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Prescription of Aerobic Exercise Training Based on the Incremental Load Test: A Model of Anaerobic Threshold for Rats

Phablo Sávio Abreu Teixeira[2], Igor Cabral Coutinho do Rego Monteiro[1], Tanes Imamura Lima[1], Aline Colares Camurça dos Santos[1], Vânia Marilande Ceccatto[1]

[1]Superior Institute of Biomedical Sciences – State University of Ceará, Fortaleza, Ceará, Brazil, [2]Institute of Biomedical Sciences - University of São Paulo, São Paulo, São Paulo - Brazil

ABSTRACT

Abreu PST, Rêgo-Monteiro ICC, **Lima TI**, **Santos ACC**, **Ceccatto VM.** Prescription of Aerobic Exercise Training Based on the Incremental Load Test: A Model of Anaerobic Threshold for Rats. **JEPonline** 2012;15(3):45-52. This study determined the efficiency of a training program based on the Incremental Load Test (ILT) to prescribe and evaluate aerobic exercise training. The maximum lactate steady-state (MLSS) was determined at 1.2 km·h^{-1} and the lactate concentration achieved at this intensity was 2.32 ± 0.33 mmol·L^{-1} ($P<0.05$). In addition, the MLSS corresponded to 60% of maximal speed achieved during the ILT. The time (78.62%), speed (74.98%), and total distance run (190.28%) during exercise training increased significantly after 6 wks when compared to the control group. Data from the ILT demonstrated a significant difference in exhaustion when compared to rest. The lactate concentrations in the control group at 27 min rose over 90.15% and in the trained group at 51 min, 6.66%. The lactate concentration at exhaustion in the trained group was significantly higher (an increase of 41.90%; $P<0.05$) than the control group. Both the detection of the lactate threshold in the ILT (the percentage of 60%) and the MLSS (showing that 1.2 km·h^{-1} as the target speed) as a valid parameter with an important metabolic meaning.

Key Words: Experimental Animal Model

INTRODUCTION

The exercise prescription for training and fitness assessment proposed for animal models differ extensively. For example, with regard to the exercise prescription and evaluation, VO_2 max (23), lactate threshold (9), maximum lactate at steady-state (20), maximum intensity testing (12), and critical speed (7) are all measurable parameters that have been adopted in order to determine the ideal training intensity. Yet, to circumvent the difficulty required of specific equipments for the determination of VO_2 max, some researchers have applied arbitrary velocities (1,14,17,18,21) while others have used blood lactate threshold (LT) (8,10,26,28). Thus, the accumulation of lactate observed during a graduated exercise test is usually interpreted as indicative of augmented contribution of the anaerobic metabolism as the main energy source (25,27).

The use of VO_2 max test is often impractical due to the high costs of the equipment in rat research. Also, given that one of the strategies used in similar studies is the evaluation of the concentration of blood lactate, the invasive technique itself often acts as a confounding variable due to the stress, waste, and trauma that leads to rejecting the exercise. Instead, researchers can use the correlations of the metabolic parameters with the use of incremental and constant tests (which are easily applied).

Some interesting correlations have already been made that correspond to a maximum lactate steady-state (MLSS) (13,20) and the value of 60% in the incremental load test (ILT) without collecting blood (12). This approach makes the application of the ILT as an important means to deriving a training prescription. The characteristics of these tests are distinct; one is a constant test and the other is an incremental test. Thus, the purpose of this study is to apply the ILT with the evaluation of blood concentrations of lactate to determine its relation with MLSS and 60% in the ILT with the expectation to applying the findings to the prescription of an endurance training program for rats.

METHODS

Animals

Twelve male Wistar rats were kept in a light/dark cycle (12 hrs/12 hrs) with water and a commercial diet ad libitum. At 60 days of age, the rats began a phase of adaptation in the treadmill for 2 wk, 5 times per wk at 0.4 $km \cdot h^{-1}$ during 10 min. After this stage, the animals were separated into 2 randomized groups: a control group (n = 6) and a trained group (n = 6).

Blood Lactate Concentration

Capillary blood samples (25 µL) were taken from the caudal vein. The rats was kept running while blood was being harvested gently. Blood was transferred to 1.5 mL tubes containing 50 µL of sodium ?uoride (1%). The lactate concentration was analyzed via an electro enzymatic method with a lactate analyzer (YSI 2300 Stat Analyzer; Yellow Springs Instruments, Yellow Springs, OH, USA).

Constant Load Test

Before the constant-load test was applied, the ILT was performed without taking blood samples. Then, 12 rats were submitted to a subsequent constant-load tests performed with intensities varying from 0.9 to 1.5 $km \cdot h^{-1}$. Blood samples (25 µL) were taken from the caudal vein each 7 min for further measurements. The highest workload that could be sustained over 28 min of running without lactate accumulation (blood lactate varying by less than 1 $mmol \cdot L^{-1}$ from 7 to 28 min) was considered the MLSS (2,15). The MLSS was calculated as the average lactate concentration measured at 7, 14, 21, and 28 min of the test. The interval between each test was 48 hrs.

Incremental Load Test

After aerobic exercise training, 12 rats were subjected to a maximum intensity test, which consisted of adding 0.2 $km \cdot h^{-1}$ at 3-min intervals with 0% incline until exhaustion (i.e., when the rat could no longer respond to mechanical stimuli applied by the researcher to maintain the physical activity). Blood samples (25 µL) were taken from the caudal vein every 3 min of running for further measurements. This test provided the total distance run and the peak workload.

Physical Training Protocol

The prescription of aerobic exercise training was designed from the ILT (60%), legitimized by the constant load test or MLSS (1.2 $km \cdot h^{-1}$). The animals trained on a treadmill (Atletic Speed 2, Atletic, Brazil), 5 days a wk for 6 wks. The training session was assembled: (a) warm-up period (5 min); (b) buffer zone (0-10 min); (c) the target zone (40 to 50 min); and (d) cool-down period (5 min).

Statistical Analysis

The data were analyzed by using the Graph Pad Prism software and graph package (V 4.0, Graph Pad Inc., San Diego, CA, USA). The findings are presented as mean ± standard error of the mean (SEM). Results were analyzed by a one-way analysis of variance (ANOVA) followed by a Tukey's post hoc test for comparison between three or more groups, and the Student's *t*-test for comparison between the two groups. A signi?cance level of P=0.05 was chosen for all comparisons.

RESULTS

Method for Determination of MLSS

The response of blood lactate concentrations over time in running rats (constant workload intensities ranged from 0.9 to 1.5 $km \cdot h^{-1}$). An apparent stabilization of lactate concentration was observed in running rats at 0.9 and 1.2 $km \cdot h^{-1}$, which corresponded to lactate changes by less than 1 $mmol \cdot L^{-1}$ after the 7 min of testing (Figure 1). At 1.5 $km \cdot h^{-1}$, lactate accumulation was observed over time and changes in lactate concentration exceeded 1 $mmol \cdot L^{-1}$ within 7 to 28 min of testing. Therefore, MLSS was determined at 1.2 $km \cdot h^{-1}$ and the lactate concentration achieved at this intensity was 2.32 ± 0.33 $mmol \cdot L^{-1}$ ($P<0.05$). In addition, the MLSS matched 60% of maximal speed achieved in the incremental exercise testing (Table 1).

Table 1. Parameters Evaluated Before and After Six Weeks of Aerobic Exercise Training: Time (min), Speed ($km \cdot h^{-1}$), and Distance (m).

Groups	All Groups **Before Training (n=12)**	Control **After Training (n=6)**	Trained **After Training (n=6)**
Time (min)	26.9 ± 1.04 min	26.2 ± 1.01 min	51.0 ± 2.0 min ***
Speed ($km \cdot h^{-1}$)	2.0 ± 0.08 $km \cdot h^{-1}$	1.9 ± 1.08 $km \cdot h^{-1}$	3.5 ± 0.10 $km \cdot h^{-1}$ ***
Distance (m)	416 ± 0.35 min	385 ± 0.12 min	1388 ± 0.76 min ***

***Significant difference between groups. Values are expressed as mean ± SEM and analyzed by the Student *t*-test with significance level ($P<0.0001$).

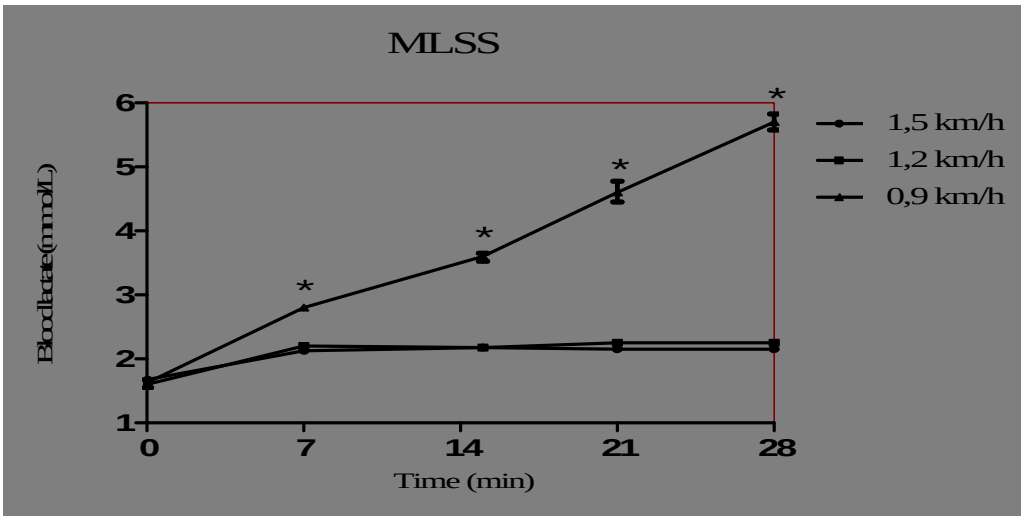

Figure 1. Time-course of alterations on blood lactate concentration assessed during submaximal constant load tests. The workload of the ?rst constant load test corresponded to 60% of maximal speed achieved during an incremental load test. Changes in blood lactate concentration between 7 and 28 min of constant load tests performed at different exercise intensities. Note that maximal lactate steady-state (MLSS) was attained at 1.2 km·h^{-1}, which was the highest workload at which blood lactate changed by less than 1 mmol·L^{-1} after 7 min of testing. Values are expressed as mean ± SEM and analyzed by one-way ANOVA (repeated measures) and a Tukey's post hoc test (*) ($P<0.05$) compared with 0.9, 1.2 and 1.5 km·h^{-1}.

Incremental load test

The time, speed, and total distance run during exercise training increased significantly after 6 wks when compared with the control group (Table 1), respectively, 78.62% (Figure 2A), 74.98% (Figure 2B), and 190.28% (Figure 2C) ($P<0.0001$). Data of the incremental load test demonstrated a significant difference in exhaustion when compared to rest (Figure 3A). The lactate concentrations in the control group at 27 min increased over 90.15% and in the trained group at 51 min to 206.66%. The lactate concentration at exhaustion in the trained group was significantly higher than the control group, which demonstrated an increase of 41.90% (Figure 3B) ($P<0.05$).

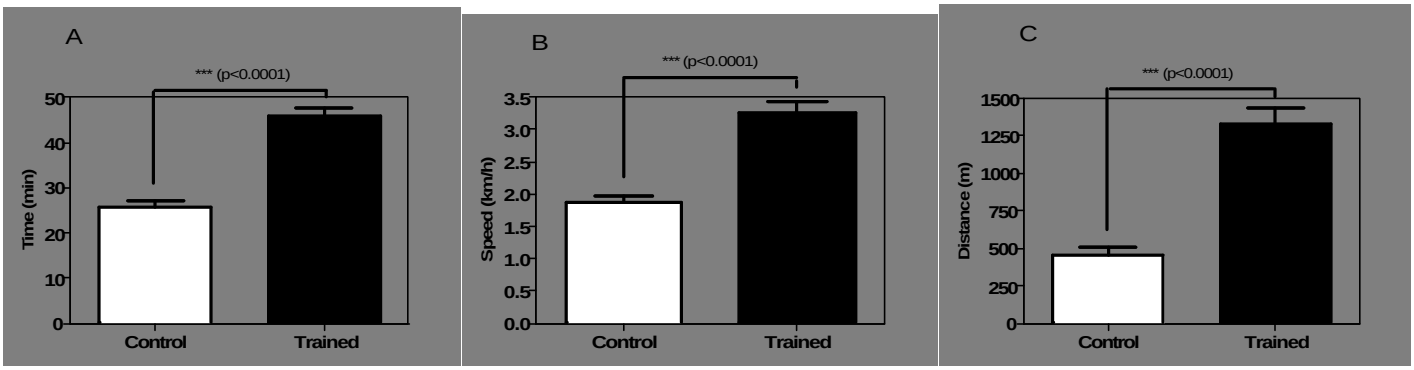

Figure 2. Effects of exercise training on (A) time, (B) speed, and (C) distance from the incremental load test (ILT) executed 48 hrs after the last training session. Control group (n=6) and trained group (n=6). ***Significant difference between groups. Values are expressed as mean ± SEM and analyzed by student *t*-test with a significance level at $P<0.0001$.

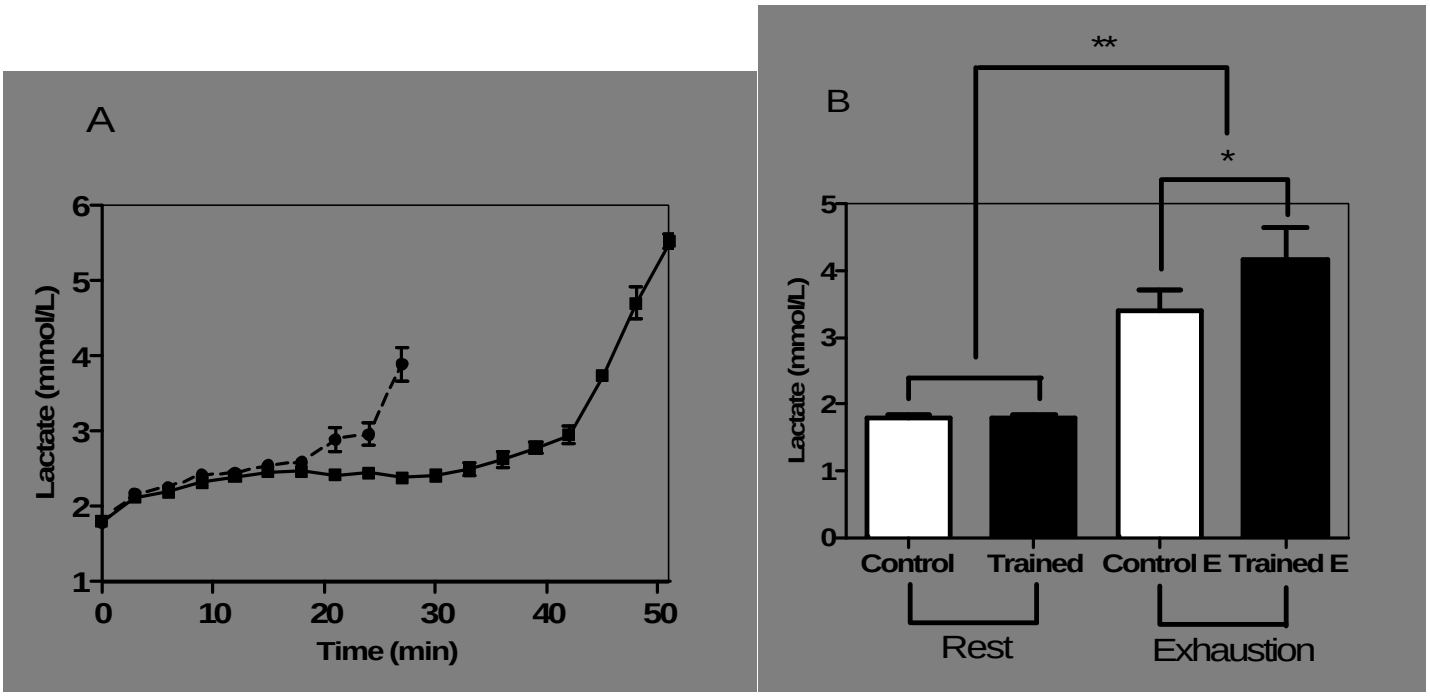

Figure 3. Effect of exercise training on blood lactate concentration (A) in the control (n=6) and the trained (n=6) groups, found in incremental load test. *Significant difference between groups. Values are expressed as mean ± SEM analyzed by a One-Way ANOVA (repeated measures) followed by Bonferroni post test with significance level set at P=0.05. Lactate (B) concentration values in rest and after exhaustion. *Significant difference between groups and **between times. Values are expressed as mean ± SEM and analyzed by a One-Way ANOVA followed by Tukey's post hoc test with significance level set at P=0.05.

DISCUSSION

This study provides a method for measuring endurance capacity based on the determination of lactate threshold by an incremental "aerobic exercise" load test (ILT) in rats. It is well established that MLSS is a good marker of endurance exercise capacity, and its determination is used to propose training programs for athletes (5). The results showed that MLSS obtained from consecutive constant-load tests was 1.2 km·h^{-1}, which occurred at 60% of maximal speed achieved in the ILT. Furthermore, exercise training increased running performance and lactate threshold.

The MLSS represents a balance between lactate transport to the blood and its removal from it (15). Billat and colleagues (7) used critical speed to assess endurance capacity in 2-month-old mice of different strains. Critical speed was determined by a regression line of a plot of the distance run and time to exhaustion in four constant-load runs (in a range of 18 to 51 m·min^{-1}). They reported that the critical speed of C57BL/6J mice was achieved at 18 m·min^{-1}.

The MLSS occurs at 60% of maximal speed achieved in an incremental exercise test, which enables its application in the prescription of aerobic exercise training and evaluation of endurance performance. Exercise training based on MLSS is known to reduce glycolytic rate, and it is associated with a minor reduction in muscle glycogen (along with an increase in the rate of fat oxidation and improved mitochondrial oxidation of pyruvate) at a given workload (3,4,6,11,24).

Carvalho and co-workers (20) showed that the kinetics of blood lactate concentration during exercise is modified by endurance training. According to Billat and co-workers (6), endurance training

increases the oxidation of fatty acids while decreasing glycogen breakdown in skeletal muscle during exercise. Metabolic plasticity of skeletal muscle tissue involves faster changes in enzyme activity via allosteric control as well as post-translational modifications that increase the control of mitochondrial content and substrate preference (16,19). In 1963, Randle and co-workers (22) demonstrated that glucose oxidation is decreased when plasma fatty acid (FA) availability is increased. According to Wende et al., (29) in order to match energy requirements with activity demands, muscle substrate utilization pathways must be tightly controlled. During intense bouts of exercise, the majority of the energy demand for muscle function is supplied by glucose. Following exercise, muscle glucose is spared via a shift towards mitochondrial fatty acid oxidation so that glycogen levels may be quickly replenished.

CONCLUSION

We found a consistent interrelation of the parameters used for the endurance training prescription for rats. The MLSS obtained from consecutive constant-load tests was 1.2 $km \cdot h^{-1}$ (as the target speed), which occurred at 60% of maximal speed achieved in the ILT. This shows that the ILT is a valid test with important metabolic significance for purposes.

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Address for correspondence: Phablo Sávio Abreu Teixeira (Msc), Institute of Biomedical Sciences, State University of São Paulo, São Paulo-SP, Brazil, Phone: +55 11 3091 7245; Email. phablofisio@gmail.com

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