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Original

Acute effects of swimming aerobic exercise on contractility and intracellular calcium handling in isolated right ventricular cardiomyocytes

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ABSTRACT

Objectives: The acute adjustments on the contractile function and the intracellular calcium (Ca^{2+}) handling in the cardiomyocytes of the right ventricle (RV) after an acute aerobic exercise session are not known. Our aim was to investigate the acute responses of the contractile function and the intracellular Ca^{2+} handling in isolated RV cardiomyocytes after a swimming exercise session.
Methods: Ten-week-old female *Wistar* rats were randomly allocated into two groups: control (C; n = 5) and exercise (Ex; n = 7). It was performed a swimming exercise session for 30 minutes. with an overload of 4% relative to the body weight attached to the tail. The rats were sacrificed after the exercise session for the analysis of the RV contractile function parameters and the intracellular Ca^{2+} handling by the isolated cardiomyocyte technique.
Results: Body and heart weights, as well as sarcomere length were similar between the groups. Also, it was not observed differences between the groups for RV cardiomyocyte contractile parameters. However, the systolic and diastolic intracellular Ca^{2+} concentration was significantly lower in the Ex group compared to C with maintenance of Ca^{2+} amplitude.
Conclusion: An acute swimming aerobic exercise session promotes cardiomyocyte contractility maintenance even with systolic and diastolic intracellular Ca^{2+} concentration reduced in the RV cardiomyocytes, reflecting an improvement in the intracellular Ca^{2+} handling.
Keywords: Acute exercise; Cardiomyocyte; Calcium handling; Right ventricle.

Efectos agudos del ejercicio de natación aeróbica sobre la contractilidad y el manejo del calcio intracelular en cardiomiocitos aislados del ventrículo derecho

RESUMEN

Objetivos: se desconocen los ajustes agudos de la función contráctil y el manejo del calcio (Ca^{2+}) intracelular en los cardiomiocitos del ventrículo derecho (VD) tras una sesión de ejercicio aeróbico agudo. Nuestro objetivo fue investigar las respuestas agudas de la función contráctil y el manejo del Ca^{2+} intracelular en cardiomiocitos aislados del VD después de una sesión de ejercicio de natación.
Métodos: se asignaron al azar ratas Wistar hembra de diez semanas de edad en dos grupos: control (C; n = 5) y ejercicio (Ex; n = 7). Se realizó una sesión de ejercicios de natación durante 30 minutos con una sobrecarga del 4% con respecto al peso corporal adherido a la cola. Las ratas fueron sacrificadas después de la sesión de ejercicio para el análisis de los parámetros de la función contráctil del VD y el manejo del Ca^{2+} intracelular mediante la técnica de cardiomiocitos aislados.
Resultados: los pesos corporales y cardíacos, así como la longitud del sarcómero, fueron similares entre los grupos. Además, no se observaron diferencias entre los grupos para los parámetros contráctiles de los cardiomiocitos del VD. Sin embargo, la concentración de Ca^{2+} intracelular sistólica y diastólica fue significativamente menor en el grupo Ex en comparación con C con el mantenimiento de la amplitud de Ca^{2+}.
Conclusión: Una sesión de ejercicio aeróbico de natación aguda promueve el mantenimiento de la contractilidad de los cardiomiocitos incluso con una concentración de Ca^{2+} intracelular sistólica y diastólica reducida en los cardiomiocitos del VD, lo que refleja una mejora en el manejo del Ca^{2+} intracelular.
Palabras clave: Ejercicio agudo; Cardiomiocito; Manejo de calcio; Ventrículo derecho.

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Efeitos agudos do exercício aeróbio de natação na contratilidade e no transiente de cálcio intracelular em cardiomiócitos isolados do ventrículo direito

RESUMO

Objetivo: os ajustes agudos na função contrátil e no transiente do cálcio (Ca^{2+}) intracelular nos cardiomiócitos do ventrículo direito (VD) após uma sessão aguda de exercício aeróbio não são conhecidos. Desta forma objetivo foi investigar as respostas agudas da função contrátil e do no transiente Ca^{2+} intracelular em cardiomiócitos do VD isolados após uma sessão de exercício de natação.
Métodos: Ratas Wistar com dez semanas de idade foram alocadas aleatoriamente em dois grupos: controle (C; n = 5) e exercício (Ex; n = 7). Foi realizada uma sessão de exercícios de natação por 30 minutos com uma sobrecarga de 4% em relação ao peso corporal preso à cauda. Os ratos foram sacrificados após a sessão de exercícios para análise dos parâmetros da função contrátil do VD e do transiente Ca^{2+} intracelular pela técnica de cardiomiócitos isolados.
Resultados: Os pesos corporais e cardíacos, bem como o comprimento do sarcômero foram semelhantes entre os grupos. Além disso, não foram observadas diferenças entre os grupos para os parâmetros contráteis dos cardiomiócitos do VD. No entanto, a concentração de Ca^{2+} intracelular sistólica e diastólica foi significativamente menor no grupo Ex em comparação com C com manutenção da amplitude de Ca^{2+}. *Conclusão:* Uma sessão aguda de exercício aeróbio de natação promove a manutenção da contratilidade dos cardiomiócitos mesmo com a concentração intracelular de Ca^{2+} sistólica e diastólica reduzida nos cardiomiócitos do VD, refletindo uma melhora no manuseio do Ca^{2+} intracelular.
Palavras chaves: Exercicio agudo; Cadiomiocito; Transiente de cálcio; Ventrículo direito

Introduction

The acute and chronic cardiac physiological effects of aerobic exercise training (AET), mainly in the structure and function of the left ventricle (LV), are widely recognized.[1-4] The long-term cardiac adaptations to AET includes the increase in ventricular ejection and filling capacities, physiological hypertrophy and reduced heart rate,[3,4] the enhanced ability of cardiomyocytes to contract and stretch;[5-7] and in the right ventricle (RV), the increase in mass and in the capacity to accommodate blood.[2,7,8]

Although it has been well established that the cardiac effects of AET in the mechanical function of cardiomyocytes results from adaptations of successive individual sessions, the acute impacts of this practice in the right ventricle (RV) are not fully understood.[4,9,10] It is known that the exercise acutely generates a cardiac overload in both ventricles, with a magnitude determined mainly by the type, intensity, and volume of the exercise.[2,8] Therefore, during exercise the LV and RV are simultaneously subjected to work overload at different degrees,[5,6,11,12] which occurs in the RV as a result of increased afterload, where the increase in the atrial pressure and limited pulmonary vascular reserve capacity leads to an increase in the pulmonary vascular and end-diastolic RV pressure.[11]

Interestingly, clinical[12-14] and experimental studies[15] have shown some negative outcomes with regard to the vigorous exercise training on the RV, including dysfunction and arrhythmias,[12-15] as well as myocardial fibrosis,[15] whereas, low-intensity exercise training seems to be unable to promote functional changes in RV cardiomyocytes.[5] However, there are few studies which highlight the acute responses of the RV cardiomyocytes to exercise.[5,11] In a previous study, it was observed that a single session of exhaustive exercise impairs the calcium handling and the mechanical function of RV cardiomyocytes,[11] nonetheless, the evaluation of these parameters after an acute aerobic exercise was not evidenced.

Therefore, the aim of this study was to investigate the contractile function and calcium handling responses after an acute swimming aerobic exercise session in cardiomyocytes isolated from RV. Our hypothesis is that the acute aerobic exercise improves the contractile function and the intracellular calcium handling in the cardiomyocytes from the RV, contributing to the understanding of the acute impact of the aerobic exercise on the right side of the heart.

Methods

Animals

Ten-week-old female *Wistar* rats (n= 12) were housed in collective cages (*4 animals per cage*), in a climate-controlled environment with a 12 h light/dark cycle and free access to food and water. All experiments were conducted according to the Guide for the Care and Use of Laboratory Animals (NIH, USA) and were approved by the University Ethics Committee for the Use of Animals under protocol number 20/2015.

Experimental protocol

The rats were randomly distributed into two groups: control (C; n= 5) and exercise (Ex; n= 7). The Ex group was submitted to a swimming exercise session, according to the protocol described below. The C group was not subjected to the exercise session; however, the rats were placed in shallow water for the same time and frequency as the Ex group.

Acute swimming exercise protocol

The exercise protocol was performed in an apparatus adapted for rats which is cylindrical in shape, with a depth of 45 cm and the water temperature was maintained between 30±1º C. Forty-eight hours before the exercise session the rats were acclimatized to the water environment in shallow water for 30 minutes. The swimming aerobic exercise was performed in a single session for 30 minutes. Araújo et al., 2009[16] found the maximum lactate steady state at an overload of 5% relative to the body weight in swimming female rats. Therefore, to ensure that all of the animals were practicing the exercise with the predominance of the aerobic sources, we chose to perform the swimming exercise with an overload of 4% relative to the body weight. After the exercise session, the animals were dried with absorbent tissue and a hairdryer.

Cardiomyocyte preparation

One hour after the exercise session or resting in the water (group C), the rats were euthanized by anesthetic overdose using ketamine and xylazine (250 mg/kg and 50 mg/kg, i.p., respectively); The hearts were quickly removed by median thoracotomy and mounted in a Langendorff system adapted to perform the retrograde perfusion by the aorta. Firstly, the hearts were perfused with Solution A (NaCl 120 mM, KCl 5.4 mM, $MgSO_4$ 1.2 mM, NaH_2PO_4 1.0 mM, $NaHCO_3$ 20 mM, Glucose 5.6 mM; pH 7.4) for approximately 3 to 5 minutes, followed by Solution B (Solution A plus 1 mg/mL collagenase, 1 mg/50mL protease; pH 7.4) for approximately 3 to 5 minutes and Solution C (Solution A plus 0.5 M $CaCl_2$, 1 mg/mL collagenase, 1 mg/50mL protease; pH 7.4) for 20 minutes. All of the solutions were oxygenated and equilibrated with 5% CO_2 and 95% O_2, and heated at 37.5°C in a coronary perfusion system. The coronary flow was maintained constant at 10 mL/min. by a peristaltic pump (Gilson, Middleton,

WI, USA). After digestion, the heart was dissected and the RV was cut into small pieces (2–3 mm^3) in Solution C at room temperature. The cells were then dissociated, resuspended and filtered. After 10 minutes, the supernatant was removed, and the pellet was washed every 10 minutes with Solutions A, B, and C, respectively.

Cardiomyocyte contractility

Briefly, isolated cells were placed in an experimental chamber with a glass coverslip base mounted on the stage of an inverted microscope (IonOptix, Milton, MA, USA), using an edge detection system with a 40x objective lens (Nikon Eclipse – TS100, USA). Cells were immersed in Tyrode's solution containing 1.8 mM $CaCl_2$ and field stimulated at 1Hz (20 V, 5 ms duration square pulses). Cell shortening in response to electrical stimulation was measured with a video-edge detection system at a 240Hz frame rate (Ionwizard, IonOptix, Milton, MA, USA) and the contractile parameters were evaluated. In addition, the sarcomere length was recorded and later analyzed using the Ion Wizard Software (IonOptix, Milton, MA, USA). Cell shortening (expressed as a percentage of resting cell length), maximal velocities of shortening and relaxation, and times to 50% shortening and 50% decay were measured in 15-20 cells per animal.

Intracellular Ca^{2+} measurements

Myocytes were loaded with 1 µM Fura2-acetoxymethyl (Fura2-AM) ester (Molecular Probes, Eugene, OR, USA) for 10 minutes at room temperature, washed with Tyrode solution and allowed to rest for an additional 10 minutes for the de-esterification of the dye. Subsequently, the cardiomyocytes were stimulated at 1Hz (Myopacer 100, IonOptix Inc., Milton, MA, USA) and fluorescence images were obtained using excitation at wavelengths from 340 to 380 nm using a Hyper Switch system (IonOptix Inc., Milton, MA, USA). Background-corrected Fura2-AM ratios reflected intracellular Ca^{2+} concentrations detected at approximately 510 nm. The Ca^{2+} transient amplitude was reported as F/F0, where F is the maximal fluorescence intensity average measured at the peak of $[Ca^{2+}]i$ transients, and F0 is the baseline fluorescence intensity measured at the diastolic phase of $[Ca^{2+}]i$ transients. Systolic and diastolic intracellular Ca^{2+}, time to peak Ca^{2+} and the time to 50% Ca^{2+} decay were also analyzed.

Statistical analysis

Data were reported as the mean ± standard error of the mean (SEM) and submitted to the Kolmogorov-Smirnov test to determine adherence to normality. The comparisons between groups were performed by the unpaired Student's t-test for independent samples. The level of significance was set at $p< 0.05$.

Results

General characteristics

The general characteristics of animals are shown in Table 1. The results indicate no statistical difference for the body and heart weight, the heart weights corrected by the body weight and the sarcomere length between the C and Ex groups ($p> 0.05$).

Table 1. General characteristics of experimental groups.

Parameters	C (n =5)	Ex (n =7)	*P-value*
Body weight (g)	200 ± 2.5	215 ± 6	0.07
Heart weight (mg)	965 ± 50	1123 ± 66	0.11
HW/BW (mg/g)	4.82 ± 0.21	5.27 ± 4.29	0.42
Sarcomere length (µm)	1.64 ± 0.02	1.69 ± 0.01	0.04

Data are expressed as the Mean ± SEM. C: Control group; Ex: Exercise group; BW: Body weight; HW: Heart weight. Unpaired Student's t-test for independent samples.

RV Cardiomyocyte contractile properties

Figure 1 shows the parameters related to the contractile function of cardiomyocytes isolated from the RV after electrical stimulation with 1Hz. It was not observed statistical differences between the groups in all of the parameters evaluated after an acute swimming aerobic exercise session ($p> 0.05$).

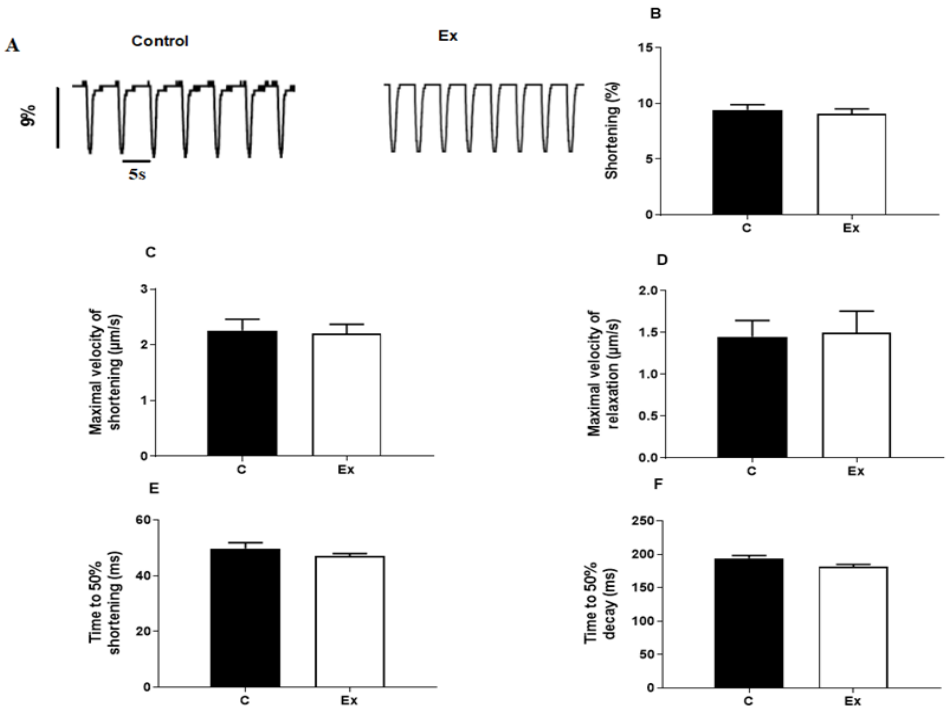

Figure 1. Contractile function of the right ventricle cardiomyocytes from control (C; n = 5; cells = 20) and exercise rats (Ex; n = 7; cells = 34). A) Representative contraction traces obtained from the cardiomyocytes of rats. Data are expressed as the mean ± SEM. Unpaired Student´s t test.

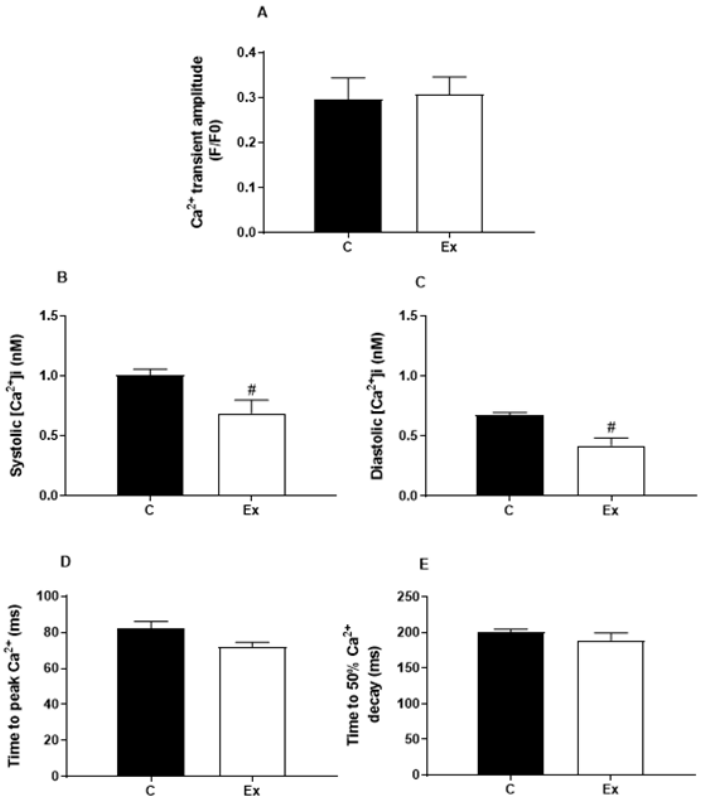

Figure 2. Cardiomyocyte calcium (Ca^{2+}) handling from control (C; n = 5; cells = 20) and exercise rats (Ex; n= 7; cells = 34). Data are expressed as the mean ± SEM. Unpaired Student´s t test. # p< 0.05 *vs.* C group.

Figure 2 shows the RV cardiomyocytes loaded with the Ca^{2+} indicator Fura2-AM after electrical stimulation with 1Hz. In Figure 2A, the Ca^{2+} transient amplitude (F/F0) was not different between the groups. As visualized in Figs. 2B and C, the cardiomyocytes from the Ex group exhibited a lower systolic and diastolic intracellular Ca^{2+} concentration compared with the C group (p< 0.05). The time to peak Ca^{2+} and Time to 50% Ca^{2+} decay (Figure 2C and D, respectively), it was not observed statistical differences between the groups.

Discussion

The aim of the current study was to analyze the contractile function and Ca^{2+} handling responses of RV cardiomyocytes after an acute swimming aerobic exercise session. Our study highlights the acute impact promoted by the aerobic exercise on the RV cardiomyocytes, which is important for the understanding of its magnitude on the main functional component of the right heart.

Acute and chronic adaptations to exercise have been extensively studied in the LV in healthy individuals,[1,3,4,17-19] whereas attention to the RV is undervalued.[2,5,7,8] The majority of the studies addressing the effects of exercise on the RV involves athletes,[2,12-14] and little scientific evidence in this field came from experimental studies with healthy animals.[5,11,15] Such studies would be important because of the impossibility to extrapolate the findings from the LV to the RV since there are substantial differences in embryology, morphology, perfusion, workload, and downstream vascular beds between the chambers.[2]

During AE, it is known that a higher relative change in RV wall stress occurs in comparison with the LV,[11,12,14] and the interventricular septum, which comprises myocardial fibers from both the LV and RV, appears to be particularly susceptible to fatigue following isolated events and periods of training.[14] In this regard, the literature shows that both acute[11,12] and chronic[12-15] exhaustive exercise, characteristic in the training of high-performance athletes, may lead to RV dysfunction and arrhythmia,[12-15] as well as myocardial fibrosis,[15] in addition to the impaired functioning of cardiomyocytes.[11]

Moreover, different exercise intensities led to distinct cardiovascular changes after exercise,[21] thus, more vigorous activities generate greater post-exercise cardiac work in order to promote the return of the individual to the rest condition. However, studies with cardiac cell isolation more accurately reflect the exercise-induced adjustments, specifically on cardiomyocytes, and not the impact on the whole cardiovascular system.

Experimental studies indicate that one of the major mechanisms related to improvements of the mechanical function of cardiomyocytes by exercise is Ca^{2+} handling, the main regulator of cardiac excitation-contraction coupling (ECC).[17,18,22] As such, the positive chronic adaptations on contractile and relaxation capacities of cardiomyocytes observed in chronic exercised individuals may be explained in part by the increased Ca^{2+} handling amplitude, the faster increase, and decay of intracellular Ca^{2+} handling and myofilament sensitivity.[23,24,25] In our study, the acute swimming exercise in rats neither increased Ca^{2+} transient amplitude nor improved the Ca^{2+} increase and decay velocities in the RV cardiomyocytes. Studies which evaluated the acute effects of exercise on this issue in RV are sparse and the majority of the literature has studied the effects of high-intensity and exhaustive exercise protocols on these parameters. In accordance, Delgado et al.[26] observed that a single exhaustive exercise session in trained and untrained rats did not change the activity of regulatory proteins of Ca^{2+} handling in the homogenate of cardiac tissue 24- and 48-hours post-exercise, however, the cardiac contractility was not measured in this study. On the other hand, Ljones et al.[11], after assessing the acute impact of an exhaustive aerobic exercise

session on the Ca^{2+} handling and its regulatory proteins in RV cardiomyocytes of untrained rats observed a reduction in Ca^{2+} transient amplitude and an increase in the time to 50% decay and diastolic Ca^{2+} removal. Therefore, since the force of contraction is mainly regulated by the amplitude and duration of intracellular Ca^{2+} handling, the absence of alterations in these parameters with our acute swimming aerobic protocol can be considered a positive result which demonstrates the maintenance of the RV cardiomyocyte function after exercise.

In this sense, a major finding of our study shows that the acute aerobic exercise induces an adjustment in the Ca^{2+} handling mechanism, causing a significant reduction of the systolic and diastolic intracellular Ca^{2+} concentration with the maintenance of Ca^{2+} amplitude and the contractile function of the RV cardiomyocytes. This result is important because it reflects an improvement in the intracellular Ca^{2+} reuptake mechanism.

Important bullets may be present such, few studies examining differential the acute responses of contractile function and calcium handling in isolated right ventricular (RV) cardiomyocytes after a physical exercise session. Understanding of acute impact from an exercise session on right side of heart and the magnitude of post-exercise response in RV cardiomyocytes to this activity is very important to the area. Finally, the effects of exercise may differ on RV based on the protocol employed.

Althought the measurement of the contractile behavior of single cardiomyocytes has made a significant contribution to our understanding of the physiology and pathophysiology of the myocardium some limitations should be mentionated in the present study. First, the techcnique of isolated cells do not permit full understood of ventricular performance, due the complex geometry of heart the mechanical performance of cadiac chambers is regulated not onlu by factors directly dependent of contractile state o myofibrils. Second, its study evaluated the cardiomyocyte contractility of right ventricule after 30 minutes by aerobic single session, in this way more studies should be conducted to clary the role of exercise type, the interdependency of volume and intensity and chronic adaptations. Third, the study did not investigate the activity and protein expression of calcium handling regulatory proteins known to affect the myocardial contraction and relaxation. In addition, the current study did not evaluate the blood samples with measures of lactate or other biological stress markers, which could indicate the actually workload performed during exercise.

In the current study promotes cardiomyocyte contractility maintenance even with systolic and diastolic intracellular Ca^{2+} concentration reduced in the RV cardiomyocytes, reflecting an improvement in the intracellular Ca^{2+} handling. Our findings confirm that exercise performed with a low to moderate intensity can be interesting for the health of RV cardiomyocytes and possibly to the RV function. These results reinforce the premise that the practice of moderate exercise training may be an important strategy for the maintenance of cardiac contractility.

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