The maximal lactate steady state is ergometer-dependent in experimental model using rats

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ABSTRACT

The maximal lactate steady state (MLSS) is considered the gold standard method for determination of aerobic/anaerobic metabolic transition during continuous exercise, but the blood lactate response at this intensity is ergometer-dependent in human beings. An important tool for exercise physiology and correlated fields is the use of animal models. However, investigation on evaluation protocols in rats is scarce. The aim of the present study was to verify if the MLSS is ergometer-dependent for the evaluation of the aerobic conditioning of rats. Therefore, 40 adult male Wistar rats were evaluated in two different exercise types: swimming and treadmill running. In both, the MLSS was obtained with 4 continuous 25 minutes tests, at different intensities, performed at 48 hours intervals. In all tests, blood samples were collected from a cut at the tail tip every 5 minutes for blood lactate analysis. The swimming tests occurred in a deep cylindrical tank, with water temperature at 31 ± 1°C. The loads used in the tests were 4.5; 5.0; 5.5 and 6.0% of the body weight tied to the animal's back. For MLSS determination in running exercise, there was selection of running rats and velocities used in the tests were 15, 20, 25, 30 $m.min^{-1}$. The MLSS was interpreted as an increase not exceeding 1.0 mM of blood lactate, from the 10^{th} to the 25^{th} minute of exercise. The MLSS in swimming exercise occurred at 5.0% of body weight (bw), with blood lactate at 5.20 ± 0.22 mM. The running rats presented MLSS at the 20 $m.min^{-1}$ velocity, with blood lactate of 3.87 ± 0.33 mM. The results indicated that the MLSS is ergometer-dependent in experimental models using animals, as it is in human beings.

Keywords: Blood lactate. Swimming. Treadmill running. Wistar rats.

INTRODUCTION

The determination of the metabolic predominance for the energy supply during an exercise presents extreme importance for the correct prescription of an activity. Accordingly, there are many proposed evaluation protocols in order to detect the intensity of transition between the aerobic and anaerobic metabolisms (Wasserman and McIlroy, 1964; Monod and Scherrer, 1965; Kinderman *et al.*, 1979; Sjödin and Jacobs, 1981; Chassain, 1986 and Tegtbur *et al.*, 1993).

Exercise physiology and correlated fields have been developing simple and complex methodologies over the years, with the purpose to determine the effort intensity. According to Gaesser and Poole (1996), the physiological responses facing the exercise trustfully signal the characteristic of the predominant metabolism of the energy supply for the given activity. Among these responses it is possible to highlight the blood lactacidemia.

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Received in 15/9/05. Final version received in 19/12/05. Approved in 14/5/06.

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The maximal lactate steady state (MLSS), defined as the highest intensity in which the anaerobic metabolism still predominates over the aerobic, is currently considered the gold standard method for the determination of the transition intensity between these metabolisms in continuous exercise performed by humans (Beneke, 1995; Beneke, 2003, Billat *et al.*, 2003). According to Beneke *et al.* (1995), the lactacidemic response in this intensity is dependent on the ergometer used by these individuals, which implies in careful generalizations of mistaken information on the training loads prescribed by the concentration of blood lactate.

The importance to precisely determine the exercise intensity is not restricted to works in which humans are objects of the study. A tool that has been considered interesting to the organism observation facing effort is the use of experimental models with animals. Such models have been elaborated in order to simulate physiopathological or training-related situations occurred with humans, and solve occasional problems derived from the observed alterations (Oliveira *et al.*, 2005; Braga *et al.*, 2004; Murdes *et al.*, 2004).

With this aim, Gobatto *et al.* (2001) evaluated the MLSS in sedentary rats adapted to the aquatic environment, submitted to the swimming exercise, as proposed by Heck *et al.* (1985) for evaluation in humans. Thereby, the animals performed 20 minutes of continuous effort in intensities correspondent to 5, 6, 7, 8, 9 and 10% of their body weight attached to their bodies, with blood samples collection being conducted at every five minutes for later lactacidemia determination. The authors observed MLSS in intensities correspondent to 6% of the body weight, with stabilization concentration of 5,5 mM. This index is different and higher than the one reported for humans in distinct exercises and for rats performing effort in rolling treadmill (approximately 4 mM). In a recent review on the maximal lactate steady state, Billat *et al.* (2003) point out the findings by Gobatto *et al.* (2001) as representative for the study with animals.

Other evaluation protocols have been developed in swimming, such as the determination of the critical load and anaerobic work ability (Marangon *et al.*, 2002) and minimal lactate test (Voltarelli *et al.*, 2002). However, due to its great importance, all of them use the MSLL as gold Standard in order to validate their procedures.

The rolling treadmill is another ergometer widely used for training in rats, and thereby, the detection of the effort intensity in running exercise is extremely important. In 1993, Pillis *et al.* proposed the application of incremental test and observation of the lactacidemic response for aerobic evaluation of runner rats, based on the anaerobic thresholds concepts (Lan) suggested for humans (Sjodin and Jacobs, 1981). Therefore, the animals performed a test characterized by the progressive velocity increase, with five minute-stages and blood samples collection at the end of each load, with later determination of the anaerobic threshold through exponential behavior analysis of the lactacidemic curve. The rats' anaerobic threshold was obtained at 25 $m.min^{-1}$, in a lactate concentration of 4 mM. The results of this study revealed blood lactate behavior

similar to the one described for humans, with the same concentration associated with the Lan, indeed. However, in the rats swimming, Gobatto *et al.* (1991) did not find this classic exponential behavior of the blood lactate curve, which suggests the need of further investigation on the lactate kinetic in animals submitted to physical exercise and the lactate responses distinction in different ergometers.

The aim of the present study was to evaluate whether the maximal lactate steady state is dependent on the ergometer used for the aerobic evaluation in rats, as it is for humans, due to this method's importance and also for prevention of mistakes in the prescription of activity for animals in different kinds of exercise.

MATERIALS AND METHODS

Animals

All experiments were conducted according to the American College of Sports Medicine politics and approved by the Biosciences Institute of the São Paulo State University – UNESP, Rio Claro. Forty-four Wistar rats, with 90 days of age, weighting 443 ± 33 g, were used. During the experimental period, the animals were kept in collective cages (five rats per cage) in a lighted room with light-dark cycle of 12:00-12:00 hs and temperature of 25°C. The rats received commercial food specific for rodents (Labina-Purina) and water *ad libitum*.

Experimental protocol

Adaptation to the water environment

Twenty animals were submitted to maximal lactate steady state tests in swimming exercise. The rats were adapted to the water environment in a standardized manner prior to the tests conduction. The adaptation occurred in a total period of 15 uninterrupted days, in a cylindrical tank with smooth surface, measuring 60 cm of diameter by 120 cm of depth (Marangon *et al.*, 2001), with water temperature kept at 31 ± 1°C (Harri and Kuusela, 1986). The purpose of the adaptation was to decrease the animal's stress without promoting physiological adaptations derived from the physical training, though.

Initially, the rats were inserted in shallow water for three days during 15 minutes. Later, the water level was increased, as well as the effort duration time and the load to be held by the animal. Thus, on the forth day, the rats swam in deep water for two minutes, with increase of two minutes at each day until the tenth day of adaptation. On the eleventh day, the animals were submitted to the swimming exercise for five minutes holding a load of 3% of their body weight, with increase of five minutes at each day, when, on the fifteenth day, the adaptation ceased.

Selection of the runner rats and adaptation to the treadmill

It was necessary to previously select the runner animals in order to conduct the tests in treadmill. The selection occurred in a seven day-period, in which the 20 rats that presented positive response to the running stimulus at least five times were chosen After the selection, the animals were submitted to an adaptation to the treadmill exercise, with progressive velocities (5 to 20 $m.min^{-1}$) and duration (5 to 15 min). The aim of the adaptation was also the reduction of stress indices presented by the animal due to the task being known without physical training promotion.

Determination of the maximal lactate steady state

The whole experimental protocol was conducted in environmental conditions identical to the ones during the adaptation period, both in swimming and in treadmill running.

In both exercises, the protocol for the MLSS determination consisted of five continuous tests with duration of 25 minutes, in different effort intensities, randomly distributed and separated by a 48-hour resting interval. In all tests there was blood collection of the animals' tails in the resting times, 5, 10, 15, 20 and 25 minutes of exercise, for later blood lactate analysis and the lactacidemic curves in each intensity.

Swimming tests

The rats performed 25 minutes of continuous effort in loads equivalent to 4,5; 5,0; 5,5 and 6,0% of their body weight, attached to their backs. There was daily load adjustment, with the animals' body mass measurement. Blood samples from the animals' tails were performed for each intensity in the times previously described, and the blood sample's treatment occurred according to a technique detailed below.

Treadmill running tests

For the MLSS evaluation in running, the selected animals performed the continuous tests in the 15, 20, 25 and 30 $m.min^{-1}$ velocities. The treadmill specific for training with rats composed of eight lanes, was kept with the electric shock off, reducing hence, the stress effect in the effort conducted by the animal. Blood samples were removed from the rats' tails as in swimming.

Blood samples and analysis

During the continuous tests, blood samples (25 µl) were removed from the animal's tail in the times already described and placed in *Eppendorf* tubes (capacity of 1,5 ml), containing 50 µl of sodium fluoride (1%). In order to avoid the blood dilution in water in the case of the swimmers, the animals were removed from the cylinder and dried, returning to the aquatic environment immediately after the blood collection. The blood lactate concentrations were determined in a lactate analyzer (Model YSI 1500 Sport, Yellow Springs, OH, EUA).

Statistical analysis

In both ergometers the MLSS was interpreted as the highest exercise intensity in which the increase of the lactacidemia was equal or lower than 1 mM, from the 10^{th} to the 25^{th} minute. The average concentrations of blood lactate in each intensity for the two ergometers were obtained through the ratio of the lactacidemic indices of the times 10, 15, 20 and 25 minutes of exercise. A one-way ANOVA was used in order to identify the differences between the blood lactate concentrations in the several durations of the continuous exercise and between distinct ergometers: swimming and treadmill running. The results are expressed in average ± standard error of the average. In all statistical procedures, the significance index was preset in $P < 0,05$ (Dawson-Saunders and Trapp, 1994).

RESULTS

The blood lactate curves of the rats submitted to the swimming exercise are expressed in figure 1. The MLSS was observed in the intensity equivalent to 5% of the body weight, in an average lactate concentration of 5,20 ± 0,22 mM.

In the running exercise, the velocity correspondent to the maximal lactate steady state was 20 $m.min^{-1}$, in lactate concentration of 3,87 ± 0,33 mM, though (figure 1). In the intensity equivalent to 30 $m.min^{-1}$ only 20% of the animals completed the test.

The one-way ANOVA identified significant difference between the maximal lactate steady state obtained in the swimming exercise and the treadmill running.

DISCUSSION

Although the lactate production occurs internally in the skeletal muscle, the blood measurements of this metabolite during exercise provide precise information about the energetic supply for the

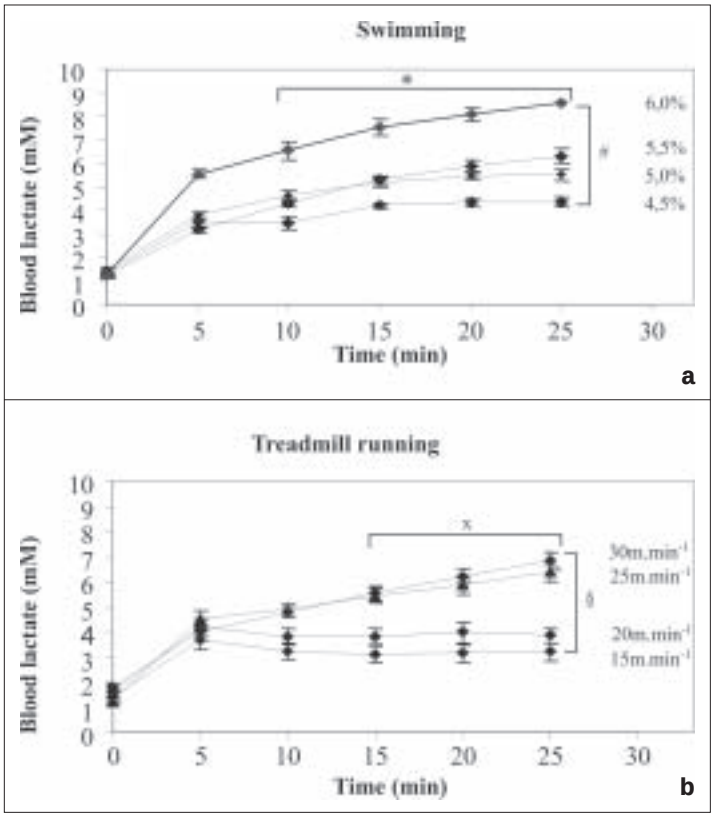

Figure 1 *– Maximal blood lactate steady state obtained in swimming exercise for rats* ***(a)*** *(n = 20) and treadmill running (n = 20)* ***(b)****. The results are expressed in average ± average standard error. The lactacidemia during the exercise was higher than the initial in all cases. * significant difference between the lactacidemia at 5 minutes and the remaining blood lactate indices in all exercise intensities. # significant difference between the blood lactate indices in the 6% intensity of the remaining intensities, from the tenth to the twenty-fifth minute of exercise § significant difference between the lactacidemia from the fifteenth to the twenty-fifth minute and the lactate indices at 5 minutes, in the velocities equivalent to 25 and 30 m.min-1. x significant difference between the lactacidemic curves in the 25 and 30 m.min-1 velocities compared to the lactate curves obtained at 15 and 20 m.min-1, from the fifteenth to the twenty-fifth minute of exercise.*

effort. Such fact implies in the use of this parameter as a prescription instrument (Mujika *et al.*, 1995) and sports training aid (Pyne *et al.*, 2001).

The maximal lactate steady state is related to the biochemical balance between appearance and removal of the blood lactate (Mader and Heck, 1986) and is considered the highest exercise intensity in which this steady state still occurs, outlining hence, a transition of aerobic-anaerobic metabolic predominance (Beneke, 1995; Billat *et al.*, 2003). Therefore, it is considered a safe method for aerobic ability identification (Jones and Carter, 2000), which corroborates so that many studies use this procedure in the evaluation of active individuals and high performance athletes (Jones and Doust, 1998; Harnish *et al.*, 2001; Beneke, 2003).

Our study is important due to the application of this individual protocol in order to evaluate laboratory animals in two different kinds of exercise: swimming and treadmill running. Moreover, there is massive scientific meaning in obtaining data about probable differences of lactacidemic responses in two ergometers widely used for training of Wistar rats.

Concerning swimming, our MLSS results are similar to the ones shown by Gobatto *et al.* (2001) and Voltarelli *et al.* (2002). The blood lactate stabilization for our sedentary rats was obtained in 5% of the body weight, as described by Voltarelli *et al.* (2002). Gobatto *et al.* (2001) reported stabilization load equal to 6% bw. Such small distinction in the load related to the MLSS may be due to the specificity of the water tank used. In the present work, it was chosen to perform the swimming exercise in a deep tank (60 cm of diameter by 120 cm of depth) with a smooth surface, avoiding thus, that the animals could lean on the sides of the tank during the test or could touch the bottom of the tank performing a jump movement. In the study by Gobatto *et al.* (2001), the tanks used were not deep (100 cm of diameter by 80 cm of depth), which possibly favored the rats' activity.

The stabilization concentration of the lactate in the maximal aerobic intensity in swimming was 5,20 ± 0,22 mM, an index very close to the one described by Gobatto *et al.* (2001) for sedentary rats (5,5 mM), which suggests reproducibility of this concentration for the species of evaluated animals. Even after aerobic training, Gobatto *et al.* (2001) did not observe alteration in the lactate stabilization concentration. In humans, the lactate stabilization in maximal aerobic intensity is usually found between 3 and 7 mM (Stegmann *et al.*, 1981; Harnish *et al.*, 2000), however, in swimming exercise, this index seems to be close to the lower threshold of the described group. Pereira *et al.* (2002) determined the anaerobic threshold of swimming athletes in progressive test and observed inflexion of the lactacidemic curve in 3,5 mM concentration. Thus, it seems that Wistar rats present higher lactate concentration indices in the MLSS in swimming exercise when compared to humans.

In the treadmill running we obtained MLSS in 20 m.min^{-1} intensity in 3,87 ± 0,33 mM concentration. Pillis *et al.* (1993) and Langfort *et al.* (1996) suggest the occurrence of the anaerobic threshold in runner rats in the 25 m.min^{-1} velocity, which is higher than in our findings. In the present study, the animals were submitted to 25 minutes of continuous exercise in the 15, 20, 25 and 30 m.min^{-1} velocities, allowing a longer observation of the blood lactate kinetic, while those authors only observed the lactate behavior related to progressive exercise.

Concerning the lactate concentration, the treadmill exercise promoted stabilization in an index significantly lower to the one observed in swimming (figures 1 and 2). The lactacidemic concentration verified in the present study is similar to the point of inflexion of the lactate curve described by Pillis *et al.* (1993) (4 mM) and to the index obtained in a classic study performed with humans in this kind of exercise (Heck *et al.*, 1985). There are no works in the literature which present aerobic training results in running in maximal lactate steady state with Wistar rats, as the one conducted by Gobatto *et al.* (2001) in swimming.

Our results clearly demonstrate the ergometer dependence of the maximal lactate steady state protocol in exercises for Wistar rats, as well as in humans. Such fact demands caution in the evaluation and prescription of physical training for these animals. Further studies should be conducted with rats in different physiopathological and training conditions in order to identify possible alterations in the evaluated aerobic parameter.

ACKNOWLEDGMENTS

This study was supported by the Foundation of Research Support of the São Paulo State (FAPESP – Proc. 07070-5/2004) and the Research Centers CAPES and CNPq (Proc. 300270/2004-16). We also thank José Roberto Rodrigues da Silva, Eduardo Custódio and Clarice Yoshiko Sibuya for the technical support.

All the authors declared there is not any potential conflict of interests regarding this article.

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