

## The Effects of Intermittent Training on Lactate Level (La) and Lactate Dehydrogenase (LDH) Enzyme Activity in Blood of Old Rat

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### Abstract:

**Purpose:** The purpose of the present study was to explore changes in blood lactate levels and LDH enzyme activity following intermittent training on old male wistar rats.

**Material and Methods:** Twenty male wistar rats (aged  $27 \pm 2$  months and weight  $389 \pm 31$  g) were selected as a case study. They matched based on their weight and assigned into the training ( $n = 10$ ) and control ( $n=10$ ) groups randomly. Training group performed intermittent exercise on a treadmill 6 times/wk for 8 wk. It consisted of 10 bouts of 4 min running interspersed by 2 min of active rest. The running speed of the treadmill were gradually increased from 18 to 30 m/min over 6 wk and maintained at this value for the rest of the exercising (2 wk) period. All rats were anesthetized with a mixture of ketamine (100 mg/kg b/wt) and xylazine (10 mg/kg b/wt) after overnight fasting. To measure lactate level and LDH activity, blood sample was obtained via cardiac puncture at rest, 24 hours after last session exercise.

**Results:** The results showed that no significant differences in blood lactate level between two groups. Although, LDH activity in the training group was higher ( $\approx 12\%$ ) than the control group, there is no differences significantly between two groups.

**Discussion and Conclusion:** These results indicate that lactate is likely decreased by intermittent training. It is benefit for lactate clearance after exercise. This potential is important advantage for muscle glycogen replenishment and may in turn favor better muscle pH regulation.

**Keywords:** Intermittent training, lactate, lactate dehydrogenase, rat

### 1. Introduction

Lactate is a dynamic substrate that has great potential as an energy source and is effective in the regeneration of ATP [1]. Normal level of blood lactate is 0.5 to 2.2 mmol/L [2]. It is thought that this value in the complete exhaustion increase to the range of 20 to 25 mmol/l [3]. Peak of blood lactate concentration occurs approximately 5 min after the cessation of intense exercise [2, 4]. This delay is assigned due to the time required for buffering and transfer of lactic acid from blood tissues [1].

In addition, LDH is an enzyme that is found in all body tissues with different concentrations and causes to increase the rate of glycolysis in converting of lactic acid to Pyruvic acid and usually its amounts gradually increases 24 to 48 hours after stimulation [5]. According to many studies the reason of this enzyme releasing is due to changes in structural of the muscle tissue followed by vigorous activity [6]. During exercise, the lactate exit from contracting muscle and use by the heart and oxidative muscle. Transport of monocarboxylates such as lactate and pyruvate across the sarcolemma is

accomplished by a family of monocarboxylate transporters (MCTs) [7]. As regards, Ageing causes modifications in body composition that alter the muscle structure and reduce the ability to perform exercises requiring strength and power [8]. Muscle fibre composition changes with a reduction in the number of type I and II fibres, coupled with the selective atrophy of type II fibres [8]. Also cross-section of skeletal muscle decreases with age. This phenomenon, which is referred to as sarcopenia, is due to reduction in the size, fiber number, or a combination of both [9]. These does may have a role in the changed production and removal of lactate observed in the elderly.

However, in studies on aging-induce muscle morphology changes, some researchers have concluded that the size of the slow-twitch fibers type does not significantly change with age, but fast-twitch fibers are recruited the selective atrophy [9]. However, the increase in intracellular lactate concentration during prolonged contraction in young subjects shows a greater increase than old ones [10]. This contradiction shows that aging is associated with reduced lactate accumulation. As a result, fatigue resistance increased in elderly skeletal muscle [10]. Some researchers suggest that reduced lactate clearance followed and during exercise in elderly people compared to younger individuals [11]. It has reported that lactate level of male and female athletes (mean age 40-88 and 35-87, respectively) reduced after competition [12]. It appears that blood lactate reducing in old age is related to factors such as muscle mass and plasma volume that are reduced with age [13]. Furthermore, it would seem, aging is also associated with a minimum efficiency of glycolytic enzymes [9, 13]. statements all enzyme activity in the elderly is lower than middle-aged when the results are expressed on muscle weight. However, when the enzyme activity is expressed relative to protein content, the lactate dehydrogenase enzyme activity was only lower in older versus middle-aged [9]. Also as age increases the activity of lactate dehydrogenase and hexokinase enzymes, citrate, and citrate synthase are reduced in muscle [14]. Moreover, the results of study on the master swimmers shown that there weren't

any significant effect in blood lactate of women (age 40-79 years) after the match. However, a significant decrease in blood lactate was observed in men with increasing age [15]. Although these empirical evidences aren't consistent together, however, show that glycolytic enzymes activities are affected by age. In addition, during high-intensity exercise, the production of lactate is associated with an increase in protons (i.e., a decrease in muscle pH) and it causes to depletion of performance [16]. By increase the muscle lactate, muscle pH dramatically decreases during high-intensity exercise ( $\text{pH} = 7.4 = > 6.6-6.4$ ) and then, consequently, in the blood [17]. Increased production of  $\text{H}^+$  inhibits the release of calcium from Sarcoplasmic network and blocks the myosin binding sites on the actin molecule.

More than this, the high acidosis conditions, numbers of sensitive enzymes to pH, including phospho-phroctokinaz (PFK) are inhibited and leads to decrease of ATP production [17]. Following the release of lactate from skeletal muscle into plasma, lactate and hydrogen ions, especially transfer inside the red blood cells through the MCT and are carried through the body [18]. Oxidative fibers uptake the amount of lactate in plasma and combine with oxygen. Part of the remaining lactate in the liver used to lactate gluconeogenesis process [19]. It is looks that physical activities, which have enough intensity, can be it through increased expression of MCT, increased mitochondrial density and accelerates gluconeogenesis assist to clearing lactate [20]. Some researcher observed lowers level of blood lactate in rat flow intermittent exercises. Moreover, it is appears the longer period of training have greater effect on the lactate clearance [21]. On the other hand, if physical activity be enough intensive or prolonged, effect on the activity of enzymes such as LDH [22]. This conclusion is supported by Carnevali et al. (2012) who reported increased lactate dehydrogenase enzyme activity and decrease in muscle lactate concentration in rats after intermittent training [23].

Taking into account that can be interpreted of the took placed research, the age and physical activity can be raised as a factor affecting lactate and lactate dehydrogenase levels, but effective

processes that are related to age and interaction between aging and physical activity is not yet fully understood. While the findings are not an end, there is still doubt and uncertainty in this case. Indeed, few studies have examined the effect of incremental intermittent training and there is little information about the effects of intermittent training on lactate clearance and its association with age. Therefore, in order to clarify ambiguities, new research is needed. In order to consider these issues, we examined the effects of intermittent training on blood lactate level and LDH enzyme activity in old male wistar rats.

## Methods

### Animals and housing

Twenty male wistar rats (aged  $27 \pm 2$  months and weight  $389 \pm 31$  g) were obtained from the Pasteur Institute of Iran. Animals were housed in a temperature-controlled room ( $22 \pm 1$  °C), kept on a 12:12 h light–dark cycle and provided with food and water ad libitum. Male rats were specifically chosen because they experience a greater rate of age-related muscle atrophy than female rats of the same strain and because this atrophy is accompanied by marked impairment in skeletal muscle contractile and metabolic function [10]. The following protocol and experimental procedures were approved by the Medical University of Isfahan, Iran. All rats matched based on their weight and assigned into the training (n=10) and control (n=10) groups randomly. Animals were habituated to treadmill exercise, where rats were allowed to rest and walk on treadmill for 10 min per day for 3 days at  $8 \text{ m min}^{-1}$ , increasing by  $2 \text{ m min}^{-1}$  on each consecutive day. Control group (CG) during exercise period were maintained in transparent polycarbonate cages. The weight of the rats was monitored throughout the experimental period. CG did not work in this time, but in other cases was kept with the training group in the same conditions. Both groups were anesthetized at the end and the desired experiments were performed on them.

### Exercise training

Treadmill running at intermittent training was performed 6 days/wk for 8 wk according to a protocol slightly modified from that described by Hafstad et al. (2011) [24]. Treadmill training

was performed, as described previously. It consisted of 10 bouts of 4 min running interspersed by 2 min of active rest. The running speed was increased gradually from 18 to 30 m/min over 6 wk and maintained at this value for the rest of the exercising (2 wk) period. In each training session, animal performed a 7 min warm-up at 30–50% of  $\text{VO}_2\text{max}$ , and 5 minute cool down at the end. This data obtained from pilot study on 4 rats.

### Biochemical analysis

All rats were anesthetized with a mixture of ketamine (100 mg/kg b/wt) and xylazine (10 mg/kg b/wt) after overnight (12 hours) fasting. To measure lactate level and LDH Activity, blood sample was obtained via cardiac puncture at rest, 24 hours after last session exercise.

For measurement of lactate, 5 min of blood using a syringe soaked heparin were directly collected from the left ventricle of animal's heart and immediately centrifuged in the speed of 3500 rpm for 10 min to separate plasma from blood cells and was transferred to the laboratory. Also 5 min of blood for the measurement of LDH enzyme activity was collected. LDH was measured in serum. To obtain serum, postoperative blood, blood was poured into the test tube and to clot formation was placed for 15 min at room temperature. Then the speed of 3000 rpm for 10 min, centrifuged to separate plasma and serum (supernatant) was maintained at a temperature of minus  $20$  °C to determine LDH enzyme activity.

### Blood lactate and LDH enzyme activity measurements

Measurement of plasma lactate and serum LDH activity was done by auto analyzer Hitachi Japan spectrophotometrically. Method for measuring lactate is that the lactate in sample by oxidase enzyme lactate converts to pyruvate and hydrogen peroxide. Hydrogen peroxide generated in the presence of peroxidase and 4 - amino Anti Perrin and an exclusive cromovan become purple material. Increase in the light absorbance that is called in the wavelength of 540–660 nm, is proportional to the amount of lactate. Also, for measuring LDH activity was used the DGKC method (standard method of Biochemical Society of Germany). In this

method, enzyme activity is determined by the rate of change in NADH concentration.

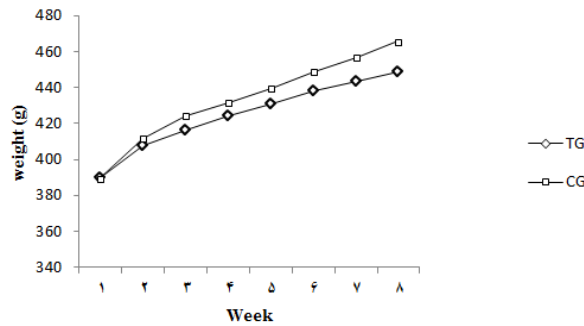
Pyruvate + NADH  $\xrightleftharpoons{\text{LDH}}$  Lactate + NAD+ H+  
 NADH is oxidized by LDH activity. Reduction value of NAD in this process has directly proportional to NADH that is measured by photometry method [5].

**Statistical analyses**

Data normality was tested by Kolmogorov-Smirnov and Comparisons between training and control groups were made by Student’s unpaired t test. All data are reported as means ± SD. The level of significance was set at p < 0.05.

**Results**

In the start and after 8 weeks of intermittent training, there was no significant difference between training and control animals’ weights (Fig. 1).



**Fig. 1.** Variation in weight during the 8-week intermittent training protocols of rats, (n = 9). Data are reported as means ± SD. \*P < 0.05 control compared to training (t test).

The results of blood lactate levels and LDH enzyme activity in the TG and CG group are presented in Table 1.

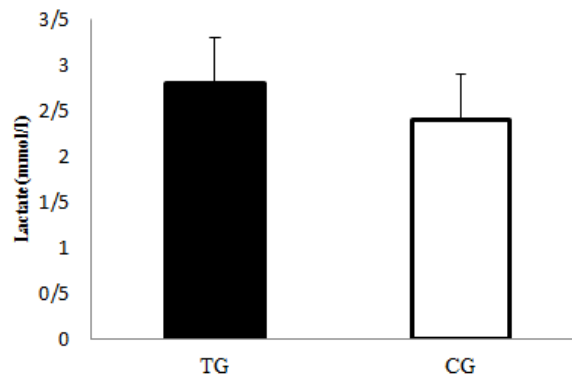
**Table 1.** Blood Lactate level and LDH activity after 8week intermittent training in rats

	TG	CG
Lactate (mmol/l)	2.8 ± /826	2.4 ± /672
LDH (U/l)	777 ± 70/3	724 ± 61/5

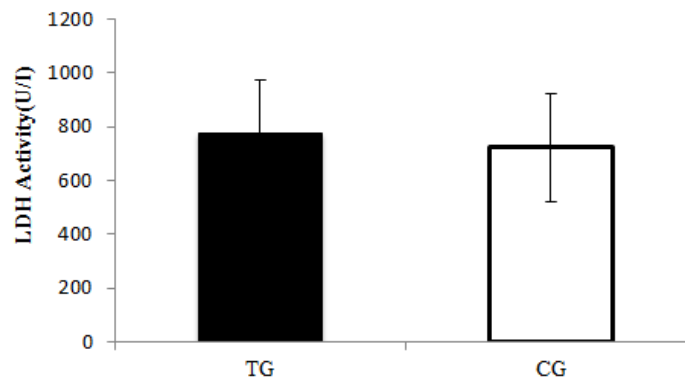
Data are reported as means ± SD. TG = Training group; CG = Control group.

The blood lactate level in the TG and CG group was 2.8 and 2.4 mmol/l respectively. The blood LDH activity in the TG and CG

group was 777.1 and 724.2 I/U respectively. There were no differences between two groups in both blood lactate levels and LDH enzyme activity (Fig. 2, 3).



**Figure 2.** Effects of intermittent training on Lactate level that analysis in the blood of TG (Training group), CG (Control group) rats. Data are reported as means  $\pm$  SD (n = 9). (t test).



**Figure3.** Effects of intermittent training on LDH enzyme activity that analysis in the blood of TG (training group), CG (control group) rats. Data are reported as means  $\pm$  SD (n = 9). t test was used for comparison between groups.

## Discussion

Our results showed that although there was a difference between means of two groups in both variables (lactate level and LDH enzyme activity), however, there was not significantly different either in lactate level or in LDH enzyme activity in both groups. Sari. et al (2013) was examined, effect of 4 and 12 weeks aerobic intermittent training on blood lactate levels of rats. They observed lactate levels were 2.11 and, 1.71 mmol/l, in 4 and 12 weeks training respectively. There was significantly different between two groups [21]. Furthermore, Carnevali et al. (2012) stated that LDH enzyme (DOI: [dx.doi.org/14.9831/1444-8939.2015/3-2/MAGNT.10](https://doi.org/10.1444-8939.2015/3-2/MAGNT.10))

activity increases and muscle lactate levels decrease in rats after the intermittent training [23]. It seems intermittent training led to a decrease in lactate level and prolonged exercise is more effective on lactate clearance. Indeed, 12 wk aerobic exercise increases oxidative fibers and reduces glycolic fibers in rat [25]. Besides, 6 wk aerobic exercises with period, time, frequency and enough intensity increase mitochondria density by 50 to %100 [26]. These findings demonstrated that lactate clearance after exercise training by increases in lactate uptake or lactate transport in muscle. In accordance with those statements, our results supported by

many of them [21, 23]. In addition, previous researches [8, 12] reported that blood lactate levels were decreased in the elderly people. They indicated that with increasing age decreases the skeletal muscles cross-section, muscle fibers and plasma volume which has led to the less production of lactate.

Moreover, studies indicate that during heavy exercise, the rapid increase in the energy demand of contracting skeletal muscles is associated with an increase in glycolysis and the subsequent production and accumulation of lactate and proton. To maintain a fast glycolytic flux during exercise, lactate production is catalyzed by the lactate dehydrogenase, which prevents pyruvate accumulation and, more importantly, maintains the supply of the proton-electron transporter nicotinamide adenine dinucleotide (NAD) to glycolysis [3]. Thus, lactate production favors the recycling of NADH from glycolysis, allowing the production of two ATP molecules per molecule of glucose utilized in glycolysis. Oxidative muscle fibers can take up lactate from the circulation, or lactate released from neighboring fibers [3]. Therefore, the subsequent metabolic use of intramuscular lactate results in ATP production without conversion to glucose in the liver. Under the condition of muscle contraction, oxidation is clean more than 80 % of the lactate produced [27].

Lack of difference in lactate of the training and control groups in the present research can be justified with the mentioned mechanisms. Therefore, it seems that execution of the intermittent training increases lactate cleaning and concentration of this metabolite doesn't increase considerably following intermittent training and is close to resting values of the control group. In ageing lactate level is reduced or unchanged after exercise with same intensity [28]. Macaluso, & De Vito (2004), reported that results of exercises showed that adaptation in response to prolonged exercise is similar between old and young people. Also, it has been stated that older sprint swimmers appeared to be capable of producing and removing lactic acid at the same rate as younger swimmers [8]. These data suggest that intensive swimming training may prevent or delay the decline with age in the

physiological factors affecting blood lactate values following a maximal sprint swim [29].

Furthermore, lactate successfully competes with glucose as carbohydrate fuel source in skeletal muscles. Therefore, low amount of blood glucose is used by other tissues during exercise [6]. However, by means of dual isotope tracers, monitoring of secondary labeling of glucose from infused  $^{13}\text{C}$ -labeled lactate, and mass isotopomer discrimination analysis it is clear that during exercise lactate is by far the most important gluconeogenic precursor in humans during exercise, as it is in fasting [30]. Thus, lactate is not only a compound that accumulates in various compartments during intense muscle activity, but it is also an important intermediary metabolite serving as an important link between energy metabolisms in different tissue [31]. Therefore, exercise training and muscle activity can increase lactate clearance. Indeed, our results showed that although there was no difference in LDH enzyme activity between training and control groups. However, LDH enzyme activity in training group was higher than the control group ( $\approx 12\%$ ).

Therefore, it seems that intermittent training protocol of this study has been effective on increase rate of blood LDH enzyme activity. To confirm these results, Carnevali et al. (2012) were demonstrated lactate enzyme dehydrogenase activity increasing after intermittent exercise in rat skeletal muscle [23]. Based on available evidence, it appears that lactate dehydrogenase enzyme has the potential to increase production by exercising [32]. Lactate dehydrogenase enzyme increases due to physical activities so that this enzyme plays effective role in energy production and lactate, creation of inflammatory conditions for muscular cells [33]. For this reason, some researchers have reported increase of LDH level due to physical activities caused by membrane of muscular fibers damage [32]. In the present study, increase of LDH probably is not due to injury of muscle. Because 8-week exercise leads to adaptation and this exercise period will not lead to muscular injury.

Influence of age and endurance training on LDH enzyme activity in young middle-aged and

old rats was studied by Lupa et al. (1994). They reported that with the increasing age, the liver LDH activity of young rats decreased, however, in the activity of this enzyme unchanged in middle-aged and old rats. These results showed that endurance training lead to a significant reduction in liver LDH enzyme activity in young rats. However, LDH enzyme activity in the skeletal muscles (soleus and EDL) unchanged with age and endurance training does not lead to significant changes in muscle LDH enzyme activity [34].

Although the metabolic changes associated with aging occur, but in lactate dehydrogenase enzyme activity was not different between the young and old rats [11]. Researcher showed that all enzyme activities were lower in older versus middle-aged adults when results were expressed as muscle wet weight. When activity was expressed relative to the protein content, only lactate dehydrogenase remained significantly lower in older versus middle-aged adults ( $p < .001$ ). As a result, some of the reduction in muscle performance in older adults may be due to lower activity of the anaerobic and aerobic enzymes as well as protein content, not solely due to a decrease in physical activity [9]. Pastorisa et al. (2000) to quantify biochemical alterations due to aging in muscular metabolic capacity in human skeletal muscles in sedentary subjects (32 men and 44 women, between 15 and 91 years), mentioned that there was no significant differences between males and females were found, but changes related to age were: a decrease in hexokinase and lactate dehydrogenase activities in the rectus abdominis; an increase in pyruvate kinase activity and a decrease in ATP and creatine phosphate concentrations in the gluteus maximus [14]. These data suggest that distinct muscles may respond differently to aging regardless of sex in sedentary subjects [14].

In summary, most studies have regarded effects of age on metabolism of lactate considerable and reported reduction of lactate and increase of LDH enzyme activity after physical activity. Although some studies didn't find any change. In general, although different experimental evidences are not compatible, they show that blood lactate level can be considerably reduced

due to relatively intensive exercise and activity of glycolytic enzymes can increase. They also reminded that relatively intensive exercise in ageing can improve factors leading to changes of lactate with age. Generally, in the present study, no change in blood lactate is probably due to desirable effects which response of intermittent exercise has on lactate clearness and increase of LDH is the factor which can accelerate cleaning trend. Promotion of lactate absorption capacity particularly for protecting lactate in blood flow is useful after exercise. In fact, this helps compensate for muscles glycogen with increasing oxidation from convention lactate into glucose to formation of glycogen. Ability to repel lactate of muscular cells into blood particularly during exercise is an advantage, which may in turn favor better muscle pH regulation.

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