young control group it was 2.16 and 1.98 mmol per liter, respectively.

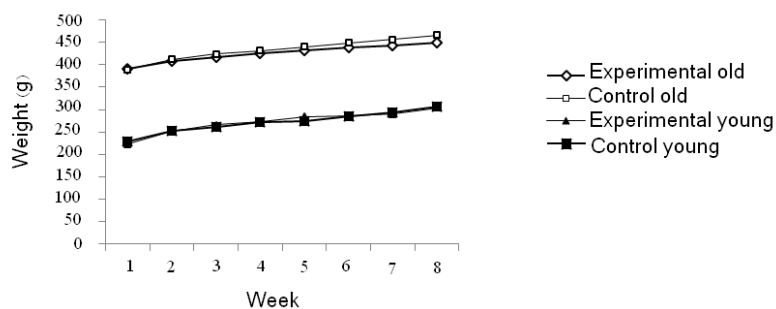


Figure 1: Change of weight in the rats during a 8-week interval training

Based on the results of the one-way analysis of variance test, there was no significant difference in blood lactate levels between groups ($p=0.115$) (Figure 2). The mean of lactate dehydrogenase levels in the old experimental group and the old control group was 777 and 724 IU,

respectively, that this difference was not significant ($p=0.32$), but the difference between lactate dehydrogenase activity in the young experimental group (753 IU) and the young control group (580 IU) was significant ($p=0.018$ and $p<0.05$) (Figure 3).

Table 1 The mean of blood lactate levels and lactate dehydrogenase activity (mean \pm SD)

	Experimental old	Control old	Experimental young	Control young
Lactate (Mmol per liter)	2.8 \pm 0.862	2.4 \pm 0.672	2.16 \pm 0.682	1.98 \pm 0.32
Lactate dehydrogenase (IU)	777 \pm 70.3	724.8 \pm 61.5	753 \pm 66	580 \pm 89.7

* Significant difference compared to the control group ($p<0.05$)

Discussion and conclusion

Important findings of this study included the difference in the means of four groups in both variables of lactate and lactate dehydrogenase and significant difference in lactate dehydrogenase between young experimental and control groups [$F=3.23$ and ($P=0.018$)] and the old experimental group and the young control group [$F=3.23$ and ($P=0.008$)] after a 8-week interval training in rats. According to evidence, during heavy exercise, energy demand increases rapidly which is associated with the increase in glycolysis and the subsequent production of lactate H^+ . In order to the rate of glycolysis be constant during the exercise, lactate production is catalyzed by lactate dehydrogenase to prevent pyruvate accumulation and, more importantly, helps to maintain the continuity of transferring nicotinamide adenine dinucleotide (NAD $^+$) to continue glycolysis (3). Oxidative muscle fibers can absorb lactate from the bloodstream or adjacent glycolytic fibers. During muscle contraction, more than 80%

of the lactate produced is cleared by the oxidation (26) and thus apparently after exercise training, lactate consumption will lead to reduced amount of this metabolite. The lack of difference in the amount of lactate in the experimental and control groups in the present study may be related to mechanisms mentioned. Lactate clearance is probably increased with interval training and its concentration does not increase much and is close to the resting values of the control group.

In this regard, Sari Dewi N et al in a study examined blood lactate levels in rats after 4 and 12 weeks of aerobic interval training (22). The results showed that the lactate level in the group that had 4 weeks training was 2.11 mmol per liter and in the other group was 1.71 mmol per liter, which is significantly lower than the first group. Carnevali Jr et al. also stated that after interval training, the lactate dehydrogenase activity is increased and muscle lactate levels are reduced in rats (24). Therefore, it seems that interval training leads to decreased lactate and

longer training has a greater effect on the lactate clearance. Díaz-Herrera et al. also showed that a 12-week aerobic exercise of rats will increase oxidative fibers and reduce glycolytic fibers (27). While Daussin et al. reported that training with appropriate time, frequency and intensity will increase 50% to 100% of mitochondria after a 6-week aerobic exercise (28). All these findings show increase in the lactate clearance after exercise training. The results of the present study indicating lactate clearance also confirm the findings of Díaz-Herrera et al, Daussin et al, Carnevali Jr et al. and Sari Dewi N et al. Also Macaluso and De Vito and Korhonen et al. reported reduction in blood lactate levels in the elderly (8,13). These researchers stated that with increasing age, transverse skeletal muscles, muscle fibers and plasma volume are decreased leading to less production of lactate.

However, Seals et al. stated that in the elderly, after training with the same intensity, the blood lactate is decreased or does not change (29). They also reported that adaptation in response to prolonged exercise is similar in the elderly and young (29). Also, Reaburn and Mackinnon reviewed the effects of age on blood lactate concentrations, lactate peak and the half-life in skilled swimmers (25 to 56 years) at the time of returning to the initial state (30). These researchers found that swift old swimmers can produce and clear lactic acid similar to young swimmers. In the present study, although the difference between blood lactate levels was not significant in the old and young group ($P=0.115$), the mean was higher in the group of old rats. These results contrasts with the results of the studies mentioned. In the present study, old rats had more weight than younger rats that may be due to the higher weight of old rats, as having more muscle mass leads to increased lactate production (31). Another reason for the decrease in lactate level after training can be the competition of lactate with

glucose as a carbohydrate fuel source in skeletal muscle; therefore, with lactate consumption, while reducing this metabolite, a small amount of blood glucose is used during exercise (6); as a result, lactate is not the only a combination that will accumulate in different parts of the body during exercise, but also is considered as an important metabolism mediator and the link between the energy metabolite in various tissues (32,33).

The results of this study about the lactate dehydrogenase also showed that although the difference was not significant in the old experimental group and the old control group ($P=0.32$), lactate dehydrogenase was significantly greater in the young experimental group than the young control group ($P=0.018$), so it seems that interval training protocol increases the lactate dehydrogenase activity. Confirming this finding, Carnevali Jr et al. reported increased lactate dehydrogenase activity after interval training in the muscle of rats (24). Clarkson and Thompson (34) also reported that with increasing the intensity of training and the conversion of activity from aerobic to anaerobic pathway, the rate of lactate accumulation is added, followed by further concentration of lactate dehydrogenase (34). Based on the available evidence, it seems that lactate dehydrogenase can be produced further due to exercise activities (35), so that this enzyme in addition to be active in the process of energy production and lactate, plays an important role in developing inflammatory conditions for muscle cells (36). Therefore, in some studies, increased levels of lactate dehydrogenase because of physical activities are due to damaged muscle fibers' membrane (36). In the present study, increased lactate dehydrogenase did not lead to muscle damage as a 8-week training will lead to adaptation and not muscle damage.

The effect of age and endurance training on lactate dehydrogenase activity in young, middle-aged and old rats was studied by Lupa VA et al. (37). They

reported that the liver lactate dehydrogenase activity decreases with increasing age in young rats, but the activity of this enzyme does not change in middle-aged and old rats. Also, the endurance training will not result in significant changes in muscles lactate dehydrogenase activity. In this regard, Masuda Shinya et al. in a study on old and young rats reported that although aging is associated with metabolic changes, there is no difference between the two groups in terms of lactate dehydrogenase activity (12). Consistent with these animal studies, Kaczor JJ in a research examined the effect of age on the aerobic and anaerobic enzymes activity in skeletal muscles of humans (10). The results showed that the activity of all enzymes (measured in this study) in the elderly is lower compared to the middle-aged group when the results are according to muscles weight, but when the enzymatic activity is according to protein content, only lactate dehydrogenase activity is significantly lower in the elderly compared to the middle-aged group. As a result, the loss of muscle function in the elderly may be due to the lower activity of aerobic and anaerobic enzymes and also protein content (10). Pastorisa et al. in a study examining the effect of age on enzymes activity and metabolites concentrations in skeletal muscle in sedentary men and women stated that aging affects metabolic capacity of skeletal muscle, particularly the glycolytic and respiratory capacities (15). They studied glycolytic and oxidative phosphorylation activities in the muscles of 76 sedentary subjects (32 men and 44 women) aged between 15 and 91 years. The results showed that parallel to increasing age, activity of lactate dehydrogenase and hexokinase, citrate and citrate synthase is decreased in muscles (15). Thus, in most

studies, age is one of the factors affecting the metabolism of lactate and decreased lactate and increased lactate dehydrogenase activity after physical activity have been reported. In the present study, the lack of significant differences in blood lactate levels between old and young rats after interval training suggests that the effect of training on the changes in lactate was similar in both groups. Also, increased lactate dehydrogenase in both age groups confirms this conclusion, although the increase was not statistically significant. In other words, the effect of training on these changes is independent of age; so it seems that although metabolic changes caused by aging is associated with weakened motor function and loss of enzymatic activity, exercise training can reduce or modify the negative effects. The results of this study and the results of the studies mentioned show reduced blood lactate levels after physical activity; so it seems that interval training has a favorable effect on lactate clearance may be due to increased absorption capacity or lactate transmission, and increased lactate dehydrogenase activity is a factor that can accelerate the clearance process.

Conclusion

The results showed that in both old and young age groups, blood lactate is decreased after interval training and lactate dehydrogenase is increased. In fact, this helps to muscle glycogen replenishment with increased oxidation from the conversion of lactate to glucose and to glycogen formation. Also, increased capability to dispose lactate in muscle cells into the blood due to physical activities especially during exercise is an advantage for intracellular pH regulation (pHi).

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