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Hemodynamic Responses during an Incremental Swimming Exercise Test in Rats

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ABSTRACT

Nunes RB, Heck TG, Alves JP, Dal Lago P. Hemodynamic Responses during an Incremental Swimming Exercise Test in Rats. **JEPonline** 2015;18(3):55-62. The aim of this study was to test the cardiovascular responses during an incremental swimming test. Rats were submitted to 25 min of incremental swimming exercise test in individual tanks filled 45 cm with water at 30°C. At the end of each stage (5 min duration each), ~25 µL of blood were obtained from the caudal vein to evaluate lactate concentration and a load increment was added to the tail (0, 2, 4, 6, and 8% of body wt). Simultaneously with exercise, hemodynamic data were recorded by a catheter filled with saline implanted (24 hrs before) into the carotid artery for direct arterial pressure measurements. Our data show that sedentary rats are able to keep a stable blood lactate concentration in workloads up to 6% of body wt. It is interest that the rats' heart rate decreased with the progression of the intensity and duration of the swimming session that was associated with a slight increase in systolic blood pressure, diastolic blood pressure, and mean blood pressure. The findings offer insight into the rats' cardiovascular responses during an incremental swimming exercise test.

Key Words: Incremental Test, Swimming Exercise, Hemodynamic Responses, Rats

INTRODUCTION

Exercise is a multifactorial activity that affects every organ and tissue in the body (4). Not only does exercise contribute to many health benefits, but lack of exercise is implicated in many chronic health problems (10). Cardiovascular improvement by exercise training has been extensively demonstrated in human, with or without preexisting disease. Today, more so than decades ago, exercise is recognized as medicine. It is not only an efficient low-cost approach to cardiac rehabilitation (9,14,15), it provides therapy for many diseases and disabilities. This is why it important to further research the benefits of exercise with animals, specially laboratory rats, in order to provide an increased understanding of the physiological alterations and mechanisms induced by acute and/or chronic exercise (8).

Benefits to the cardiovascular system are constantly studied in different exercise protocols in rats, like treadmill running (3), voluntary wheel running (1), and swimming (2,11). Rodents have the innate ability to swim. Hence, swimming exercise in rats is a method frequently used in exercise physiology and behavior research (12,13). Although swimming requires less expensive and less elaborate equipment than treadmill and spontaneous wheel running, the investigator must carefully select the container in which rats will swim, as well as the temperature and depth of the water. In addition, when performed correctly, swimming can provide a more uniform type of physical exercise that avoids the “stop and go” behavior such as found in treadmill protocols (8).

In humans, heart rate (HR) is used to prescribe the intensity of aerobic exercise because of its relationship with oxygen consumption (VO_2). In swimming protocols in rats, the assessment of blood lactate levels is a physiological measure that is usually used as an indication of exercise intensity (5). The fact that swimming protocols for rats do not have cardiovascular parameters described as is common with human subjects suggest the need to do so, especially since many studies use this method to investigate physiological adaptations of exercise training.

METHODS

Animals

Seven male Wistar rats aged 90 days from the Animal Breeding Unit of Universidade Federal de Ciências da Saúde de Porto Alegre (UFCSPA) were studied. The animals were kept in plastic cages (47 cm x 34 cm x 18 cm) under controlled humidity (75 to 85%) with a temperature of 22 ± 2°C, a dark-light cycle of 12 hrs with lights on from 7 am to 7 pm. They were fed a conventional laboratory diet (Supra-lab, Alisul Alimentos S.A, Brazil) with water ad libitum. All protocols were approved by the Universidade Federal de Ciências da Saúde de Porto Alegre (UFCSPA) Ethical Committee for Research (CPA 017/05).

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 10 min in shallow water at 30°C to reduce the rat behavior stress related to water contact. Physical training adaptations were not allowed with this intervention (6,7).

Measurement of Exercise Intensity by Blood Lactate Concentration

Caudal venous lactate concentrations were determined before and after exercise by Lactate Analyzer (Accutrend®Plus System, Roche). The results were expressed as $mmol \cdot L^{-1}$ (6,7).

Hemodynamic Data Acquisition and Analysis

One catheter (PE-10) filled with 0.06 mL saline was implanted in anesthetized rats (85 mg·kg^{-1} ketamine and 15 mg·kg^{-1} xylazine) into the carotid artery for direct arterial pressure measurements. After surgery the rats were separated in individual cages and received food and water ad libitum. At the day of experiment the arterial cannula was connected to a strain-gauge transducer (P23Db, Gould-Statham), and blood pressure signals were recorded by a microcomputer equipped with an analog-to-digital converter board (CODAS, 2-kHz sampling frequency; Dataq Instruments, Inc). The recorded data were analyzed on a beat-to-beat basis to quantify changes in systolic arterial pressure (SAP), diastolic arterial pressure (DAP), mean arterial pressure (MAP), and HR (11). The hemodynamic data were also evaluated during the exercise test. We used the following surgery procedure: Dopalen® (Ketamine 1.16 g·10 mL^{-1}, Agribrands Ltda, Brazil) and Anasedan® (Xylazine 2.3 g·100 mL^{-1}, Agribrands Ltda, Brazil).

Experimental Design of Incremental Swimming Exercise Test

One day after the water adaptation period, the carotid of the rats was cannulated (as described above). Twenty-four hours after cannulation of the rats, resting hemodynamic data were recorded and blood samples were obtained for lactate concentration measurement. The rats were submitted to 25 min of incremental swimming exercise test in individual tanks filled 45 cm with water at 30°C. At the end of each stage (with 5 min duration each), a load increment was performed, adding weight to the tail 0, 2, 4, 6, and 8% of body wt of each rat). At the end of each stage, blood samples (~25 µL) were obtained from caudal vein. The hemodynamic parameters were recorded simultaneously during exercise.

Statistical Analysis

One-way ANOVA followed by Student-Newman-Keuls *post hoc* test were used for the statistical analysis in blood lactate concentration and hemodynamic parameters (SAP, DAP, MAP, and HR). The results are expressed in mean ± standard deviation. The level of statistical significance was set at 5%. All statistical analyses were conducted using the SPSS (V.18) Software for Windows.

RESULTS

All data measured during the swimming exercise test protocol are represented in Figure 1. Animals presented a progressive increase in blood lactate concentration during the swimming exercise test in response to the incremental workloads. Blood lactate in the 6% and 8% overload stages presented higher levels when compared to the first stages of the incremental swimming test. These levels remained higher than the rest values in both the 2-min and the 5-min stages of recovery after exercise ($P<0.001$). When compared to the rest condition, HR increased during the first minutes of the swimming exercise without a load (represented by 0%). Then, HR decreased continuously during the swimming test (when weight was added to the tail), but remained above the resting HR until the 6% overload stage. In the 8% overload stage and in the first 2 min of recovery HR was not different from the resting values ($P=0.942$). The start of exercise session results in higher levels of SAP compared to the rest condition ($P=0.04$). Simultaneously, the SAP, DAP, and MAP were not influenced during the incremental exercise test.

Our data showed a negative correlation ($r = -0.249$; $P=0.047$) between blood lactate concentration and heart rate during all protocol, analyzing all stages of the swimming test. During the exercise test (excluding the rest and recovery values from correlations analyses) there were a strong negative correlation ($r = -0.672$; $P<0.001$) between HR and blood lactate concentration (figure 2).

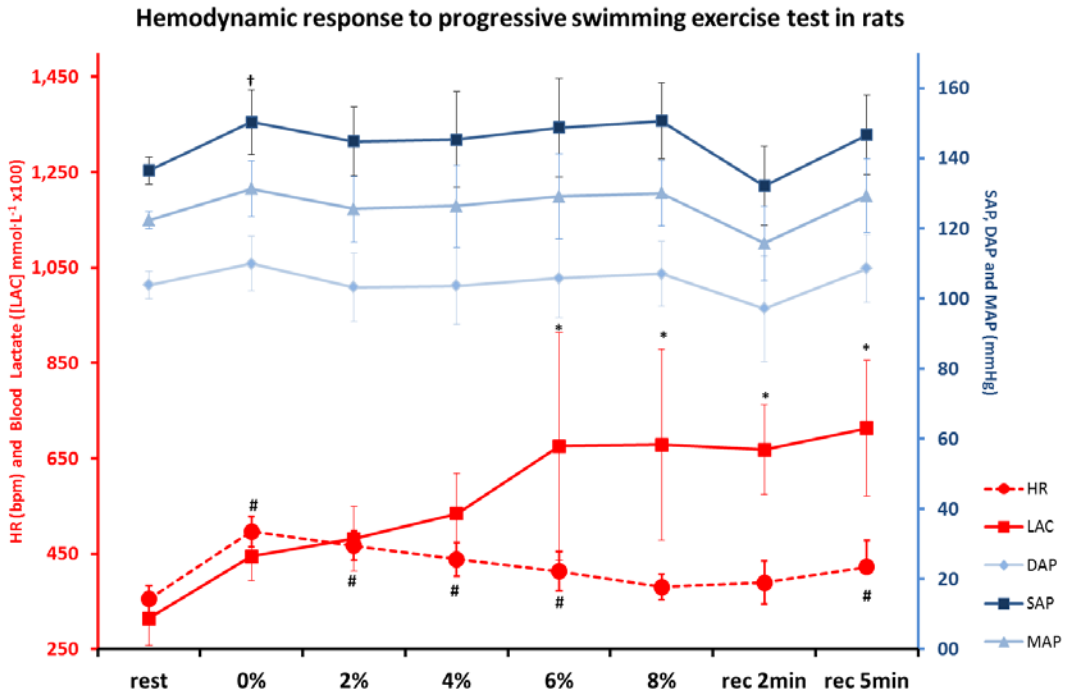

Figure 1. Hemodynamic Response to Progressive Swimming Exercise in Rats. The rats were submitted to 25 min of incremental swimming exercise test with hemodynamic (HR, SAP, MAP, and DAP) and lactate (LAC) data acquisition before (rest), during 5 workloads at 0%, 2%, 4%, 6%, and 8%, and after the exercise period (rec 2 min and rec 5 min). #P<0.001 compared with rest condition. †P = 0.04 compared with rest condition. *P<0.001 compared rest, 0%, 2% and 4% stages.

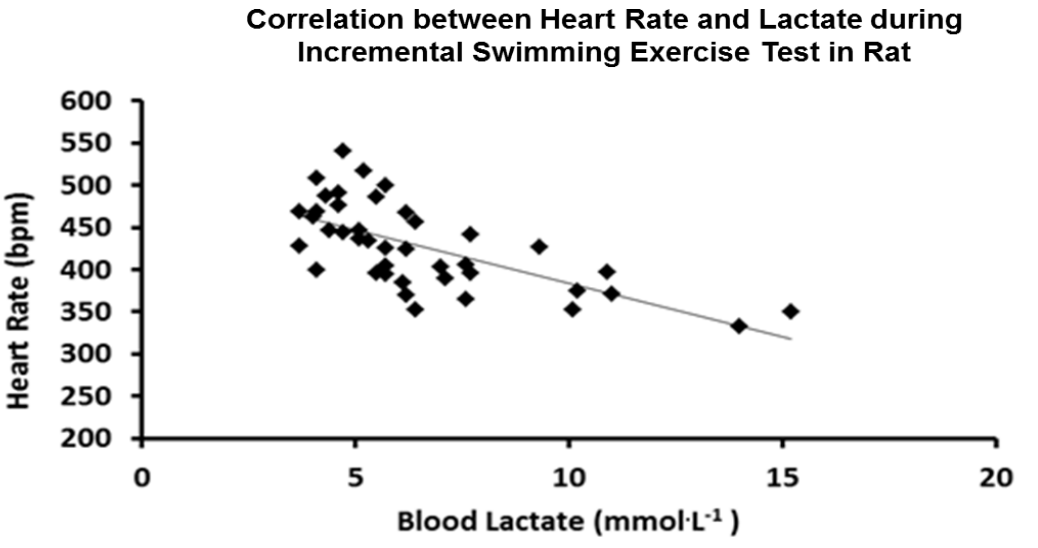

Figure 2. Heart Rate and Blood Lactate Concentration Correlation. Negative relationship between HR and blood lactate concentration during the 5 stages of incremental swimming exercise test expressed by a linear regression line (r = - 0.672) (P<0.001).

DISCUSSION

Swimming exercise in rats is used for purpose of physical conditioning and/or the analysis of acute physiological adaptations (14). While cardiovascular research with different swimming exercise protocols has examined novel molecular, biochemical, and pathological aspects of rats, the purpose of this study was to test the cardiovascular responses during an incremental swimming test in *rattus norvegicus*. The findings are expected to be of some help with the future studies of capability test of rats with hypertension or heart failure.

When we analyzed the hemodynamic responses during the swimming exercise, it is important to take into consideration water temperature, loads, swimming apparatus, muscle recruitment, and others stress factors. For example, the temperature of the water during the exercise test and the swimming apparatus can influence the hemodynamic response. That is why we proposed a swimming protocol with a water temperature at 30°C. Also, the frequency and duration of submerging during exercise are both a function of the number of animals that swim simultaneously. When animal swims without competition there is a decrease in exercise intensity that is prevented by loads attached to the base of tail.

While it is common for rectal temperature to increase in runners immediately after exercise, it is uncommon to see the same in rats. In fact, this does not occur in swimming rats during similar experimental conditions. Also, in human subjects, immersion results in a 50% increase in cardiac output at a temperature range between 30 to 34.5°C. The increase is the result of an increase in stroke volume more so than HR (15). These physiological adaptations may represent a beneficial treatment strategy to patients with chronic heart failure by producing a positive effect on the training response during warm water (3).

From a stress perspective, it is likely that animal exercise experiments may bias the physiological responses. Thus, acute swimming exercise is associated with the metabolic stress response of high serum adrenocorticotrophic and corticosterone hormones concentrations. Activation of the stress response leads to metabolic alterations such as the elevation of glucose, fat free acids, and lactate serum concentrations in animals that swam in relation to animals at rest. Also, the more intense the exercise the more pronounced the response in animals (4). In anticipation of the stress oriented influence, the present study minimized the potential for a confounding factor by scheduling three exercise sessions to help the rats adapt to the water before the exercise test.

Continuous swimming exercise produces a significant recruitment of both the forelimb and hindlimb muscles. Rats that swim continuously without the addition of weight to the body or tail do so at a metabolic rate of 2 to 3 METs with a VO_2 of 46 to 63 $mL \cdot kg^{-1} \cdot min^{-1}$, which is considered a moderate intensity of 45 to 65% of VO_2 max. When a weight equal to 4% of the rat's body weight is attached to the base of the tail, the intensity is increased to 65 to 75% of VO_2 max (11). At this intensity (4%), the rats in the present study reached a HR of ~438 $beats \cdot min^{-1}$ with a blood lactate concentration of 5.3 $mmol \cdot L^{-1}$.

Maximal lactate steady-state for sedentary rats submitted to acute swimming exercise occurs at blood lactate concentrations of 5.5 $mmol \cdot L^{-1}$ (9). This value represents the blood lactate concentration that is considered an indicator of the endurance exercise capacity. Our data show that sedentary rats are able to keep a stable blood entry/removal ratio in workloads up to 6% of body wt. The blood lactate concentration increased with higher loads, which indicated higher production in relation to removal. Thus, the loads below 6% of the body wt. can be considered 'sub-threshold'. Treadmill protocols use

lactate as intensity parameter as well, but the lactate levels for runners are substantially higher than in rats swimming after 60 min of exercise (7).

The rats' HR increased when they started to swim and, then, it decreased with the progression of the intensity and duration of swimming session. Heart rate increased from ~355 beats·min^{-1} at rest to ~496 beats·min^{-1} (~1.4 fold increased) during the first 5 min of exercise. Previously, we evaluated the hemodynamic parameters in rats during continuous 20 min of free swimming exercise without an overload and observed that exercise increased HR from ~370 beats·min^{-1} at rest to an average of ~432 beats·min^{-1} (~1.17 fold increased) (10). In the present study, the rats swan 5 min in each stage, and there was a decrease in HR after the first 5 min of exercise, despite the increase in the overload. The load attached to the tail was progressively increased from 2% to 4%, 6%, and 8% and the HR decrease progressively (467, 438, 413, 381 beats·min^{-1}, respectively). Since the mean HR during the first 20 min of exercise (i.e., from 0% to 6% stage) is similar to the rats that swam 20 min without an overload (~453 beats·min^{-1} vs. ~432 beats·min^{-1}, respectively), the time of the submersion and/or the adaptation to the environment influenced the hemodynamic response in the cannulated rat model.

The swimming exercise HR response in rats is different from the treadmill hemodynamic response (6), which may be related to the vagal effects of partial submersion. Additionally, the swimming induced increased in HR is markedly less than the HR response during running protocols (7). Both running and swimming had little effect on mean blood pressure response during exercise session in rats (7). In our study, the incremental exercise test shows slight increase in SAP, DAP, and MAP, similarly, to others protocols that showed MAP increases after 20 min of swimming exercise when compared with the rest condition. Curiously, 4 and 8 wks of training abolished these effects on blood pressure (7,10).

One comparative study (6) showed that HR and cardiac output increased only with treadmill exercise, while blood pressure remained stable during both protocols. Coronary blood flow increased only with treadmill protocol. Skeletal muscle flow, determined in six muscle groups, increased more in runners (97 to 587%) than swimmers (-44 to 260%, with marked decreases of blood flow in quadriceps group during the swimming exercise). Both protocols induce a decrease in blood flow to the skin and splanchnic regions and increases in cerebral regions. Blood gas analyses suggest lactic acidosis and hyperventilation only during running protocols. These findings indicate that the cardiovascular effects of acute, exhaustive bouts of swimming and running protocols for rats are not comparable (6).

CONCLUSIONS

Our data show that sedentary rats are able to keep a stable blood lactate concentration in workloads up to 6% of body wt. It is interest that the rats' HR decreased with the progression of the intensity and duration of the swimming session that was associated with a slight increase in systolic blood pressure, diastolic blood pressure, and mean blood pressure. The findings offer insight into the rats' cardiovascular responses during an incremental swimming exercise test protocol. We propose this protocol to provide a method for future studies about cardiovascular functions and analyses in chronic diseases rat models (such as hypertension and chronic heart failure). An incremental test may represent a diagnostic parameter for cardiovascular deficit during several heart diseases.

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References

1. Allen DL, Harrison BC, Maass A, Bell ML, Byrnes WC, Leinwand LA. Cardiac and skeletal muscle adaptations to voluntary wheel running in the mouse. ***J Appl Physiol.*** 2001;90(5): 1900-1908.

2. Arad M, Schwalb H, Mahler Y, Appelbaum YJ, Uretzky G. The effect of swimming exercise on spontaneous ventricular defibrillation and ventricular fibrillation threshold in the isolated perfused rat heart. ***Cardioscience.*** 1990;1(4):295-299.

3. De Angelis KL, Oliveira AR, Dall'Ago P, Peixoto LR, Gadonski G, Lacchini S, et al. Effects of exercise training on autonomic and myocardial dysfunction in streptozotocin-diabetic rats. ***Braz J Med Biol Res.*** 2000;33(6):635-641.

4. Gill JM, Cooper AR. Physical activity and prevention of type 2 diabetes mellitus. ***Sports Med.*** 2008;38(10):807-824.

5. Gobatto CA, de Mello MA, Sibuya CY, de Azevedo JR, dos Santos LA, Kokubun E. Maximal lactate steady state in rats submitted to swimming exercise. ***Comp Biochem Physiol A Mol Integr Physiol.*** 2001;130(1):21-27.

6. Heck TG, Nunes RB, Petry MR, Maslinkiewicz A, Saldiva PHN, Dal Lago P, Rhoden CR. ROFA Instillation promotes lung and heart oxidative stress without hemodynamic effects in exercising rats. ***JEPonline.*** 2014;17(1):78-89.

7. Heck TG, Petry MR, Maslinkiewicz A, Goettems-Fiorin PB, Ludwig MS, Saldiva PHN, Dal Ago P, Rhoden CR. Effects of ambient particles inhalation on lung oxidative stress parameters in exercising rats. ***JEPonline.*** 2014;17(3):58-69.

8. Kregel KC, Allen DL, Booth FW, Fleshner M, Henricksen E, Musch TI, et al. ***Resource Book for the Animal Exercise Protocols***. American Physiological Society, 2006.

9. Leung FP, Yung LM, Laher I, Yao X, Chen ZY, Huang Y. Exercise, vascular wall and cardiovascular diseases: An update (Part 1). ***Sports Med.*** 2008;38(12):1009-1024.

10. Mostarda C, Rogow A, Silva IC, De La Fuente RN, Jorge L, Rodrigues B, et al. Benefits of exercise training in diabetic rats persist after three weeks of detraining. ***Auton Neurosci.*** 2009;145(1-2):11-16.

11. Nunes RB, Tonetto M, Machado N, Chazan M, Heck TG, Veiga AB, et al. Physical exercise improves plasmatic levels of IL-10, left ventricular end-diastolic pressure, and muscle lipid peroxidation in chronic heart failure rats. ***J Appl Physiol.*** 2008;104(6):1641-1647.

12. Perrot-Sinal TS, Kostenuik MA, Ossenkopp KP, Kavaliers M. Sex differences in performance in the Morris water maze and the effects of initial nonstationary hidden platform training. ***Behav Neurosci.*** 1996;110(6):1309-1320.

13. Perrot-Sinal TS, Gregus A, Boudreau D, Kalynchuk LE. Sex and repeated restraint stress interact to affect cat odor-induced defensive behavior in adult rats. ***Brain Res.*** 2004;1027(1-2): 161-172.

14. Salvetti XM, Oliveira JA, Servantes DM, Vincenzo de Paola AA. How much do the benefits cost? Effects of a home-based training programme on cardiovascular fitness, quality of life, programme cost and adherence for patients with coronary disease. ***Clin Rehabil.*** 2008;22(10-11):987-996.

15. Yung LM, Laher I, Yao X, Chen ZY, Huang Y, Leung FP. Exercise, vascular wall and cardiovascular diseases: An update (Part 2). ***Sports Med.*** 2009;39(1):45-63.