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Effects of an acute swimming session until exhaustion in myocytes isolated from Wistar rats

Efeitos de uma sessão aguda de natação até exaustão sobre miócitos isolados de animais Wistar

Efectos de una sesión aguda de natación hasta el agotamiento en miocitos aislados de animales Wistar

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Maria Cecília Teles
ORCID: https://orcid.org/0000-0002-1490-7012
Federal University of Ouro Preto, Brazil
E-mail: maria.magalhaes@aluno.ufop.edu.br
Lucas Rogério dos Reis Caldas
ORCID: https://orcid.org/0000-0002-7155-6319
Federal University of Minas Gerais, Brazil
E-mail: lucasrrcaldas7@gmail.com
Luciana Fernandes Barros
ORCID: https://orcid.org/0000-0003-0018-8755
Federal University of Ouro Preto, Brazil
Email: luciana_sorrab@hotmail.com
Bianca Iara Campos Coelho
ORCID: https://orcid.org/0000-0002-3632-8978
Federal University of Ouro Preto, Brazil
E-mail: bianca.campos@aluno.ufop.edu.br
Franciany de Jesus Silva
ORCID: https://orcid.org/0000-0002-9836-9820
Federal University of Viçosa, Brazil
E-mail: francianysilva@hotmail.com.br
Mauro Cesar Isoldi
ORCID: https://orcid.org/0000-0003-4961-5332
Federal University of Ouro Preto, Brazil
E-mail: mauro.isoldi@ufop.edu.br

Abstract
The role of regular physical exercise is recognized for the prevention, control and treatment of cardiovascular diseases. However, studies show that after strenuous aerobic exercise sessions in healthy individuals can lead to cardiac damage, due to the high degree of stress imposed on myocardial structures. This study verifies the effects of swimming to exhaustion on contraction and relaxation velocities, as well as the velocity of calcium release and reuptake in cardiomyocytes isolated from the left ventricle. Wistar animals aged 16 weeks were submitted to a protocol of swimming until exhaustion with a load of 5% of body weight and later the cardiomyocytes were isolated. It was observed that an acute session of swimming until exhaustion promoted an increase in the velocity of contraction and relaxation and an increase in the velocity of calcium release. Exercise to exhaustion promotes adverse effects on the myocardium, however more studies are needed to explain these effects and demonstrate the molecular mechanisms involved in the process.
Keywords: Acute exercise; Myocardium; Swimming.

Resumo
O papel da prática regular de exercício físico é reconhecido para prevenção, controle e tratamento de doenças cardiovasculares. Entretanto, estudos mostram que após sessões de exercício aeróbio extenuante em indivíduos saudáveis podem levar a dano cardíaco, devido ao alto grau de estresse imposto sobre as estruturas do miocárdio. Este estudo tem como objetivo verificar os efeitos da natação até exaustão nas velocidades de contração e relaxamento, bem como a velocidade de liberação e recaptação do cálcio em cardiomiócitos isolados do ventrículo esquerdo. Animais Wistar com 16 semanas foram submetidos a um protocolo de natação até exaustão com carga de 5% do peso corporal e posteriormente os cardiomiócitos foram isolados. Foi observado que uma sessão aguda de natação até a exaustão promoveu aumento na velocidade de contração e relaxamento e aumento na velocidade de liberação do cálcio. O exercício até a exaustão promove efeitos adversos no miocárdio, entretanto mais estudos são necessários para explicar esses efeitos e demonstrar os mecanismos moleculares envolvidos no processo.
Palavras-chave: Exercício agudo; Miocárdio; Natação.

Resumen
Se reconoce el papel del ejercicio físico regular para la prevención, control y tratamiento de las enfermedades cardiovasculares. Sin embargo, los estudios muestran que después de sesiones de ejercicio aeróbico extenuantes en individuos sanos puede provocar daño cardíaco, debido al alto grado de estrés impuesto a las estructuras miocárdicas. Este estudio tiene como objetivo verificar los efectos de nadar hasta el agotamiento en las velocidades de contracción y relajación, así como la velocidad de liberación y recaptación de calcio en cardiomiocitos aislados del ventrículo izquierdo. Animales Wistar de 16 semanas de edad fueron sometidos a un protocolo de natación hasta el agotamiento con una carga del 5% del peso corporal y posteriormente se aislaron los cardiomiocitos. Se observó que una sesión aguda de natación hasta el agotamiento promovió un aumento en la velocidad de contracción y relajación y un aumento en la velocidad de liberación de calcio. El ejercicio hasta el agotamiento promueve efectos adversos sobre el miocardio, sin embargo, se necesitan más estudios para explicar estos efectos y demostrar los mecanismos moleculares involucrados en el proceso.
Palabras clave: Ejercicio agudo; Natación; Miocardio.

1. Introduction

The role of regular physical exercise is recognized for the prevention, control and treatment of cardiovascular diseases (Lee, 1993; Okely et al., 2021; Thompson et al., 2003). However, studies show that after strenuous aerobic exercise sessions in healthy individuals, they can elevate biomarkers related to cardiac damage, due to the high degree of stress imposed on myocardial structures (Contrepois et al., 2020; Middleton et al., 2008; Perk et al., 2012; Scharag et al., 2008; Shave et al., 2007, 2010).

Also, according to Elliott (2015), the accumulation of evidence suggests that acute episodes of prolonged strenuous exercise can cause transient cardiac dysfunction, including a reduction in the handling of calcium in cardiomyocytes, which leads to questions about the consequences of exercise to exhaustion for health. cardiovascular.

Oláh et al. (2015) demonstrated that the exhaustive physical activity has an adverse effect on the heart, where the observed functional impairment is associated with increased oxidative stress, increased apoptotic signaling and dysregulation of the metalloproteinase system.

Ljones et al. (2017) demonstrated that acute exercise is associated with a reduction in Ca^{2+} amplitude, an increase in diastolic Ca^{2+} removal time and reduced fractional shortening in the right and left ventricle, as well as an increase in right ventricular reluctance time. The expression levels of phosphorylated phospholamban (Ser16 and Thr17) increase in the left ventricle, but not in the right ventricle. Elevated levels of reactive oxygen and nitrogen species have been found in humans after excessive physical exercise (Radák et al., 2003; Vollard, Shearman & Cooper, 2005), the generation of reactive oxygen species after exhaustive exercise in rats causing damage to the myocardium has also been reported (Muthusamy et al., 2012).

The production of reactive oxygen species leads to protein and lipid peroxidation, DNA damage and cellular dysfunction, such processes lead to irreversible cell damage with consequent death, and these damages are also found in cardiovascular pathologies (Caniffi et al., 2020; Tsutsui, Kinugawa & Matsushima, 2011).

Therefore, the aim of study is to analyze whether there is a difference in the contraction and manipulation speeds of calcium in animals submitted to a protocol of swimming until exhaustion.

2. Methodology

Animals and exhaustion protocol

Wistar animals from the Animal Science Center of the Universidade Federal de Ouro Preto were used, which were kept in collective cages, where they received water and commercial food ad libitum, being kept in an environment with an average temperature of (25 ± 1° C) and cycle light - dark (12 h-12 h), from 4 weeks of age, and only at 16 weeks the experiments were carried out. The project was approved by the Ethics Committee for the Use of Animals (CEUA) of the Universidade Federal de Ouro Preto (opinion nº 10/2018).

The animals were randomly divided into two groups of 7 animals each: control (C) and exhaustion (E). All animals were submitted to adaptation to the liquid medium in a swimming pool, for three consecutive days and at alternate times, for 10 min of swimming (water temperature 32 ± 1º C) without overload. Before the swimming protocol, the animals were weighed for the preparation of loads, corresponding to 5% of the body mass, which was affixed to the animals' tails. When the animal was placed in the water, the stopwatch was started and the swimming time was recorded. When the animals could not rise to the surface of the water to breathe within a period of 10 seconds, they were removed from the pool in the supine position, if the position was maintained, it was considered exhaustion (Casimiro-Lopes et al., 2012). At the end of the exercise protocol, the animals in the exhaustion group were immediately euthanized by decapitation, while the animals in the control group were placed in water for 5 min and subsequently euthanized.

Isolation of Cardiomyocytes

After euthanasia, the heart was removed and left ventricular myocytes were enzymatically isolated as previously described by Natali et al. (2002, 2015). In this technique, after the heart is removed and weighed, it is mounted in a Langendorff system and perfused for 3-5 min with a calcium-free solution and then for another 15-20 min digestion with collagenase type II is performed and to finish the isolation is performed mechanical dispersion of the cells that are stored until analysis.

Cell contractility and calcium transient

Cell contractility was measured as previously described by Locatelli et al. (2017), briefly, the contractions of cardiomyocytes isolated from the left ventricle were measured using the cardiomyocyte length alteration technique using the edge detection system (Ionoptix, USA) mounted on an inverted microscope (Nikon Eclipse – TS100, USA) equipped with an oil immersion objective lens (S Fluor, 40x, Nikon, USA). Cardiomyocytes were externally stimulated at a frequency of 7 Hz, which corresponds to the in vivo animal in exercise, with voltage pulses of 5 ms duration and an intensity of 20 V using a pair of steel electrodes, coupled to the two internal sides. chamber, using an electrical stimulator (Myopacer, Field Stimulator, Ionoptix, USA).

Left ventricular $[Ca^{2+}]_i$ transient measurements were performed as described by Rodrigues et al. (2018), briefly, an inverted microscope (Nikon Eclipse – TS100, USA) equipped with an oil immersion objective lens (S Fluor, 40x, Nikon, USA) was used. Myocytes were incubated with the plasma membrane permeable Ca^{2+} fluorescent indicator, Fura-2 aminopolycarboxylic acid (Fura-2AM, ThermoFisher, Waltham, USA). Stimulation was performed at a frequency of 7Hz and the fluorescence emission was detected between 340 and 380 nm, by a photomultiplier tube.

Statistical analysis

Statistical analysis were performed using the GraphPad Prism 8.0® software. After testing the normality of the data, the t test was performed to compare the means. A statistical significance level of 5% was adopted.

3. Results

Table 1 presents the general characteristics of the animals included in the study, where it can be observed that the animals body mass, as well as the weight of the ventricles and the weight of the left ventricle were similar, which shows the homogeneity of the sample. The animals in the exhaustion group managed to keep swimming for 13.98 ± 3.41 minutes.

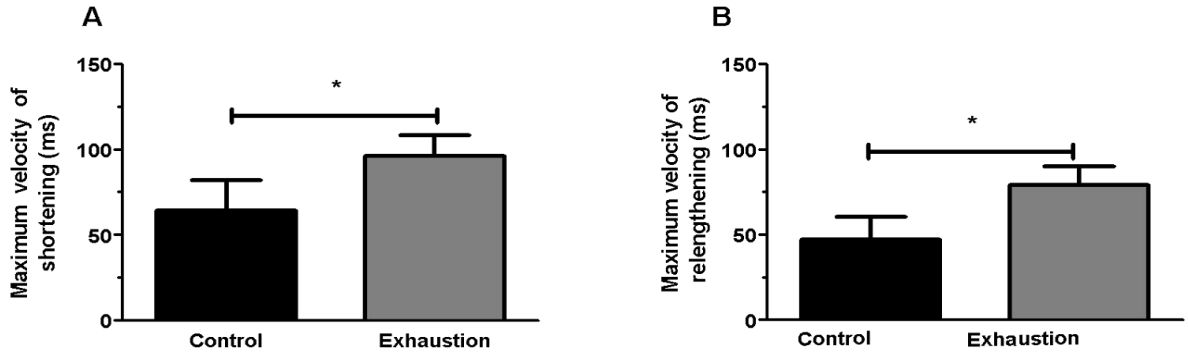
Table 1. General and cardiovascular characteristics.

Variables	Control	Exhaustion	*p*
Body mass (g)	436,3±36,90	436,6±29,08	0,66
Ventricle weight (g)	1,30±0,09	1,33±0,16	0,85
Left ventricle weight (g)	0,837±0,07	0,848±0,11	0,96

Comparison of the general characteristics of animals. Data expressed as mean ± standard deviation. n=7 animals per experimental group. The t test was used to compare groups. * Indicate statistical difference between groups. P<0.05. Source: Prepared by the authors (2022).

Figure 1 shows that exercise until exhaustion leads to greater speed and shortening and relaxation in isolated myocytes from the left ventricle of the animals submitted to the protocol until exhaustion.

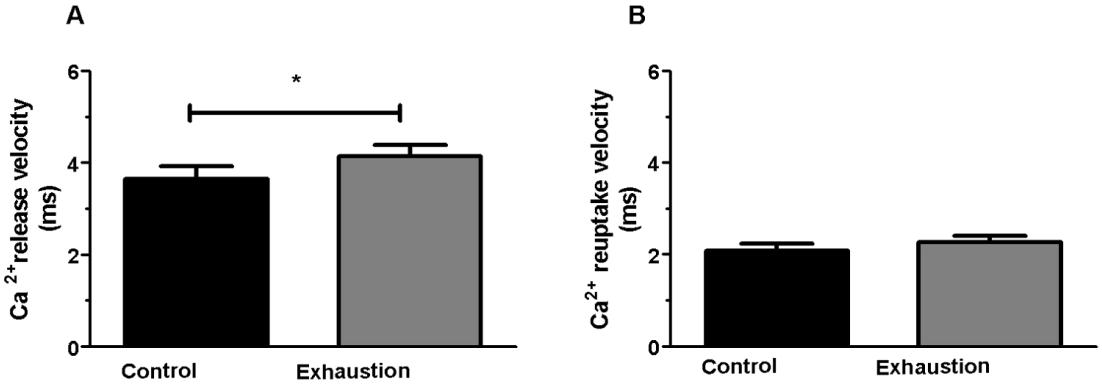
Figure 1. Maximum velocity of shortening (A) and maximum velocity of relengthening (B).



Effects of an acute swimming session until exhaustion on the contraction and relaxation velocity of cardiomyocytes isolated from the left ventricle of Wistar animals. t test was used for between group comparisons. Data are reported as means ±SD for 80 - 90 cells per animal in each group. * Indicate statistical difference between groups. P<0.05. Source: Prepared by the authors (2022).

Furthermore, it can be observed that there was an increase in the rate of calcium release (Fig. 2A) in the exhaustion group, however the rate of calcium reuptake did not show a significant difference (Fig. 2B). The increase in shortening velocity was accompanied by an increase in calcium release velocity, however the increase in relaxation velocity was not accompanied by an increase in calcium reuptake velocity.

Figure 2. Ca^{2+} release velocity (A) and Ca^{2+} reuptake velocity (B).



Effects of an acute swimming session until exhaustion on the rate of calcium release and reuptake in cardiomyocytes isolated from the left ventricle of Wistar animals. The t test was used for comparisons between groups. Data are presented as means ± SD for 80 - 90 cells per animal in each group. * Indicates statistical difference between groups. P<0.05. Source: Prepared by the authors (2022).

4. Discussion

In our study, we found a swimming time of 13.98 ± 3.41 min, in addition to increases in contraction and relaxation velocities, as well as in the velocity of calcium release after an acute session of swimming to exhaustion load.

Corroborating our findings, studies carried out previously by Casimiro-Lopes et al. (2008), with Wistar animals in an acute swimming protocol with 5% body weight, the animals could keep swimming for approximately 4.16 min, and in a later study, the animals kept swimming for approximately 5 min, the difference was that in the latter they were female rats (Casimiro-Lopes et al., 2008). A study by Oláh et al. (2015) contradicts our results where the animals kept swimming for 3 h, however they were forced to swim and an increase in plasma levels of creatine kinase and cardiac troponin T was found, in addition to myocardial fragmentation and presence of inflammatory cells, contractility impaired, increased end-systolic volume and reduced ejection fraction.

When the animal was subjected to exercise until exhaustion, there was an increase in the speed of shortening, which can be explained by the increase in the speed of calcium release. However, the increase in relaxation velocity was not accompanied by an increase in calcium reuptake velocity, which, despite having presented an increase, did not present a significant difference. Although we did immeasure in this study, calcium-managing proteins are responsible for controlling the influx and efflux of calcium necessary for the process myocardial contraction and relaxation (Bers, 2014). When L-type channels are opened, calcium entry into the cell is allowed (Aronsen, Louch & Sjaastad, 2016; Eisner et al., 2013). The type 2 ryanodine receptors (RyR2) are activated by increasing intracellular calcium concentration and release more of these ions that are contained in the sarcoplasmic reticulum (SR), further increasing its concentration. Ca^{2+} binds to troponin C, present in actin filaments, tropomyosin moves and releases the actin binding site allowing the formation of cross-bridges with myosin and subsequent muscle contraction (Aronsen, Louch & Sjaastad, 2016; Eisner). et al., 2013). This phase is related to ventricular systole and may explain the increase in myocardial contraction velocity under a 7 Hz stimulus caused by the increase in calcium release velocity, and the more calcium is released, the more cross-bridges will be formed and thus generate a greater force of contraction and consequently greater cardiac output, which is necessary to maintain the demands imposed during exercise (MacArdle 2016; Silverthorn, 2017).

For the relaxation process to occur, Ca^{2+} needs to be recaptured to the RS, for which the phospholamban (PLB) protein is phosphorylated, thus stopping inhibiting the Ca^{2+}ATPase pump (SERCA2), which pumps Ca^{2+} to the reticulum, where

remains stored. To reduce Ca^{2+} levels in the cytosol, SERCA2 works with the Na^{2+}/Ca^{2+} exchanger (NCX), which pumps three Na+ ions into the intracellular medium and takes one Ca2+ ion to the extracellular medium, thus reducing the levels of Ca^{2+}. In this way, tropomyosin moves and blocks the binding sites between actin and myosin again, leading to muscle relaxation (D. Eisner et al., 2013; D. A. Eisner et al., 2017).

What we observed in our study is that there is an increase in the rate of relaxation of cardiomyocytes that was not accompanied by an increase in calcium reuptake into the reticulum, when the animal was exposed to the stress of strenuous exercise, so we can infer that exercise to exhaustion can alter the phospholamban, SERCA and NCX proteins, which are responsible for calcium reuptake and release to the external environment so that the heart enters the diastole phase, this may indicate an adverse effect of strenuous exercise in cardiomyocytes, however they are further studies are needed to confirm these mechanisms.

The increases found in the speed of contraction and relaxation under 7Hz stimulation in our study were consistent with those demonstrated in a study Carneiro-Júnior et al. (2013) where they performed training for 8 weeks, however the stimulation was only 1Hz, despite this, when the calcium management proteins were analyzed, they did not show a significant difference. With these findings, we can infer that acute exercise performed until exhaustion exerts an effect on cardiomyocytes when stimulated at 7Hz and these effects are similar to the effects of training for 8 weeks, however when cardiomyocytes are stimulated at 1Hz as demonstrated by Carneiro Junior et al. (2013). Studies on acute exercise protocols are scarce, it would be interesting to conduct studies where the effects of acute and chronic exercise on the contractile mechanisms of the heart are compared.

5. Conclusion

The swimming exercise performed until exhaustion promotes changes in the contractile mechanisms of cardiomyocytes isolated from the left ventricle under 7Hz stimulation, however, further studies are necessary to be able to accurately state the mechanisms involved in the process.

Studies using other stimulation frequencies and analyzing the functioning of calcium-management proteins are necessary to precisely explain the proposed mechanism.

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