

# Análises das capacidades aeróbia e anaeróbia de ratos adultos e idosos

## Analysis of aerobic and anaerobic capacity in adult and elderly rats

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**RESUMO:** O objetivo do presente estudo foi comparar a capacidade aeróbia e anaeróbia de ratos adultos e idosos. Foram utilizados 27 ratos Wistar distribuídos em dois grupos em função da idade, sendo 13 animais no grupo Adulto (GA) - 126 dias; e 14 animais no grupo idoso (GI) - 465 dias. Os animais foram submetidos ao Teste do Lactato Mínimo (Lacmin) em meio líquido, para a determinação de parâmetros Anaeróbios e Aeróbios. Os animais do grupo adulto apresentaram limiar anaeróbio de  $3,47 \pm 0,52$  mmol/dl; e os idosos de  $3,44 \pm 0,47$  mmol/dl. Para a capacidade anaeróbia, os animais adultos apresentaram  $69,77 \pm 12,40$  segundos; e os animais idosos  $56,43 \pm 7,84$  segundos, denotando diferença estatisticamente significativa ( $p < 0,05$ ). Pode-se concluir que os animais adultos apresentaram um desempenho semelhante aos idosos, o que sugere que o envelhecimento não tenha afetado a capacidade aeróbia dos animais. Porém, houve diferença quando comparada a capacidade anaeróbia, tendo o grupo adulto com maiores valores no teste.

**Palavras-chaves:** Capacidade Aeróbia; Desempenho anaeróbio; Ratos, Adulto; Idoso.

**ABSTRACT:** The aim of this study was to compare the aerobic and anaerobic capacity of adults and elderly rats. Twenty seven Wistar rats were used, distributed into two groups according to age; there were 13 animals in the adult group (GA) and 14 in the elderly group (GI). The animals were submitted to a lactate minimum test (Lacmin) in the liquid medium, for determination of Anaerobic and Aerobic parameters. The animals in the adult group presented an anaerobic threshold of  $3.47 \pm 0.52$  mmol/dl, and  $3.44 \pm 0.47$  mmol/dl for the elderly group. For anaerobic capacity, the adult animals showed  $69.77 \pm 12.40$  seconds and the elderly animals  $56.43 \pm 7.84$  seconds, indicating a statistically significant difference ( $p < 0,05$ ). It is concluded that the adult animals demonstrated similar aerobic performance to the elderly, suggesting that aging did not affect the aerobic capacity of the animal. However, when comparing the anaerobic capacity, the adult group presented higher values compared to the elderly.

**Key Words:** Aerobic Capacity; Anaerobic performance; Rats; Adult; Elderly.

## Introduction

Blood lactate (Lac) has been used as a parameter to measure physical performance, since it indicates the capacity of the body's anaerobic energy system in relation to exercise<sup>1</sup>. Lac is an energy substrate which results from the metabolic process and energy production<sup>2</sup>.

Of the methods proposed for estimating the maximal lactate steady state (MLSS), the lactate minimum test (LACmin) is quick and reliable<sup>3</sup>. The LACmin test was developed by Tegtbur *et al.*<sup>2</sup> and later adapted for use in animals by Voltarelli *et al.*<sup>3</sup>. It was reproduced by Araujo *et al.*<sup>4</sup>, obtaining satisfactory results.

Consequently it has become useful as a method of prescribing exercises in certain physical training programs<sup>5</sup>, particularly when there are differences among the athletes, such as age. It is known that physical exercise results in a number of changes in the body of the individual and, therefore, physical capacity and performance<sup>6</sup>.

Another point to be considered is the age of the evaluated individual. A number of modifications occur in the functionality of the organism as part of the aging process, which may affect the physical performance of the individual, especially in sports and activities that result in the generation of muscular strength.

Among the physiological changes brought about by aging is the decrease in strength and cardiorespiratory fitness which directly affects functional independence<sup>7</sup>. However, despite the identification in the literature of some findings related to the aging process, it can be seen that information about the fatigue process is still being discussed<sup>8-9</sup>.

Accordingly, experimental studies may be a way to test the use of physiological variables in the aging process, since there is a drawback to exposing the elderly to the performance of tests at full load or which result in exhaustion of the individual. In view of the above, the objective of this study was to compare both the aerobic and anaerobic capacity of adult and elderly rats.

## Methods

### Sample

In total, 27 male Wistar rats were used (*Rattus norvegicus*, albino), distributed into two groups according to age, 13 animals in the adult group (GA) - 126 days; and 14 animals in the elderly group (GI) - 465 days. The animals were housed in plastic collective cages (five animals per cage), in a temperature controlled room (22 ± 2°C), with a relative humidity of 60% ± 10%, a light-dark cycle of 12 hours, starting at 7a.m., and received food and water *ad libitum*.

All procedures complied with the Ethical Principles in Animal Research adopted by the Brazilian Society of Science in Laboratory Animals (SBCAL) and received approval from the Ethics Committee for Research of the Faculty of Science and Technology UNESP – Presidente Prudente Campus, no. 5/2010.

### Experimental Groups

#### *Adaptation of the animals*

The adaptation was carried out in a tank of 80 cm in diameter and 75 cm in height. The animals were separated by cylindrical tubes of 25 cm in diameter by 120 cm in length. For the first three days the animals were placed in the tank with a water depth of 10 cm and water temperature of 31 ± 2°C, for 15 minutes. On the fourth day the animals were subjected to swimming, with the water at a depth of 70 cm, for 2 minutes. From the fifth to the tenth day, the swimming time was increased by two minutes each day. On the 11th day swimming was carried out for five minutes with an added load of 3% of body weight.

### 13 Anaerobic capacity in rats

The load was added by means of lead weights, placed in a cloth bag and tied with elastic to each animal's chest. Starting on the 11th day, the exercise period was extended by five minutes per day, until the 15th day. The adaptation was performed in order to reduce the animals' stress but without promoting physiological adaptations in response to the physical exercise<sup>10-12</sup>.

#### Lactate Minimum Test

On the 16<sup>th</sup> day the animals were submitted to a lactate minimum test (LACmin), which allowed determination of the Anaerobic and Aerobic parameters,<sup>4-13</sup>. This test consists of two phases: hyperlactacidemia induction (HI), which assesses the anaerobic capacity through Tlim, and an incremental test (IT), which verifies aerobic capacity through Lan. For the HI, the animals performed two efforts with an overload of 13% of body weight, with an interval of 30 seconds between efforts. The first effort lasted for 30 seconds, and the second was performed until exhaustion of the animal (Tlim), considered when the animal was unable to remain on the surface of the water for 10 seconds. The rats were then given 9 minutes of passive recovery, during which time blood samples (25µL) were collected for measurement of; blood lactate concentration immediately after exercise (LAC peak), peak concentration of blood lactate at 3min (Lac 3), 5min (5 Lac), and 7min (Lac 7).

After the recovery time the animals proceeded to the IT, which consisted of a sequence of 3 minute swimming stages, starting with a load of 2% of body weight, with a load increase of 0.5% at each stage up to a maximum of 7% of body weight (progressive stages). There was an interval of 1 minute between stages for load adjustment and blood sample collection. The test was stopped when the animal was not able to proceed with the swimming exercise, regardless of the stage they had reached.

#### Blood Samples and Analysis

Blood samples were collected from a cut in the distal end of each animal's tail in graduated capillary tubes, for subsequent analysis of blood lactate concentration.

To avoid the blood being diluted with water, the blood sampling region was dried prior to collection. The samples (25µL) were placed in plastic microtubes, transparent, graduated with lids, with a volume of 1.50mL, containing 50µL of Sodium Fluoride (1%).

#### Analysis of blood lactate concentration

Blood lactate concentrations were determined in a lactate analyzer, model YSI 1500 Sport (Yellow Springs, OH, USA). From these values, a curve of blood lactate vs. workload was obtained for each rat using a second-order polynomial fit, obtained with the aid of Excel software (Microsoft®). The concentration relating to LACmin was obtained through the derivative of the zero polynomial fit (Figure 1).

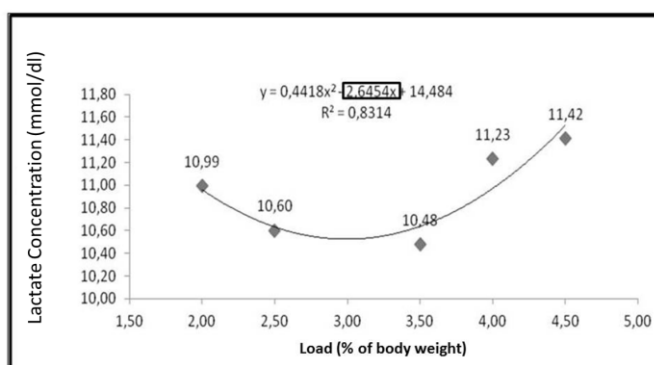


Figure 1. Example of the curve determining the lactate minimum of one rat.

## Statistical Analysis

The results are expressed as mean and standard deviation. Data normality was confirmed using the Shapiro-Wilk test and the comparison between groups was performed using the Student t test for independent samples with a standard error of ( $p < 0.05$ ).

## Results

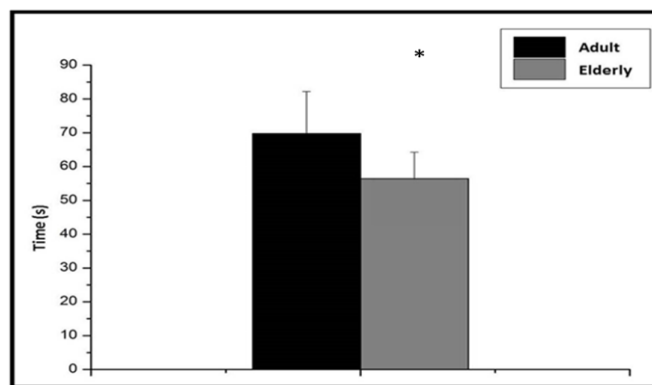
There was a significant difference ( $p < 0.05$ ) in the evaluation of body weight between the adult and elderly groups (Table 1).

**Table 1.** Mean and standard deviation of the weights of the animals.

| Group   | Weight (g)         |
|---------|--------------------|
| Adult   | 356.9 $\pm$ 41.23* |
| Elderly | 514.2 $\pm$ 61.88* |

(\*): Statistically significant difference between the groups ( $p < 0.05$ ).

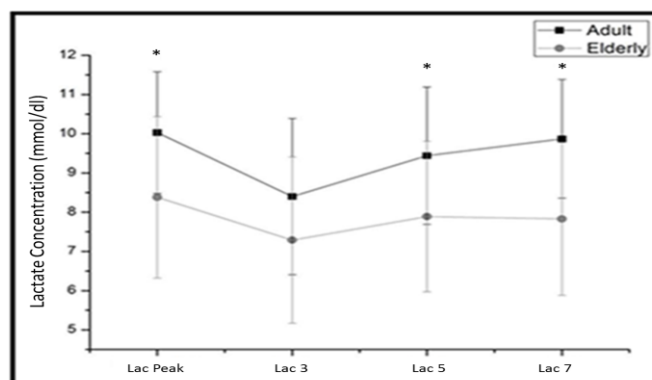
The rats in the adult group performed better in the Tlimf (Figure 2), compared to the elderly group, with a statistically significant difference ( $p < 0.05$ ).



**Figure 2.** Mean values of time to exhaustion for the adult and elderly groups.

(\*): Statistically significant difference between the groups ( $p < 0.05$ ).

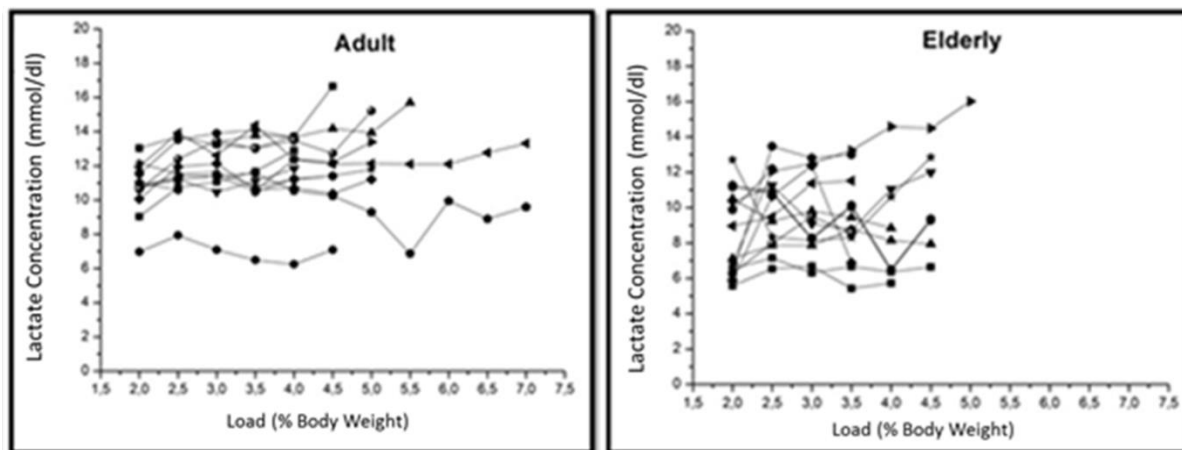
The values of lactate peak, Lac 5 and Lac 7 presented significantly different values ( $p = 0.028$ ;  $p = 0.039$  and  $p = 0.006$ , respectively), with higher values in the adult group, but this difference was not observed in Lac 3 (Figure 3). Moreover, it is noted that the adult group presented a mean anaerobic threshold value of  $3.47 \pm 0.52$  mmol/dl, while the elderly group value was  $3.44 \pm 0.47$  mmol/dl.



**Figure 3.** Mean values of blood lactate concentration (mmol/dl).

(Lac Peak): hyperlactacidemia induction; (Lac 3): after three minutes; (Lac 5): after five minutes; (Lac 7): after seven minutes. (\*): Statistically significant difference between the groups.

The values of blood lactate concentration in the IT are shown in the scatter plot (Figure 4).



**Figure 4.** Scatter plot of the results of the blood lactate concentration obtained from each animal in the incremental test.

Lan was determined from the IT by means of a graph of the relationship between lactate concentration and load (% of body weight). The mean values of Lan were  $3.47 \pm 0.52\%$  of body weight for the adult group, and  $3.44 \pm 0.47\%$  of body weight for the elderly group; these values were not statistically different.

## Discussion

In the present study the lactate minimum test was used as the protocol for assessing the aerobic and anaerobic capacities of adult and elderly rats. No significant difference was observed in relation to aerobic capacity. However, the mean values of anaerobic capacity presented were higher and statistically different in the adult animals ( $p < 0.05$ ). The lactate minimum test was used in this study because it is a rapid, easily applied test which accurately identifies the point at which there is a balance between the production and removal of blood lactate, with the added advantage of measuring both aerobic and anaerobic capacity in the same test. The method considered to be the "gold standard" for assessing aerobic capacity is the maximal lactate steady state (MLSS)<sup>14-17</sup>.

The MLSS test protocol, although effective, takes time and is unfeasible during the competitive season. This fact prompted some researchers to propose a way to estimate MLSS with a faster and more practical protocol, in this case the LACmin test<sup>2</sup>.

The finding that there was no difference in aerobic capacity between the adult and elderly groups could be explained by the significantly higher body weight of the elderly animals. This may have influenced the results, due to the fact that the accumulation of adipose tissue promotes greater buoyancy in elderly animals, which therefore may have allowed for reduced efforts and possibly caused overestimated results<sup>18</sup>. The choice of an evaluation protocol performed on a treadmill could be a means of identifying aerobic capacity wherein the accumulation of body fat does not favor animals with a higher percentage of adipose tissue.

In any case, the inclusion of elderly animals in a training program may lead to improved physical abilities. In a study of Cunha *et al.*<sup>18</sup>, it was found that four weeks of swimming training at an intensity corresponding to the anaerobic threshold resulted in improved aerobic fitness and body weight maintenance in elderly rats. Therefore, physical training could still be a contributing factor to the control of body weight.

However, when analyzing anaerobic capacity in adult and elderly animals, it was found that the former group presented superior physical performance to the latter. It was also found that the LAC peak was lower in the adult animals, and from this it can be inferred that the elderly animals developed reduced energy production compared to the adults.

The aging process promotes considerable damage in the anabolic pathway of the skeletal muscle metabolism<sup>19</sup>. Such factors may explain physical decline with advancing age. Muscle tissue tends to lose its configuration, which affects the muscular system (disorders of the myofilaments and Z-lines) and mechanisms (reduction of satellite cell activation / proliferation and changes in mitochondrial function and muscle cells) that reduce muscle strength<sup>20-21</sup>.

However, exercise can lessen the degenerative processes caused by aging. In a study using elderly animals, Palomero *et al.*<sup>22</sup> showed that the muscles of these animals showed attenuation in the processes of acute stress and regulation of reactive oxygen species during the contractile activity of the skeletal muscle. It could be, therefore, that the stimulus caused by training can trigger adaptations of the contractile function.

Assessment of anaerobic capacity was evaluated by Tlim, the stage where, after hyperlactacidemia induction, the animals performed an effort until exhaustion. In this variable the adult animals presented a better performance than the elderly ones, indicating a loss of anaerobic capacity with increasing age.

For practical purposes, the Lan was applied by subjecting the subjects to efforts with progressively greater loads and with a concomitant assessment of blood lactate concentration. Determination of Lan can be made based on the deviation in the baseline circulating lactate or the work intensity corresponding to a fixed concentration of circulating lactate. The first method is based on the fact that the nonlinear increase in blood lactate concentration in relation to exercise intensity indicates the MLSS of the metabolism, as defined by Wasserman and McIlroy<sup>23</sup> and Simões *et al.*<sup>24</sup>.

The second procedure assumes the principle that until a certain concentration of circulating lactate, a balance occurs between muscle production and removal of this substrate from circulation<sup>4</sup>. Its accumulation in the blood is an indication that the aerobic system is not supporting the demand for energy needed to perform the exercise. The measurement of the amount of lactate produced is considered a reliable measure of physical effort generated by exercise<sup>25</sup>.

In clinical studies, animal models have provided important information regarding the performance of physical exercise under various experimental conditions, including obesity, diabetes and malnutrition<sup>1-29</sup>. Thus, this study contributes to the literature by identifying the aerobic and anaerobic performance in adult and elderly animals; and demonstrating the decrease in this capacity with increasing age. This finding may contribute to the knowledge gap in the literature, since the difficulty of exposing elderly humans to the performance of maximum tests can be observed, as well as verification of aerobic and anaerobic parameters in the liquid medium.

However, limitations should be pointed out, since the evaluation was performed in the liquid environment which may have influenced the performance of the elderly animals, overestimating their performance. Future studies using a treadmill or a ladder could complement the findings presented in this work.

## Conclusions

It can be concluded that the adult animals presented similar performances to the elderly, which suggests that aging did not affect the aerobic capacity of the animals. However, there were differences when comparing anaerobic capacity, with the adult group achieving higher values in the test. Is worth emphasizing that the form of evaluation performed in the liquid medium may have contributed to the success of the older group when evaluating aerobic capacity.

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