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Swimming Physical Training in Rats: Cardiovascular Adaptation to Exercise Training Protocols at Different Intensities

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ABSTRACT

Guerreiro LF, Pereira AA, Martins CN, Wally C, Gonçalves CAN. Swimming Physical Training in Rats: Cardiovascular Adaptation to Exercise Training Protocols at Different Intensities. **JEPonline** 2015; 18(1):1-12. There have been many studies of physical exercise in laboratory animal models. However, these studies are limited by their experimental protocols. The aim of the present study was to develop physical training protocols of low, moderate, and high intensity and to compare the cardiovascular acclimation of Wistar rats to these protocols. Forty male rats 60 days old at the beginning of the experiment and weighing 310 ± 10 g were divided into four groups (n=10): control, low intensity (LI), moderate intensity (MI), and intermittent high intensity (HI). The training was divided into an acclimation phase and a maintenance phase and took place 5 times a week over 16 wks. After the training, the animals were euthanized. Creatine kinase and heart morphometry analyses were conducted. Lactate values were 4.0 \pm 0.5 mmol L⁻¹ for LI, 5.5 \pm 0.4 mmol L⁻¹ for MI, and 9.2 \pm 1.0 mmol·L⁻¹ for HI. Heart rate was significantly reduced in the LI (339 \pm 20 beats min⁻¹) and MI (344 \pm 20 beats min⁻¹). Morphometric analyses showed that the thickness of the free wall of the left ventricle was greater in the HI group (2.233 \pm 0.29 mm) (P≤0.05). The thickness of the interventricular septum was greater in the HI group (2.158 \pm 0.71 mm) and the area of the left ventricle chamber significantly greater in the MI group (25.657 \pm 3.6 mm²) (P≤0.05). Our results show that these protocols induce different metabolic pathways and promote distinct adaptations in the cardiovascular system to the type of physical training experienced.

Key Words: Exercise, Cardiovascular, Swimming Intensity

INTRODUCTION

Studies on exercise physiology use animal models to simulate conditions of physical stress commonly observed in humans, with the aim of better understanding changes that occur in humans at the systemic, cellular, and molecular levels (3,5,27). Many studies of physical exercise in laboratory animal models have been conducted (14,15,20,22,28,30,31). However, these studies are limited by their experimental protocols. Creating physical exercise interventions in experimental physiology is not that simple because some factors, such as training duration, frequency, session duration, exercise intensity, and metabolism used during the physical activity, cannot be controlled (5). These factors make it difficult to simulate physical activity experienced by humans in experimental models (12). The transposition of experimental data and physical training protocols to experimental physiology is frequently inadequate, leading to misinterpretations in data before publication (5). Therefore, it is necessary that protocols for physical exercise in animals properly simulate the situations under investigation (18).

Swimming protocols are frequently used with rats, mainly because rats have an innate swimming ability and acclimate well to training, which can be done for little cost compared to treadmill running (17). Studies of swimming in rats have shown acclimations similar to those observed in humans (17,29). Studies using animal models should use physical exercise protocols that simulate situations to which humans are exposed daily, such as continuous aerobic exercise of low and moderate intensity, in addition to intermittent exercise of high intensity as observed in team sports. The intensity of the exercise can differentiate acclimation to training by determining the metabolic pathway, the energetic substrate and the energy source used, as well as the tension overload on the cardiovascular system (19).

Studies of aerobic and anaerobic metabolism have been developed to improve physical training in rats (6,10,17,23,25). The intensity of exercise can be controlled by the production, removal, and accumulation of lactate in the bloodstream. The maximal lactate steady state (MLSS) is the highest concentration of lactate and workload that can be maintained over time at the anaerobic threshold (12,16,23). MLSS intensity can be determined during continuous exercise, considering an exercise intensity equivalent to a concentration of 4 mmol·L⁻¹ of lactate in humans (4). Lactate concentrations at MLSS have been studied in rats and values of 5.5 nmol·L⁻¹ observed (6,17,23). Blood lactate levels higher than MLSS during physical exercise indicate that anaerobic metabolism predominates over aerobic metabolism (17,23). We hypothesized that the intensity of exercise can promote different adaptations in the cardiovascular system. The aim of the present study was to develop protocols for long-term physical training (swimming) in Wistar rats (*Rattus norvegicus*) and to compare cardiovascular acclimation to this training below (low intensity), at (moderate intensity), and above (intermittent high intensity) MLSS intensity.

METHODS

Subjects

All experiments were conducted in compliance with the Ethics Commission of Animal Use (Comissão de Ética em Uso Animal CEUA-FURG), process number 052/2011 and Guidelines on Animal Ethics and Welfare for Veterinary Journals. Forty male Wistar rats were used. The rats were 60 days old at the beginning of the experiment and weighed 200 to 300 g. The animals were kept in collective cages (5 animals/cage), fed a pelleted diet for rodents (15 g/rat/day) and given fresh water *ad libitum*, and maintained under a 12-hr light:dark photoperiod (lights on at 07:00) at a temperature of $22 \pm 2^{\circ}$ C.

Experimental groups:

Below MLSS intensity (Low Intensity [LI]):

This low-intensity swimming protocol took place in the individual swimming system (Figure 1) without load, for 60 min, 5 times·wk⁻¹, for 8 wks. The purpose of this methodology was to simulate exercise without controlling intensity by overload, thus allowing the animals to exercise daily at an intensity below MLSS. This protocol aimed to simulate common human physical activity, such as walking or running at low intensity, with controlled frequency and duration.



Figure 1. Swimming Tank. (A) Swimming tank for control, low, and moderate intensity protocols. **(B)** Swimming tank with pulled system for high intensity protocol. Internal cylinder submerged to intermittent swim, and pulled out to recovery, each movement lasting 15 sec (swim/recovery).

MLSS Intensity (Moderate Intensity [MI])

This swimming protocol, which also took place in the individual swimming system (Figure 1), comprised 60 min of swimming, 5 times wk⁻¹ with a workload equivalent to 5% of the animal's body weight. The workload was provided by pieces of lead attached to the back of the animal and was equivalent to a moderate MLSS intensity.

Above MLSS Intensity (Intermittent High Intensity [HI])

The rats in this group were submitted to intermittent individual swimming up to 60 min, 5 times wk⁻¹ for 16 wks. Each 60-min session alternated periods of vigorous swimming for 15 sec with a workload equivalent to 15% of the animal's body weight at intensities above MLSS followed by recovery for 15 sec. The entire session comprised 30 min of intermittent vigorous swimming and 30 min of rest.

No-Training Control (C)

The control animals were placed in a liquid environment to simulate the stress under experimental conditions for 1 min, 5 times wk⁻¹ for 16 wks.

Procedures

Training Tanks

The physical training period lasted for 16 wks. The animals exercised 5 times wk⁻¹ between 19:00 and 22:00, respecting the animals' natural active period. The continuous swimming system used in the LI and MI conditions consisted of a rectangular tank (90 cm width \times 100 cm length \times 80 cm height) filled with water up to 50 cm. Ten polyvinyl chloride (PVC) pipes 20 cm wide and 50 cm long were placed inside the tank. The water temperature was kept close to 32°C. The animals were able to exercise

individually, each in a different PVC pipe. The intermittent swimming system used in the HI condition consisted of a 60-cm wide cylinder covered at the bottom with mesh-galvanized aluminum and containing 10 PVC pipes. The cylinder, which was on pulleys, was immersed in the tank described previously for 15 sec, forcing the animals to swim vigorously. Then the cylinder was lifted by the pulleys, allowing the animals to recover for 15 sec outside of the water (Figure 1).

Training Protocol

The 16-wk swimming program to which the animals submitted comprised two phases: progressive acclimation and maintenance (Figure 2A and 2B). In the first 8 wks, the rats acclimated to various intensities of exercise. In Week 1, the animals were progressively acclimated to the liquid environment for 20 min, after which the training load and duration of swimming increased progressively (from 20, 25, 30, and 40 min in Week 1 to 40, 45, 50, 55, and 60 min in Week 2). Starting in Week 2 swimming loads were applied (1% for MI and 6% for HI, increasing progressively to a maximum of 5% [MI] and 15% [HI] in Week 8). This phase lasted for 8 wks.



Figure 2. Training Program Protocol. This Protocol last 16-wk and is divide into two periods: 1. Progressive Acclimation (8 wks) and 2. Load Maintenance (8 wks) (n=10/group). **(A) Progressive Acclimation and Maintenance to the Workload** (% of body weight – BW). **(B) Progressive Acclimation and Maintenance to the Total Volume of Exercise** (Time in minutes). **LI:** low-intensity swimming, without workload. **MI:** moderate-intensity swimming with load equal to 5% BW. **HI:** high-intensity swimming with load equal to 15% BW.

After this initial acclimation phase, once the animals were able to swim for 60 min, the animals were acclimated to different intensities of swimming (Figure 2A, 2B). This phase was characterized by the maintenance of higher loads and longer duration of exercise (60 min and a workload of 5% [MI] or 15% [HI]) for 8 more weeks. The load was adjusted weekly according to the animal's body weight.

Determination of Lactate Levels

Lactate levels was determined by analysis during exercise. Animals in all groups were removed from the water 10, 20, 30, and 40 min into the exercise session and blood samples (25 μ I) collected according to Voltarelli et al. (29). The lactate concentration was determined in mmol·L⁻¹ with a lactate analyzer (Accutrend).

Determination of Muscle Damage

Creatine kinase levels as determined using a Labtest Diagnóstica SA specific kit were used as markers of muscle damage. The dosage was defined based on the enzymatic system with final point reaction in serum samples from the animals by spectrophotometry expressed in U/L (8).

Blood Pressure and Heart Rate Measurements

Blood pressure and heart rate were measured using a cuff-tail plethysmograph (Leitica 5001). The rats were contained in a properly heated system at 30°C to facilitate caudal artery vasodilation, from which systolic and diastolic arterial blood pressure and heart rate were measured based on arterial pulse pressure. Measurements were taken once every 30 days for 16 wks.

Heart Morphometric Analysis

The animals were euthanized by decapitation and their hearts removed by the end of the study. Hearts were weighed and processed histologically by hematoxylin and eosin staining. Afterward, the slides were photographed using a digital camera coupled with a stereo microscope. The thickness of the free wall of the left ventricle (mm), the thickness of the interventricular septum (mm), and the total area of the left ventricle chamber (mm²) were measured using the software *Image J*.

Statistical Analyses

The data are expressed as means \pm standard deviations (M \pm SD).Tests for the normality of the data distribution (Kolmogorov–Smirnoff test) and homoscedasticity of variances (Levene's test) were performed to verify the assumptions of the analysis of variance. Parametric data were analyzed using analysis of variance followed by Tukey's post hoc test, and nonparametric data were analyzed using the Kruskal–Wallis test. The statistical significance was set at P≤0.05.

RESULTS

The swimming training lasted 16 wks, and in that time, no stress behavior (e.g., cannibalism, aggressiveness, or mortality) was observed. The MI and HI groups at maximum load (5% and 15%, respectively) reached the maximum volume of swimming (60 min) at the end of the progressive acclimation period (Week 8) and sustained that load and volume until the end of the maintenance period (Week 9; Figure 3). The C animals had a lactate concentration of $2.5 \pm 0.4 \text{ mmol} \cdot \text{L}^{-1}$ during the MLSS test. The LI group had a concentration of $4.0 \pm 0.5 \text{ mmol} \cdot \text{L}^{-1}$, which was not significantly different from C. The MI group had a blood lactate concentration of $5.5 \pm \text{ mmol} \cdot \text{L}^{-1}$, which was significantly higher than C (P≤0.05). The HI group had a blood lactate concentration of $9.2 \pm 1.0 \text{ mmol} \cdot \text{L}^{-1}$, which was significantly higher than all other groups (P≤0.05; Figure 3).



Figure 3. Lactate Levels after the Progressive Acclimation Period (Weeks 9) at 10, 20, 30, and 40 min of Exercise. C: control. LI: low-intensity swimming, without workload. MI: moderate-intensity swimming, 5%. HI: high-intensity swimming, 15%. Data are means \pm SD (mg·dL⁻¹). Different letters indicate significant differences between groups at the 5% significance level.

The results for muscle damage were 320 ± 20 U/L for C, which was not different from LI (340 ± 69 U/L) or HI (380 ± 59 U/L). However, the MI group had a significant reduction in damage of 15% (250 ± 15 U/L) compared with the other groups (P≤0.05; Figure 4).



Figure 4. Creatine Kinase Values (U/L) at the End of the Load Maintenance Period (16 wks). C: control. LI: lowintensity swimming, without workload. MI: moderate-intensity swimming, 5%. HI: high-intensity swimming, 15%. Data are means \pm SD (mg·dL⁻¹). Different letters indicate significant differences between groups at the 5% significance level.

The results for cardiac morphometry showed that the thickness of the left ventricle free wall was greater (P≤0.05) in HI (2.233 ± 0.29 mm) compared with C (1.992 ± 0.29 mm) and LI (2.433 mm) (Figure 5A). The thickness of the interventricular septum was greater in HI (2.158 ± 0.71 mm) than in all other groups (P<0.05) (Figure 5B). Finally, the left ventricle chamber was significantly larger in MI (25.657 ± 3.6 mm²) than in C (19.551 ± 3.8 mm²), and HI (14.858 ± 3.7 mm²) had a significantly lower value than the other groups (P≤0.05; Figure 5C).



Figure 5. Heart Morphometry. (A) Left ventricle free wall thickness (mm). (B) Interventricular septum thickness (mm). (C) Left ventricle chamber area (mm²). **C:** control. **LI:** low-intensity swimming, without workload. **MI:** moderate-intensity swimming, 5%. **HI:** high-intensity swimming, 15%. Data are means \pm SD (mg·dL⁻¹). Different letters indicate significant differences between groups at the 5% significance level.

Heart rate was significantly reduced in the LI (344 ± 20 beats·min⁻¹) and MI (350 ± 20 beats·min⁻¹) groups compared with C (420 ± 25 beats·min⁻¹) and HI (378 ± 23 beats·min⁻¹; P≤0.05). Systolic and diastolic blood pressure values were not altered by any training protocol (Table 1).

Groups	Heart rate (beats-min ⁻¹)		Systolic Pressure (mmHg)		Diastolic Pressure (mmHg)	
Control	Initial 365 ± 26	Final 392 ± 22 ^ª	Initial 136 ± 31	Final 158 ± 29	Initial 107 ± 22	Final 105 ± 20
Low Intensity	360 ± 30	339 ± 15 ^b	155 ± 32	165 ± 24	118 ± 21	115 ± 22
Moderate Intensity	368 ± 18	344 ± 19 ^b	137 ± 23	150 ± 23	115 ± 16	110 ± 24
High Intensity	358 ± 23	378 ± 21 ^ª	162 ± 26	172 ± 31	115 ± 13	120 ± 23

Table 1. Heart Rate, S	ystolic and Diastolic Blood Pressure.
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Different letters a,b indicate significant differences between treatment groups at the 5% significance level.

DISCUSSION

The swimming training protocol investigated here was effective at developing cardiovascular alterations in response to physical exercise. The LI group had lactate levels below MLSS, supporting a low-intensity protocol. However, the rats in the MI group, which bore a workload equivalent to 5% of their body weight, had blood lactate levels (5.5 mmol·L⁻¹) similar to MLSS (17,29). These latter results are similar to those reported by Voltarelli et al. (29) using subthreshold workloads (4% of body weight) and threshold equivalent loads (5% of body weight).

Swimming training protocols with intensities above MLSS and using loads equivalent to more than 6.5% of body weight resulted in an increase in blood lactate production by means of the anaerobic glycolytic pathway. Such protocols are exhaustive and enable animals to perform only a few minutes of physical exercise. Because of this, experimental protocols of high-intensity intermittent training that biochemically simulates team sports are rare. Rats in our high-intensity (HI) group bore a maximum load of 15% of their body weight, resulting in an increase in blood lactate up to 11 mmol·L⁻¹. This was efficient at promoting intense predominantly anaerobic exercise above MLSS during a training volume of 60 min, which is equivalent to the practice of team sports (4,7,11).

The results of the present study show that aerobic exercise at intensities below MLSS (LI) and at MLSS (MI) reduces resting heart rate after 16 wks. Such bradycardia may be due to a possible increase in sympathovagal balance after continuous training, as confirmed by the classical literature on sport science (24,26,27). According to the data presented here, the training protocols did not have any additional effects on systolic or diastolic pressure (which is understandable, as the rats used in the present study were normotensive), confirming the efficiency of acclimation to progressively greater loads.

Cardiac remodeling is a physiological condition that is a morphological consequence of systematic physical training involving an increase in maximum cardiac production, increase in systolic volume,

and decrease in resting heart rate (9). Volume overload and an increase in filling pressures result in an increase in elongation strength on the myocardium (1). The present training protocols resulted in two very distinct types of cardiac remodeling. The continuous aerobic swimming of moderate intensity (MI protocol) increased the left ventricle chamber area, promoting eccentric hypertrophy. Eccentric hypertrophy is an elongation of the cardiac muscle fibers of the left ventricle chamber caused by increased blood flow due to aerobic exercise, which increases blood demand in the left ventricle hemodynamics (13). Similar to the results of the present study, Evangelista et al. (15) found an increase in the cardiac chambers using a moderate protocol of two daily sessions over 4 wks and without overload.

In contrast, the intermittent high-intensity swimming (HI protocol) resulted in an increase in the thickness of the left ventricle wall and interventricular septum, leading to concentric hypertrophy. Because of the post-load increase, the highest intarventricular pressure during the ejection phase in high-intensity exercise can increase stress on the myocardium wall (1,13,21), with a consequent increase in wall thickness, characterizing concentric hypertrophy (2). This cardiovascular adaptation is similar to that observed in our HI group and is determined by the elevated MLSS values observed here. In line with the present results, Kemi et al. (22) investigated high-intensity training on a treadmill for 2 hrs a day over 8 wks and reported that animals developed concentric cardiac hypertrophy with an increase in the thickness of the posterior wall of the left ventricle and an increase in the thickness of the posterior wall of the left ventricle and an increase in the thickness of the interventricular septum.

Many studies have found concentric hypertrophy in the hearts of trained rats and mice, although their training protocols are controversial. For example, studying low-intensity but high-volume and highfrequency exercise (90 min of swimming for 6 wks), Evangelista et al. (14) demonstrated concentric hypertrophy in rats. Lemitsu et al. (20) observed that rats exercised on a treadmill at moderate intensity for 8 wks increased the mass index and the thickness of the left ventricle wall, indicating that moderate-intensity exercise causes concentric cardiac hypertrophy. Using a protocol of moderate loads of 5% body weight but of long duration, Sun et al. (28) also found concentric hypertrophy. Authors who observed that moderate- and low-intensity physical training can promote concentric hypertrophy could be misinterpreting their results if they compare these protocols to high-intensity training. Even though low- and moderate-intensity workloads were used, they were used over 8 wks without progressive acclimation, with sessions 120 min in duration, and up to 2 times d⁻¹, characterizing an exhaustive effort. Indeed, Woodiwiss and Norton (31), analyzing the hearts of rats submitted to volunteer exercise at running wheels in their cages, found physiological hypertrophy associated with an increase in the left ventricle wall, concluding that low-intensity exercise might lead to concentric hypertrophy. However, the authors did not control the intensity of the physical exercise, and the exercise alternated between moments of high and low activity followed by rest, similar to intermittent high-intensity anaerobic exercise.

The swimming protocols tested here promoted cardiac development compatible with physiological cardiac hypertrophy, but the manner in which the remodeling happened varied according to the intensity, volume, and frequency of the physical exercise, which was eccentric in the MI training and concentric in the HI training.

CONCLUSIONS

The protocol of swimming physical training for Wistar rats validated in the present study is a proper method of studying experimental sport science, considering the responses to continuous aerobic exercise of low and moderate intensity as well as high-intensity intermittent anaerobic exercise. The protocol induced different metabolic pathways (according to the MLSS levels observed) and promoted distinct myocardium hypertrophy, bradycardia, and no change in blood pressure in normotensive rats. The success of this protocol is found in its progressive acclimation period, in which animals are acclimated to increasingly greater swimming loads and volumes in such a manner as to guarantee that they can be trained without significant muscle damage, fatigue, or mortality. Therefore, the use of this protocol is suggested because of its safety in terms of training intensity levels. We urge future experimental studies in sports science to properly define all protocols regarding adequate acclimation to loads and exercise volume, therefore avoiding misinterpretations.

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