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# Incremental Swimming Exercise Test: A Protocol to Evaluate Physical Performance in Rats

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## ABSTRACT

**Santos AB, Domanski E, Sulzbacher MM, Basso EGP, Goettems-Fiorin PB, Frizzo MN, Ludwig MS, Heck TG.** Incremental Swimming Exercise Test: A Protocol to Evaluate Physical Performance in Rats. **JEPonline** 2018;21(4):1-9. Animal models of swimming exercise have been used to investigate the effects exercise on many physiological variables in rats. The purpose of this study was to determine whether an incremental swimming exercise test (ISET) can discriminate trained and untrained rats. Eight 90 days old female rats were submitted to the ISET in individual tanks filled with 45 cm of water at 30°C. At the end of each 5-min stage, a weight was attached to the tail of the rats (0, 2, 4, 6, 8%, 10%, and 12% of body weight) and blood samples (~25 µL) were obtained to determine blood lactate concentration. Fatigue was considered when the rat was unable to swim with the nostril above the water surface for 10 sec. The trained rats improved the performance in the ISET (Time to fatigue: 24.4 ± 1.25 min vs. 27.48 ± 2.17 min, $P<0.05$). Also, trained rats showed lower levels of blood lactate concentration in each stage of the ISET and presented no alteration in lipid peroxidation in the heart, gastrocnemius, and soleus muscles. The data can be used in analyzing similar protocols and physiologic responses in future studies of swimming performance in rats.

**Key Words:** Lactate, Rat, Swimming Exercise Test

## INTRODUCTION

Animal models of swimming exercise have been used as a research strategy to investigate the effects of acute and chronic exercise on many physiological adaptations in rats (6,11,12). In this way, different swimming exercise protocols are used to describe the benefits of exercise training regarding oxidative stress (12). Also, it is an adequate model to show acute effects of exercise on redox balance (18). The purpose of the present study was two-fold. The first objective was to determine the role of the incremental swimming exercise test (ISET) in discriminating trained and untrained healthy rats. Second, the second objective was to determine the effects of the exercise training adaptations on oxidative stress markers after the ISET performance. The findings should be helpful in further understanding the use and application of the swimming performance test in rats.

When rats are submitted to a free swim session without loads attached in their bodies, there is a natural behavior of buoyancy that decreases exercise intensity during the exercise session. This effect may be prevented by inclusion of loads attached to the base of tail or thorax (4,9). Rats that swim without the addition of weight to the body or tail perform a moderate intensity exercise (~45 to 65% $VO_2$ max). When weights are attached to the base of the tail representing 4% of the rat total body weight, the animal performs at an exercise intensity of ~65 to 75% of $VO_2$ max and reaches a blood lactate concentration as ~5.3 $mmol \cdot L^{-1}$ (9). Since maximal lactate steady state for sedentary rats submitted to acute swimming exercise occurs at blood lactate concentrations of 5.5 $mmol \cdot L^{-1}$ (4), the measurement of blood lactate concentration represent an indicator of the endurance exercise capacity in rats.

The increase in exercise intensity is related to the increased formation of hydrogen peroxide, superoxide, and hydroxyl radical by successive electron additions to oxygen in the respiratory mitochondrial metabolism. In this way, exercise increases tissue rates of oxygen consumption, and thus, an acute exercise as a maximal exercise performance test may cause an increase in the heart oxidative stress biomarkers (10). On another hand, chronic exercise training modifies the pro-oxidant/antioxidant equilibrium and can modify the acute exercise response regarding oxidative stress parameters, avoiding oxidative stress (11,12,20). Thus, oxidative stress markers can be used as additional analysis to evaluate physiological adaptations to exercise training after an acute exercise performance test session.

This study was designed to propose a simple incremental swimming exercise test protocol to evaluate adaptations to exercise training in rats. The data obtained in our study may be applied as one protocol in future animal models of exercise physiology research.

## METHODS

### Animals

We used 8 female Wistar rats, aged 90 days, from the Animal Breeding Unit of Regional University of Northwestern State's Rio Grande do Sul (UNIJUI). The animals were kept in semi-metabolic cages, under controlled conditions of temperature (22 ± 2°C) and light-dark cycles (light from 7:00 a.m. to 7:00 p.m.). The animals received water and diet ad libitum. This protocol was approved Animal Ethics Committee of UNIJUÍ (017/15).

## Procedures

### *Adaptation to the Water*

All rats were adapted to the water before the beginning of the study. The adaptation protocol consisted of 3 consecutive days of keeping the animals for 10 min in shallow water at 30°C to reduce the rat stress behavior related to water contact.

### *Measurement of Exercise Intensity by Blood Lactate Concentration*

Caudal venous lactate concentrations were determined before and after exercise by a Lactate Analyzer (Accutrend®Plus System, Roche). The results were expressed as $mmol \cdot L^{-1}$ (5,6,8).

### *Incremental Swimming Exercise Test (ISET)*

Before and after the training period, blood samples were obtained for the lactate concentration measurement. The rats were submitted to the ISET in individual tanks filled with 245 cm of water at 30°C. At the end of each 5-min stage, a load increment of 0%, 2%, 4%, 6%, and 8%, 10%, and 12% of body weight of each rat was added to the tail. At the end of each stage, blood samples (~25 µL) were obtained from the caudal vein. The rat was considered fatigue when it was unable to swim with the nostril above the water surface for a 10-sec period. At that point the animal was removed from the water and dried. The duration of the test was recorded.

### *Exercise Training*

After the first swimming exercise test, 4 of the 8 rats started the training protocol. The remaining 4 rats were in the Sedentary Group. Rats in the Training Group swam 20 min with a 2% workload attached to the tail, $5\ d \cdot wk^{-1}$. In the following week, the animals swam for 20 min with a 4% workload. The swimming time was extended to 30 min in the 3rd wk, 40 min in the 4th wk and 50 min in the 5th wk. The tanks in which physical training was carried out presented a capacity of 50 L at a water temperature between 30 and 32°C. The swimming program followed the recommendations of the American Physiological Society (9).

### *Determination of Lipid Peroxidation*

After the last swimming exercise test, the rats were euthanized. The gastrocnemius and soleus muscles and the heart were removed from each rat and washed in saline solution and quickly frozen in liquid nitrogen. For the determination of lipid peroxidation levels (3), the tissues samples were homogenized (1:7 w/v) in 120 mmol KCl, 30 mmol sodium phosphate buffer (pH = 7.4) added with protease inhibitor 0.5 mmol PMSF (Phenylmethanesulfonyl Fluoride) at 0 to 4°C. The suspensions were centrifuged at 600 g for 10 min at 0 to 4°C to remove nuclei and cell debris. The pellets were discarded and the supernatants were used as homogenates. Homogenates were precipitated with 10% TCA, centrifuged, and incubated with thiobarbituric acid (T5500-Sigma) for 60 min at 100°C. TBARS were extracted using butanol (1:1 V/V). After centrifugation, the absorbance of the butanol layer was measured at 535 nm (6). The amount of TBARS formed was expressed in nanomoles of malondialdehyde per milligram of protein (Measured by Bradford method) (2). Malondialdehyde standard was prepared from 1,1,3,3,-tetramethoxypropane (Fluka, USA) (5,11,13).

## Statistical Analysis

A One-way ANOVA with repeated measures was used for the statistical analysis of the blood lactate concentration. Time to fatigue between the two groups was compared by the Student *t*-Test. The results are expressed in mean ± SD. The level of statistical significance was set at 5%. All statistical analyses were conducted using the SPSS (V.18) Software for Windows.

## RESULTS

Before the exercise training program got underway, animals from the Sedentary Group and the Trained Group presented a similar performance in the ISET. The rats swam until fatigue, which was ~24 min with no difference between the groups (P>0.05) (Table 1). After training, the rats in the Trained Group improved performance in the ISET, reaching ~28 min of swimming until fatigue; whereas, the Sedentary Group had no improvement in performance (Table 1).

**Table 1. Time to Fatigue in Incremental Swimming Exercise Test (ISET) in Rats.**

| | Time to Fatigue (min) | |
|---|---|---|
| | **Sedentary** (n=4) | **Trained** (n=4) |
| **Before Training** | 23.24 ± 0.57 | 24.00 ± 1.08 |
| **After Training** | 24.42 ± 1.25 | 27.48 ± 2.17* |
| **delta** | 1.18 | 3.48* |

All animals showed similar blood lactate concentration during the first ISET. As expected, the incremental workload attached to the tail promoted an increase in the lactate concentration without a significant difference between the groups before exercise training (P>0.05, Figure 1A). All animals in both groups before training reached the 8% workload until fatigue. After 5 wk of training, all rats in the Trained Group reached the 12% workload; whereas, only one rat from the Sedentary Group reached the 10% workload. The others rats (i.e., 3 of the 4) from the Sedentary Group reached fatigue at the 8% stage, which was a similar swimming performance in comparison with the 1st ISET (Figure 1B).

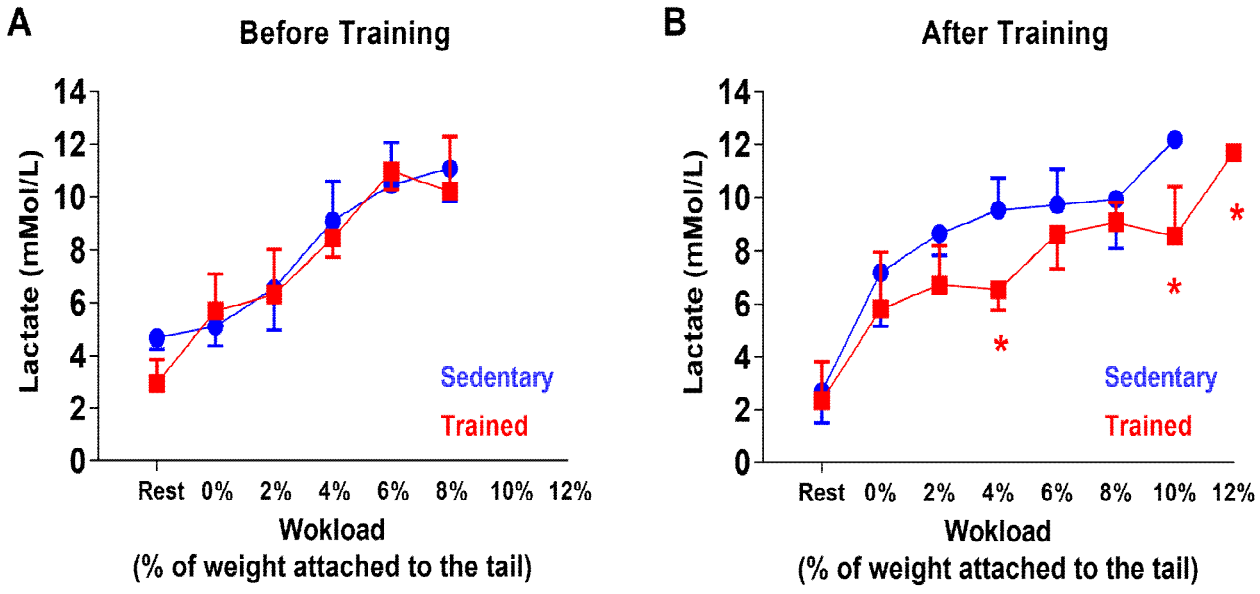


**Figure 1. Blood Lactate Concentration during Incremental Swimming Exercise Test in Female Rats Before and After Training.**

After the 2nd ISET, the rats' heart (Figure 2A), gastrocnemius (Figure 2B), and soleus (Figure 2C) were extracted to determine the lipid peroxidation levels of which no alterations in these tissues were found.

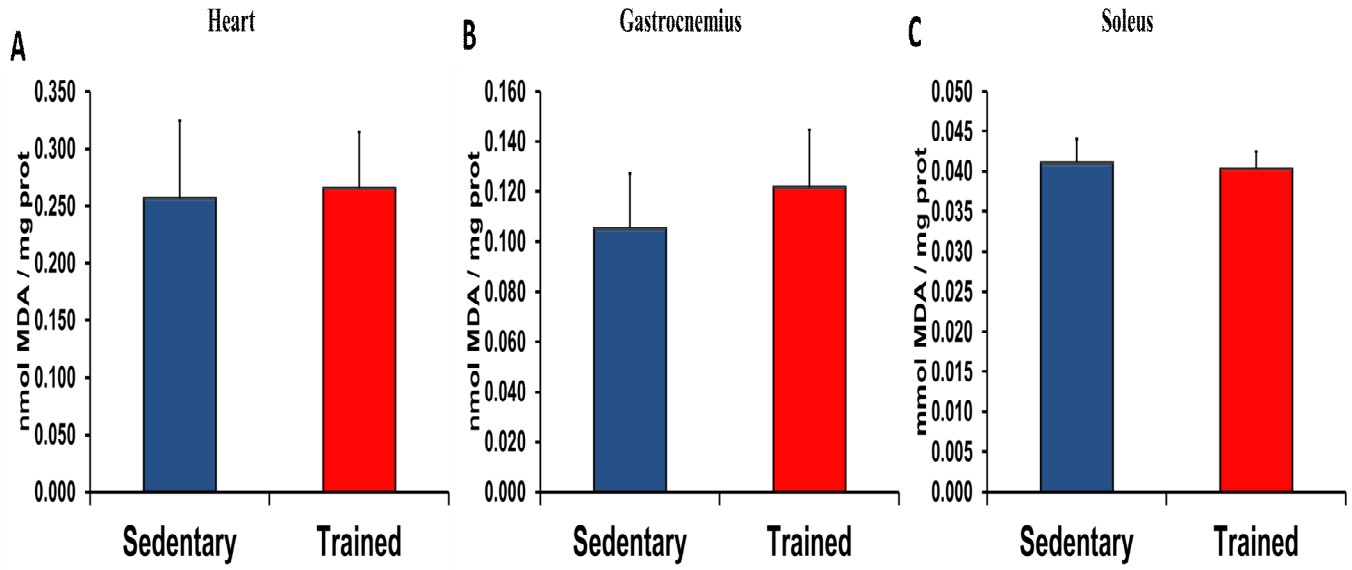


**Figure 2. Lipid Peroxidation Levels after Incremental Swimming Exercise Test in Tissues from Trained Female Rats.**

## DISCUSSION

The application of exercise tests in animal models represents a scientific opportunity for exercise physiologists to better understand the impact of incremental exercise programs on the physiology of performance. The extension of exercise physiology research worldwide is dependent on the availability and use of less expensive yet scientifically productive models. The present study is a good example of using a simple exercise performance test in rats. As used in this study, the Incremental Swimming Exercise Test (ISET) discriminated the trained rats from the untrained rats using three variables: (a) time until to fatigue; (b) blood lactate levels; and (c) oxidative stress markers. These findings should be helpful in future studies of swimming performance test in rats.

Today, there is a significant body of evidence to support the hypothesis that physical exercise has the potential to increase free radical production and lead to oxidative stress in many tissues and plasma. The oxidative stress is a result of high intensity or long duration exercise sessions that appears to lead to a high increase in free radical production that overwhelms the antioxidant defense (1,15,16). The present demonstrated in an animal model that moderate-intensity aerobic training is sufficient to improve the physical performance without modifying a parameter of oxidative stress in skeletal muscle and/or heart.

Exercise training promotes several protective effects in skeletal muscle by increasing the activity of antioxidant enzymes. Regular exercise training can result in an increase in both Cu/Zn superoxide dismutase (SOD1) and superoxide dismutase 2, mitochondrial (SOD2) expression by ~50% and a decrease by ~25% in the muscle carbonyl content, a marker of

oxidative protein stress in muscle. This effect is sufficient to protect the skeletal muscle against oxidative stress and protease activation (19). The muscle protection induced by regular exercise against oxidative stress is also attributed to the increase in cytoprotective molecules, as heat shock protein 70kDa (HSP70) (7,14)

Since reactive oxygen species (ROS) contribute to muscle fatigue, counteraction of the oxidative muscle stress is associated to improve exercise performance in experimental animal models and humans. Administration of antioxidant (e.g., N-Acetyl-cysteine) prevents oxidative stress in skeletal muscle and increases performance from 15 to 50% (15). Increased ROS concentration can affect calcium regulation flux and the myofilament function. Exposure of skeletal muscle fibers and isolated sarcoplasmic reticulum proteins to ROS reveals that regulatory proteins and calcium release channels are oxidized.

Additionally, almost half of the ROS effects involve members of the mitogen-activated protein kinase (MAP kinase) and nuclear factor κB (NF-κB) pathways, that are intracellular “signals” that can be inhibited by increasing in the muscle HSP70 expression induced by exercise (7). Also, the release of cytokines and HSP70 to extracellular milieu (eHSP70) are dependent on exercise intensity (6), and eHSP70 levels can be influenced by training (11). Thus, an exercise adapted organism may not increase cytokine and chaperokine fatigue signaling to the central nervous system during a maximal exercise effort, avoiding early fatigue behavior (7). Together these data can explain the adaptive response to exercise training observed in the present study (i.e., the increase in (muscle) performance without an increase in lipid peroxidation levels) (15).

In cardiac tissue, similar adaptations to exercise training can be observed. Exercise training based on a swimming protocol similar to the present study can result a positive hemodynamic response, increased anti-inflammatory cytokines levels, and improved the levels of muscular lipid peroxidation (decrease) (12). The lowest level of lipid oxidation in cardiac tissue can be observed in high-frequency moderate training (5 $d \cdot wk^{-1}$), which suggests that moderate exercise at this frequency induces no change or reduces myocardial oxidative stress. On the other hand, 1 $d \cdot wk^{-1}$ results in no antioxidant improvement or modification in SOD and catalase (CAT) activities. Thus, the routine of exercise at submaximal capacity prevents oxidative stress during a new exercise session (21).

Measurements of blood lactate during exercise provided information the energy necessary for the execution effort in the animals of our study. A maximum lactate steady state concentration is postulated between 3% and 6% of the workload, and a moderate intensity range of 60 to 75% of $VO_2$ max at a 4 to 4.6% workload (4,9,17). Additionally, an 8% workload may represent a high-intensity swimming exercise that is estimated to represent more than 90% of $VO_2$ max (6,22). Thus, assuming an energy equivalent of $O_2$ consumed as ~100 $mL \cdot kg^{-1} \cdot min^{-1}$ as rat $VO_2$ max in workload range from 6 to 8%, our ISET protocol may be considered adequate to represent a maximal effort test (6,9). Also, the exercise training progression used in the present study characterizes adequately the aerobic exercise training for rats.

## CONCLUSIONS

The findings in the present study support the use of the simple incremental swimming exercise test protocol to discriminate the training adaptations of trained rats from untrained rats. The trained rats improved the ISET performance (i.e., Time to Fatigue in minutes: 27.48 ± 2.17 vs. 24.42 ± 1.25, $P<0.05$). Also, the trained rats showed lower levels of blood lactate concentration

in each stage of the ISET and presented no alteration in lipid peroxidation in the heart, gastrocnemius, and soleus muscles. The data obtained in our study may be applied as one protocol in future animal models of exercise physiology research.

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