Short Communication

Determining the range of aerobic exercise on a treadmill for male Wistar rats at different ages: A pilot study

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Abstract

The purpose of this study was to investigate the range of aerobic exercise on a treadmill for male Wistar rats at different ages. Twelve male Wistar rats were divided to three groups of immature, adult, and old (n= four in each). In the first session, the rats began to run at a rate of 2 m/min to perform the fatigue test, and the treadmill speed increased by 2 m/min every 2 minutes. The process of acceleration continued until the rats were no longer able to continue moving on the treadmill and were exhausted. Then, the blood lactate of each subject was measured immediately and their maximum speed was recorded. After 48 hours of recovery, the subjects performed maximum recorded speed on a treadmill in three 10-minute steps of 25%, 50% and 75%, respectively. Immediately after each step, the blood lactate was measured and recorded. Immature rats at an average speed of 18 m/min reached to their maximum speed with an average lactate concentration of 8±1.8 mmol/l, the adult rats at an average speed of 36 m/min reached to their maximum speed with an average lactate concentration of 6.8±0.4 mmol/l while the old rats reached to their maximum velocity with an average of 30 m/min and an average lactate concentration of 6.95±0.9 mmol/l. Therefore, it is recommended that aerobic exercise start at low speed for untrained rats, i.e. 25% of their maximum speed, which is lower than the lactate threshold, and gradually increase up to 50% of their maximum speed.

Key Words: **Aerobic exercise, Blood lactate, Lactate threshold, Wistar rat**

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Introduction

In general, an activity or movement that lasts for more than a few minutes requires the use of an aerobic system to provide the energy needed for that physical activity and animal specimens such as Wistar rats are no exception. Influential factors in using the aerobic system are physical fitness level, especially aerobic power (maximum oxygen consumption), lactate threshold, activity economics, gender, and the age of subjects (Beneke et al., 2011). Moreover, assessing aerobic fitness is very important for prescribing exercise in exercise physiology and rehabilitation (Gitt et al., 2002).

Determination of aerobic range, the intensity at which ATP production is predominantly aerobic, is performed using a gas analyzer, but due to its high cost and lack of easy access, it is not common for everyone. Instead, researchers use heart rate in rest, during exercise and post-workout recovery to predict aerobic power. However, measuring the heart rate of animals like Wistar rat is not as easy as that of human species (Brito Vieira et al., 2014). Some studies have reported that lactate threshold and maximum oxygen consumption can be used to determine aerobic capacity in rat models (Gonzalez et al., 1998; Kumagai & Nishizumi, 1986; Shepherd & Gollnick, 1976; Tsumiyama et al., 2012). Therefore, the use of blood lactate can be a good predictor parameter for determining the aerobic range relative to other variables.

Furthermore, VO_{2max} measurement requires special equipment and it is difficult to measure the respiratory gases of rats during exercise (Gonzalez et al., 1998; Tsumiyama et al., 2012). In many studies instead of aerobic exercise training, the term endurance training is used. In this regard, researchers are always trying to find protocols for determining the intensity of exercise activity in rats by applying the principles of human tests (Voltarelli et al., 2002) and they have applied a variety of methods to determine the intensity of exercise in animal studies (Manchado-Gobatto et al., 2011). In this regard, Voltarelli et al. (2002) used the minimum lactate test in rats to determine the anaerobic threshold. The results showed that changes in blood lactate in rats during experiments have a similar pattern to those in humans. In addition, the results of their study showed that maximal lactate steady state (MLSS) can be used to determine the maximum aerobic intensity in Wistar rat (Voltarelli et al., 2002). Tsumiyama et al. (2012) showed that the lactate threshold in rats could be determined from successive concentrations of blood lactate during increasing exercise (The average running speed was 18 m/min). The results showed that lactate thresholds can be accurately determined by taking consecutive blood samples during increasing exercise tests (Tsumiyama et al., 2012). However, most studies have determined the intensity of running on a treadmill in one or two age groups. Therefore, in this study, we sought to provide an intensity of running based on the maximum speed of each rat on a treadmill in three age groups of immature, adult, and old, which was measured in the aerobic range.

Materials and Methods

Animals

Twelve male adult Wistar rats were purchased from Pasteur Institute of Iran's animal care and production facility. Rats were divided to three age groups of 2 weeks (immature, 100-150 g), 8 weeks (adult, 220-250 g), and 22-24 months (old, 380-420 g) (n=4 in each). In an environment with a temperature of 23 ± 2 ° C, humidity was maintained at 45 to 55% and the dark cycle to light was maintained at 12:12 h. During the study, standard food pellets and water were provided freely. NIH instructions were followed in all phases and were approved by the Institutional Animal Care and Use Committee (IACUC) at Islamic Azad University, Iran (Rasht Branch).

Exercise test

After being acquainted with the laboratory environment and treadmill (5-7 days), the subjects were divided to three groups: immature, adult, and old. Resting lactate was measured on the first day by rat tail scraping (Christensen et al., 2009). The rats then began to run at a rate of 2 m/min to perform the fatigue test, and the treadmill speed increased by 2 m/min every 2 minutes. The process of acceleration continued until the rats were no longer able to continue moving on the treadmill and became exhausted. Treadmill tests performed during the subjects' active period (Christensen et al., 2009).

Blood Lactate

Blood was sampled (0.2 μL) and measured by a portable blood lactate device (Lactate Scout+, EKF diagnostics, Germany) at distal end of the rats' tail vein. Blood lactate of rats were measured immediately and their maximum speed was recorded. Finally, the mean values obtained from the speed of rats in each age group were recorded as the maximum intensity of the exercise. After 48 hours of continuous recovery, the subjects ran on the treadmill in three stages of 10 minutes with intensity of 25%, 50%, and 75% of their maximum recorded speed, respectively. Lactate count was measured immediately after each step.

Statistical Analysis

All data are presented as mean ± standard deviation. First, the Shapiro–Wilk test was used to determine the normality of the data distribution. Homogeneity of physical characteristics of the subjects at the onset of the study and the analysis of the dietary data were done using one-way analysis of variance (ANOVA). The analysis was performed using the IBM SPSS Statistics 29.0.

Results

Rats at different ages showed different concentrations of blood lactate at different running speeds compared to increasing intensity exercise (Table 1).

The results show that in 8 weeks, the lactate concentration of immature rats with the speed of 18 m/min (100% speed) was 8±1.8 mmol /l while its concentration for adult rats in the speed of 36 m/min (100% speed) was 6.8±0.4 mmol/l. In addition, the lactate concentration in old rats with the speed of 30 m/min (100% speed) was 6.95±0. 9 mmol /l. The blood lactate changes in the three groups are portrayed in figure 1.

According to figure 1, the adult group had the highest intensity of training compared to other groups and lactate concentration in this group gradually increased. The immature group reached its highest lactate threshold at less than 20 m/min. Therefore, the intensity of training in samples of rats at different ages should be considered with care. Some stages of the exercise test are shown in figure 2.

Discussion

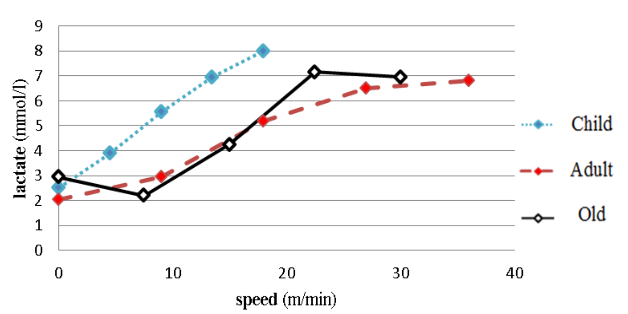

Figure 1. Blood lactate changes in immature (child), adult and old rats at 25%, 50% and 75% of their maximum speed.

Figure 2. Different stages of testing in Wistar rats and measuring blood lactate with a lactometer.

Aerobic fitness is one of the main capabilities for success in many sports. Therefore, determining the intensity of exercise activities in the aerobic range is important for prescribing exercise (Booth et al., 2010). However, determining the intensity of a sport activity in which energy production is achieved through the aerobic system is particularly difficult, especially in animal specimens. The findings showed that treadmill performance varied in male rats in each age group. This means that rats experienced exhaustion and lactate peak in the age groups of immature, adult, and old at the speeds of 18, 36, and 30 meters per minute, respectively, after increasing the training protocol on the treadmill. Therefore, it can be claimed that the intensity of aerobic exercise in different age groups based on lactate threshold should be different.

Due to the sensitivity of blood lactate concentration to intense exercise, extensive research has been performed to determine the transition time of aerobic metabolism to anaerobic metabolism by measuring the blood lactate concentration of rats (Contarteze et al., 2008; Manchado-Gobatto et al., 2011). Performing maximal lactate training protocols is a tool that allows researchers to determine the maximum intensity of exercise (Voltarelli et al., 2002). Several researchers have referred to the term lactate threshold as the onset of blood lactate accumulation (lactate threshold 4 mmol / L or LT4) during an incremental test assessing endurance-aerobic performance (Faude et al., 2009). Kawanishi et al. trained rats with the age of 4 to 12 weeks for 12 weeks at a speed of 21 to 31 m/min for 30 to 60 minutes, 5 sessions per week on a treadmill as the endurance exercise training (Kawanishi et al., 2018). However, in their research, the range of aerobic exercise measured using lactate concentration up to reaching the anaerobic threshold was not mentioned.

Table 1. Lactate concentration of male rats at different rates in three age groups (Mean ± SD)

Age	Rest		25 % maximum speed		50 % maximum speed		75 % maximum speed		100 % maximum speed	
	Speed (m/min)	Lactate (mmol/l)	Speed (m/min)	Lactate (mmol/l)	Speed (m/min)	Lactate (mmol/l)	Speed (m/min)	Lactate (mmol/l)	Speed (m/min)	Lactate (mmol/l)
Immature	0	3±0.4	4.5	3.6±0.7	9	5.7±1.2	13.5	6.1±2.59	18	8±1.8
Adult	0	2±0.07	9	3±0.50	18	5±2.4	27	6.2±2.8	36	6.8±0.4
Old	0	2.4±0.52	7.5	2.2±0.1	15	4.1±2.57	22.5	7.2±1	30	6.95±0. 9

Tsumiyama et al. took consecutive blood samples (every two minutes) from adult rats that started running on a treadmill at an increasing protocol of 2 m/min and ran up to a maximum speed of 26 m/min. The lactate threshold was set at 3.5 mmol/L at 18 m/min (Tsumiyama et al., 2012). However, the samples were limited to one age group; therefore, in the present study, the intensity of aerobic exercise in three age groups of male rats, immature, adult and old, was evaluated. Moreover, Manchado-Gobatto et al. performed a study on adult untrained rats, and reported the lactate threshold of 3.9 mmol/L at 20 m/min (Manchado-Gobatto et al., 2011). In general, studies on rats have reported average lactate thresholds between 4.4 and 5.1 mmol/l (Beneke et al., 2011).

It seems that in animal studies, training protocols can be adjusted to the aerobic range by measuring blood lactate concentration and lactate threshold (Carvalho, 2005). Therefore, if we consider experiencing fatigue when running on a treadmill at increasing speed for rats is equal to the maximum intensity of training based on the peak blood lactate, by recording the maximum speed at this point for each rat regardless of its age, we can quadruple the speed of each rat were by dividing the training intensity.

Conclusion

The present study aimed at determining the range of aerobic exercise intensity for male rats in the age groups of immature, adult, and old. According to the recorded concentrations of lactate at different speeds, it is recommended that aerobic exercise in untrained rats start from light intensity, i.e. 25% of their maximum speed, which is less than the lactate threshold level, and after physiological adaptation resulting from training, gradually increase to moderate intensity, i.e. 50% of the maximum speed until the end of the training protocol.

What is already known on this subject?

The use of blood lactate can be a good predictor parameter for determining the aerobic range relative to other variables.

What this study adds?

The present study provided appropriate data for determining the range of aerobic exercise intensity of immature, adult, and old male rats.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval The Islamic Azad University Animal Welfare and Ethical Review Body approved the study.

Informed consent Animal study.

Author contributions

Conceptualization: MR. FCh., E.A.; Methodology: MM. BT., A.E.; Software: MR. FCh., E.A.; Validation: MM. BT., A.E.; Formal analysis: E.A.; Investigation: MM. BT., A.E.; Resources: MR.FCh.; Data curation: E.A.; Writing - original draft: MM., A.E.; Writing - review & editing: MM. BT.; Visualization: MM. BT.; Supervision: MR. FCh.; Project administration: A.E.; Funding acquisition: E.A.

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