Blood lactate level in Wistar rats after four and twelve weeks intermittent aerobic training

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Abstrak

Latar belakang: *Latihan fisik aerobik selain dilakukan secara kontinu, juga dapat dilakukan secara intermiten yang bertujuan agar kadar laktat lebih rendah. Konsentrasi laktat dalam tubuh merupakan salah satu faktor yang menentukan performa subjek terlatih. Tetapi, hingga saat ini, sedikit penelitian mengenai latihan fisik aerobik yang dilakukan secara intermiten dan kadar laktat darah. Oleh karena itu, penelitian ini bertujuan untuk mengukur kadar laktat darah pada tikus Wistar setelah latihan fisik aerobik intermiten selama 4 dan 12 minggu.*

Metode: *Enam belas tikus Wistar dibagi menjadi dua kelompok, yaitu kelompok kontrol dan aerobik. Tiap kelompok dibagi menjadi dua subgrup, yaitu subgrup 4-minggu dan 12-minggu. Pada kelompok aerobik dilakukan latihan fisik mengunakan* treadmill *T-6000 dengan kecepatan 20 m/menit selama 20 menit, dan setiap 5 menit diistirahatkan selama 90 detik. Pengukuran kadar laktat dilakukan dengan kit L-lactate (PAP) Randox (LC 2389).*

Hasil: *Kadar laktat kelompok 4-minggu aerobik ialah 2,11 mmol/L sedangkan kelompok kontrolnya 1,82 mmol/L (p > 0,05). Kadar laktat pada kelompok 12-minggu aerobik (1,71 mmol/L) berbeda signifikan dibanding kelompok kontrolnya (3,03 mmol/L, p < 0,05).*

Kesimpulan: *Penelitian ini menunjukkan bahwa kadar laktat setelah 12 minggu latihan fisik aerobik intermiten lebih rendah daripada kelompok 4-minggu latihan aerobik intermiten maupun kelompok kontrol 12-minggu.* ***(Med J Indones. 2013;22:141-5. doi: 10.13181/mji.v22i3.582)***

Abstract

Background: Aerobic training can be done not only continuously, but also intermittently. Intermittent aerobic training aimed to get blood lactate level lower than continuous aerobic training. Blood lactate concentration in one of the various factors that determine training performance. However, until recently, little studies about intermittent aerobic training and blood lactate levels have been done. Therefore, this study aimed to measure blood lactate levels in Wistar rats after 4 and 12 weeks of intermittent aerobic training.

Methods: 16 Wistar rats were divided into two groups, control and aerobic group. Every group was divided into two subgroups, 4-week and 12-week subgroup. Aerobic group performed training using T-6000 treadmill with a speed of 20 m/minute for 20 minutes, with resting period for 90 seconds every 5 minute. Measurements of lactate level was done with L-lactate (PAP) Randox kit (LC2389).

Results: Blood lactate level in the 4-week aerobic group was 2.11 mmol/L, while that of the 4-week control group was 1.82 mmol/L ($p > 0.05$). Meanwhile, lactate level in 12-week aerobic group was 1.71 mmol/L ($p < 0.05$), and significantly lower than in 12-week control group, which was 3.03 mmol/L.

Conclusion: This study showed that lactate level after 12-week intermittent aerobic training was the lowest compared to 4-week intermittent aerobic and 12-week control group. ***(Med J Indones. 2013;22:141-5. doi: 10.13181/mji.v22i3.582)***

Keywords: *Blood lactate, intermittent aerobic training, Wistar rat*

In the body, pyruvic acid is converted to lactic acid by lactate dehydrogenase (LDH). Normally, lactate level in the body at rest is low. At the time of exercise, lactate level will rise in accordance with the load of work done. Glandden[1] stated that an increase in lactate at training as a potential stimulus for the occurrence of glycolysis. Lactate is produced in training not only as the result of anaerobic but also aerobic metabolism, which occurs in adequate oxygen condition.[1-4]

In aerobic training, blood lactate concentration is one of the factors that determine training performance, besides vO_2 maximal, fuel utilization, and types of muscle fiber. Blood lactate accumulation will happen when production rate of lactate is higher than its clearance rate. Lactate accumulation can cause problems such as pain, muscular fatigue, metabolism fatigue, and performance decrease. Hence, the purpose of aerobic training is to lower lactate level when doing exercise.[1,2,4]

In aerobic training, we usually determine maximal lactate steady state (MLSS), which is the highest concentration during exercise at which lactate entry in

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blood equals its removal. MLSS is a standard method to determine the metabolic transition from aerobic to anaerobic training.[1-6] The study of Kindermann that was cited by Manchado, et al[5] showed that metabolic transition between aerobic and anaerobic training occurs at lactate level 2-4 mmol/L.

In a study by Manchado, et al[5] continuous aerobic training in Wistar rats showed that MLSS was achieved at a speed of 20 m/minute for 25 minutes, with lactate concentration stabilized at 3.9 mmol/L. This result was the same with the value observed in human studies although at a different age.[6] This study aimed to measure blood lactate levels in wistar rats after 4 and 12 weeks of intermittent aerobic training.

METHODS

Study design

This research was an experimental *in vivo* study, which was done at the laboratory of Biochemistry and Molecular Biology, Faculty of Medicine, Universitas Indonesia, from October 2010 until April 2011. Speed of animal treadmill (T-6000) was 20 m/minute, which could be used by 6 rats in a single treadmill.

Subjects

Sixteen rats were divided into two groups, control and aerobic groups. Then, each group was divided into two subgroups, 4-week and 12-week group. Inclusion criteria were outbred strain albino Wistar rats, *Rattus novergicus*, healthy, male, weight range: 100-250 grams, 8-week old at the beginning of study.

Before and during research, except the exercise, rats were treated equally. The rats were given a standard diet including vitamines and drink *ad libitum*. Ambient temperature around the cage was 23 ± 1°C. The cages were kept clean with 12 hours of light and 12 hours of dark room setting. The protocol of this study has been approved by Health Research Ethics Committee, Faculty of Medicine, Universitas Indonesia (Approval letter no. 76/PT02.FK/ETIK/2011).

Lactate measurement

In the aerobic group, blood was collected from the inferior vena cava in the cavity of the right atrium immediately after aerobic training was finished by using a 3-mL syringe, which contained heparin as anti-coagulant. In the control group, blood sampling was performed at the time of surgery in resting condition. Then, the blood was immediately stored at 4°C and centrifuged at 3,500 rpm for 10 minutes to separate plasma from blood cells.

Meanwhile a blank solution was made by mixing 10 μL H_2O with 1,000 μL L-Lactate reagent (PAP) Randox (LC 22 389) in the cuvette. To make standard solution, we mixed 10 μL of standard in the lactate kit with 1,000 μL L-lactate reagent, in the cuvette.

Then, we made sample solutions by mixing 10 μL blood plasma with 1,000 μL L-lactate reagent. Finally, all solutions were incubated for 10 minutes at room temperature and then, the absorbance of the samples, blank, and standard solution were examined using spectrophotometry with a wavelength of 550 nm.

Measurement of blood lactate formula:

$$\frac{(\text{AS-AB}) \times \text{SC}}{\text{Ast - AB}}$$

AS: absorbance of sample
AB: absorbance of blank solution
SC: standard concentration = 4.4 mmol/L
Ast: absorbance of standard

Analysis

All data were analyzed by using independent t-test to compare the average means of the intermittent aerobic training and control groups. $P < 0.005$ was considered statistically significant.

Running speed protocol

Animals were acclimatized for one week to adapt with the environment. Environmental acclimatization was performed in all groups, whereas training acclimatization was made to intermittent aerobic groups with acclimatization stages as follows: Day-1, rats were placed in a turned off animal treadmill for 5 minutes; day-2, rats were placed in the animal treadmill for 5 minutes in a speed of 5 m/minute; day-3, rats were placed in the animal treadmill for 10 minutes in a speed of 5 m/minute, rested 90 seconds every 5 minutes of training; day-4, rats were placed in the animal treadmill for 15 minutes in a speed of 5 m/minute, then rested 90 seconds every 5 minutes of training; day-5, rats were placed in the animal treadmill for 5 minutes in a speed of 10 m/minute; day-6, rats were placed in animal treadmill for 10 minutes in a speed of 10 m/minute, rested 90 seconds every 5 minutes of training; day-7, rats were placed in the animal treadmill for 10 minutes in a speed of 15 m/minutes, rested 90 seconds every 5 minutes of training.

This training was performed with a frequency of 5 times a week for 4 weeks and 12 weeks with 2 days off each week. In the 12-week intermittent aerobic training group, after acclimatization and age of the rats reached 8 weeks, intermittent aerobic training was performed with a speed of 20 m/minute for 20 minutes with a rest of 90 seconds every 5 minutes of running.

RESULTS

Figure 1 shows blood lactate levels on intermittent aerobic training and control groups.

Lactate levels in Wistar rats after 4 weeks intermittent aerobic training was 2.11 mmol/L (higher compared to its control, $p > 0.05$). After the intermittent aerobic training was performed for 12 weeks, the level of lactate was 1.71 mmol/L, which was significantly lower than the 12-week control group (3.03 mmol/L, $p < 0.05$).

The results also showed that the longer the period of intermittent aerobic training (12 weeks) in Wistar rats, lactate level that was produced immediately after training was lower than when the training was just carried out for 4 weeks.

DISCUSSION

Rat is one of the animals that is often used in researches on the effects of aerobic training because of the limitation in using human, and lower maintenance cost of the animal. This study used Wistar rats due to their behaviour, which are more active compared to other types of strain, such as Sprague Dawley.[7,8]

When using rat as experimental animals, their age, sex, strain, and the intensity of exercise should be considered because those factors may influence the effect and the rat's performance in training.[7,8] Therefore, the study protocol such as speed, duration, and intensity during aerobic training is the most important component in this research. Based on the research of Manchado, et al,[5] our study used 20 m/minute running speed because the highest workload can be reached at this intensity without accumulation of blood lactate, while at higher intensities (25, 30, 35 m/minute) there were progressive increases in lactate level. Thus, this study did training for 20 minutes, intermittently.

The research consisted of four periods, each period was 5 minutes with 90 secondorest every 5 minutes running. This training was performed intermittently to decrease blood lactate levels. In intermittent training, each period was interspersed with rest periods for at least 30 seconds to 6 minutes, or less running speed for a certain period of time. This study used intermittent aerobic training to get lower lactate level. Daussin, et al[9] stated that the maximum oxygen uptake would be increased by 15% in the intermittent aerobic training, while only 9% maximum oxygen uptake was increased if the aerobic training was done continuously. This study was done in 4 weeks. Many studies regarded 4 weeks of training as "short period" for animal training, while 12 weeks was regarded as "long period".[9,10]

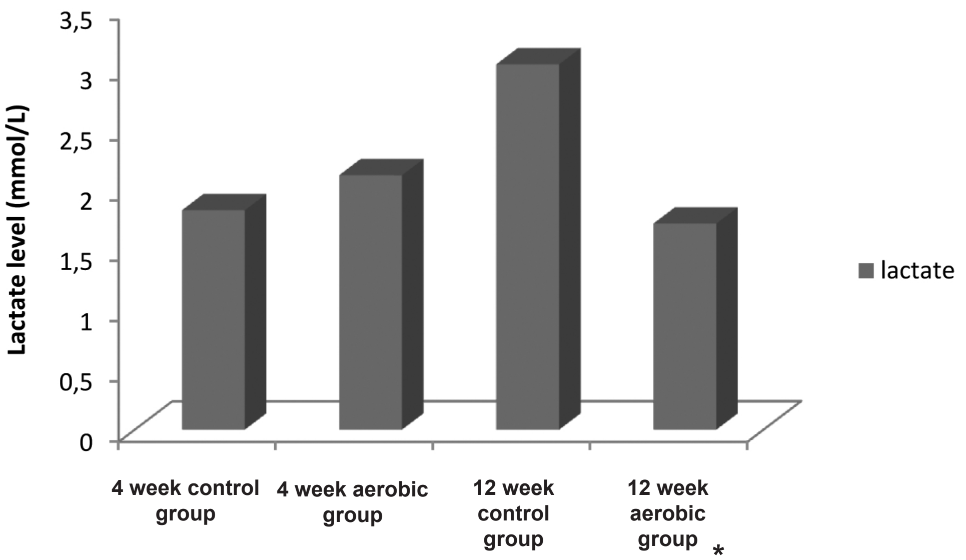

Figure 1. Lactate levels in 4 and 12 week aerobic training and control group (mean ± SD, 1.82 ± 1.07; 2.11 ± 0.33; 3.03 ± 0.78; 1.71 ± 0.1 mmol/L)
* $p < 0.05$ vs 12 week control group

The most dominant metabolic system used in aerobic training is oxidative metabolism system (aerobic), which is used as long as supplies of nutrients are available. If sufficient oxygen is available, pyruvate will be catalyzed into lactic acid by LDH in a small and constant amount in the blood (10 mg/ 100 mL of blood). Lactate level is normally 0-4 mmol/L. Lactate concentration is low at rest and increass at training in accordance with the load of work done. At training, blood lactate accumulation occurs when the rate of lactate production is higher than the rate of lactate clearance.[1-4,11]

Immediate lactate levels after 12-week intermittent aerobic training was lower than the 12-week control group (Δ = -1.32 mmol/L, $p < 0.05$). Lactate in the 12-week intermittent aerobic training group was also lower compared with lactate levels at 4-week intermittent aerobic training group (Δ = -0.40 mmol/L, $p > 0.05$). Our study showed that lactate levels after 4 weeks in aerobic training were still higher than 4-week control group (Δ = -0.40 mmol/L, $p > 0.05$). Lactate levels immediately after 12-week aerobic training was lower than 12-week control group (Δ = 1.32 mmol/L, $p < 0.05$).

In a study by Manchado, et al,[5] lactate concentration in Wistar rats at continuous speed of 20 m/minute was 3.9 mmol/L. In this study, lactate level after 4 weeks intermittent aerobic training had reached 2.11 mmol/L, and were still higher than control group. This discrepancy could happen because of different protocol; they measured lactate level in the first day, while our study measured after 4 weeks, so there may be an adaptation to training.

Donovan and Brooks[12] found lower lactate level in aerobic training was due to lactate clearance that was higher than lactate production. After aerobic training had been done for 12 weeks, lactate level was the lowest compared to 12-week control group and 4-week intermittent aerobic group. Our results suggest that the longer aerobic training is performed regularly with the same intensity and duration, the lower is the production of lactate until minimum level. Lower lactate level in aerobic exercise in trained compared to untrained animal is the aim in aerobic training. Our results showed that the performance of Wistar rats was increased along with the duration of aerobic training.

Lactate level in 12-week control group was higher compared with 4-week control and 12-week aerobic group. This fact might be due to increased lactate level with aging, which normally happens in untrained rats, as also happens in untrained people.

Diaz-Herrera, et al[13] showed that aerobic training for 12 weeks increased oxidative fibers in rats, such as fiber type I 24%, type IIA 8%, and type IIX 16%, and decreased glycolytic fiber (type IIB: 20%). Bonen[14] showed that aerobic training increased monocarboxylate transporter (MCT) I in heart and skeletal muscles. Siu[15] found increases in mitochondria and oxidative enzymes in skeletal and heart muscles of rats after 8 weeks of aerobic training. In human studies, Evertsen, et al that was cited by Gharbi[16] stated that intermittent aerobic training also maintained MCT I concentration. Daussin, et al[9] stated that aerobic training, in specific duration, frequency, and intensity of training could improve mitochondria 50 until 100% in 6 weeks after training.

In conclusion, blood lactate level in 12-week intermittent aerobic was lower compared to 4-week intermittent aerobic training and 12-week control group. We consider that decrease in blood lactate in Wistar rats in intermittent aerobic training needs longer time to happen. This result also showed that the performance of trained Wistar rats was better after long adaption time (12 weeks).

Acknowledgments

We would like to thank the head of DRPM UI who provided funds for the study through *Riset Awal Universitas Indonesia*. We are gratefully obliged to dr. Ermita, MS., head of Physiology Department, Faculty of Medicine, Universitas Indonesia, dr. Minarma Siagian, MS., head of physiology magister programme and also all of the lecturers. We also thank Dr. Rostika Flora for her animal treadmill.

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