Morphology and Contractility in Cardiomyocytes of Rats with Low Exercise Performance

Judson Fonseca Quintão-Júnior[1], Antônio José Natali[1], Miguel Araújo Carneiro-Júnior[3], Cynthia Aparecida de Castro[1], Lucas Rios Drummond[1], Victor Neiva Lavorato[1], Leonardo Bonato Felix[1], Jader dos Santos Cruz[2], Thales Nicolau Prímola-Gomes[1]

Universidade Federal de Viçosa[1], Viçosa, MG; Universidade Federal de Minas Gerais[2], Belo Horizonte, MG; Universidade Federal do Espírito Santo[3], Vitória, ES, Brazil

Abstract

Background: **Aerobic capacity is essential to physical performance, and low aerobic capacity is related to the triggering of various cardiovascular diseases.**

Objective: **To compare the morphology and contractility of isolated rat cardiomyocytes with low performance and standard performance for exercise.**

Methods: **Wistar rats with 10 weeks of age underwent a protocol of treadmill running until fatigue, and were divided into two groups: Low Performance (LP) and Standard Performance (SP). Then, the animals were sacrificed, the heart was quickly removed and, by means of enzymatic dissociation, left ventricular cardiomyocytes were isolated. The cell and sarcomeres length and width of cardiomyocytes were measured using an edge detection system. The isolated cardiomyocytes were electrically stimulated at 1 and 3 Hz and cell contraction was measured by registering the change of their length.**

Results: **The cell length was shorter in the LP group (157.2 ± 1.3 μm; $p < 0.05$) compared to SP (161.4 ± 1.3 μm), and the same result was observed for the volume of cardiomyocytes (LP, 25.5 ± 0.4 vs. SP, 26.8 ± 0.4 pL; $p < 0.05$). The time to peak contraction (LP, 116 ± 1 vs. SP, 111 ± 2 ms; $p < 0,05$) and total relaxation (LP, 232 ± 3 vs. SP, 143 ± 3 ms; $p < 0.05$) were higher in the LP group.**

Conclusion: **We conclude that left ventricular myocytes of animals with low performance for exercise are smaller than animals with standard performance. In addition to that, they present losses in contractile capacity. (Arq Bras Cardiol 2012;98(5):431-436**

Keywords: **Cardiac myocytes; rats; exercise; fatigue; myocardial contraction; animal physical conditioning.**

Introduction

Aerobic capacity is essential not only for physical performance, but it is also related to the outbreak of various diseases. A low aerobic capacity is associated with the incidence of cardiovascular diseases and greater mortality[1,2].

Aerobic capacity is a complex variable that is under the influence of genetic and environmental factors[3]. Genetic factors may represent 70% to 90% in the variations observed in the aerobic capacity[4]. The term intrinsic aerobic capacity has been used to refer to the set of genes that determine changes in aerobic capacity when there is no prior physical training (untrained state)[5-8]. The main determinant of intrinsic aerobic capacity is the capacity of the heart to maintain adequate blood flow to body tissues. Previous studies demonstrated that rats with High Intrinsic Aerobic Capacity (HIAC) showed gains in cardiac function[4], higher maximum oxygen consumption (VO_{2max})[3] and, at the cellular level, higher morphological and contractile parameters compared to rats with Low Intrinsic Aerobic Capacity (LIAC)[9]. These results show the importance of cardiac contractile activity in the determination of intrinsic aerobic capacity.

At the cellular level, the contractile capacity of cardiomyocytes is directly related to the Ca^{2+} homeostasis. The Ca^{2+} is involved in the process of excitation-contraction coupling, both at the time of excitability of cardiomyocytes and in the final activation of contractile filaments causing contraction[10,11]. Previous results of our study group showed that cardiomyocytes isolated from rats with high-performance for exercise (HP) showed gains in contractile function (both systolic and diastolic), and a larger global intracellular transient of Ca^{2+} ($[Ca^{2+}]_i$ transient) compared to standard performing rats (SP)[12].

Studies on the morphology and contractile function of cardiomyocytes of animals with low performance and standard performance are scarce. Hence, the objective of this study was to compare morphological and contractile parameters of isolated rat cardiomyocytes with LP (low performance for exercise) and SD (standard performance for exercise). According to our hypothesis, cardiomyocytes of LP animals showed significant reductions in the morphology and contractile capacity.

Mailing Address: Thales Nicolau Prímola-Gomes •
Universidade Federal de Viçosa, Departamento de Educação Física, Laboratório de Biologia do Exercício - BIOEX - Av. Ph. Rolfs, s/n – Campus Universitário – 36570-000 – Viçosa, MG - Brazil
E-mail: thales.gomes@ufv.br
Manuscript received November 01, 2011; revised manuscript received November 03, 2011; accepted January 24, 2012

Methods

Animals

For these experiments, we used male Wistar rats (Rattus norvegicus), with 10 weeks of age, weighing 224 ± 5 g at the beginning of the trial, from the animal colony of the Universidade Federal de Viçosa (state of Minas Gerais). The animals were kept in polyethylene boxes with a maximum of five rats per box in a room at 23°C with commercial chow and water ad libitum and a cycle of 12-12 h light/dark (6:00 a.m. - 06:00 p.m.). This study followed the standards established in the Guide for the Care and Use of Laboratory Animals (Institute of Laboratory Animal Resources, National Academy of Sciences, Washington, D.C., 1996) and respected the Ethical Principles in Animal Experiments of the Brazilian College of Animal Experimentation (COBEA). The study was approved by the Ethics Committee on Animal Experimentation of Universidade Federal de Minas Gerais - Cetea/UFMG (protocol nº 015/08).

Exercise protocol

The animals were subjected to a protocol based on the total exercise time until fatigue (TET)[12]. One week before the test, the animals went through an adjustment period on the treadmill (Insight Instrumentos - Ribeirão Preto, Brazil) for five consecutive days (5 min/day; 5% slope) and daily increases of the treadmill speed (10, 10, 11, 13, 15 m/min). After one week of adaptation, each animal performed, on three alternate days, three progressive exercise tests until fatigue (initial speed of 10 m/min, 5% slope). During testing, the treadmill speed was increased by 1 m/min to 3 min each. The test was stopped when the animal entered in fatigue. The moment of fatigue was defined when the rat could not keep running at the treadmill speed[12,13].

Selection strategy

The selection strategy used in this study was based on the TET for each rat[12]. Previous data from our laboratory have shown that by using this selection strategy, three groups can be determined: Low Performance (LP), Standard Performance (SP) and High Performance (HP). Based on these data, the animals were divided into two groups: LP (n = 10) and SP (n = 10).

According to the testing methodology adopted, the LP group consisted of animals with TET below 16.63 min, i.e., a TET that is smaller than at least one standard deviation of the population. In turn, the animals with TET between 16.33 and 46.57 min were included in the SP group, i.e., those which had a TET not higher or smaller than a standard deviation of the population[12].

Body mass, euthanasia, heart and ventricular mass, cardiomyocyte isolation

The animals' body mass was measured immediately prior to euthanasia by means of an electronic scale (Mars - Brazil). The heart was removed, washed in a solution containing 750 μM of $CaCl_2$ to remove excess blood, and weighed on a precision scale (Gehaka - Brazil). After weighing the heart, the aorta was cannulated and the heart was perfused with a solution of isolation, containing: [composition (mM): 130 Na^+; 5.4 K^+; 1.4 $MgCl_2$; 140 Cl^-; 0.75 Ca^{2+}; 5.0 Hepes; 10 glucose; 20 taurine; and 10 creatine; pH 7.3 at room temperature][14,15]. Then, perfusion was changed to a Ca^{2+}-free solution containing 0.1 mM EGTA for 6 min for the destruction of cardiomyocytes scalariform bands. Then, the heart was perfused with a solution containing a 1mg.mL^{-1} collagenase type 2 (Worthington, USA) and 100 μM of $CaCl_2$ for 25 min, for the destruction of extracellular collagen fibers. All solutions used in the procedure were oxygenated (O_2 100% - White Martins, Brazil) and maintained at a temperature of 37°C. At the end of the infusion, the ventricles were separated from the atria and weighed[14]. Thereafter, the left ventricle was separated into fragments that were placed in vials containing 5 mL of enzyme solution (collagenase) supplemented with 1% bovine serum albumin. The vials were shaken moderately for 5 min in "bain marie" at 37°C. Next, the contents of the vials were filtered and centrifuged (3000 rpm) for 30 s. The supernatant was removed and the cells were suspended in 750 μM solution of $CaCl_2$. The cells were stored for a period of up to 6 hours after the procedure of isolation[14]. The indices of ventricular and cardiac hypertrophy were calculated for ratios of heart and ventricle weight, respectively, to body weight.

Cell contraction

The cell contraction was measured by the technique of changing the length of cardiomyocytes using an edge detection system mounted on an inverted microscope (Ionoptix, USA) as described previously[14,15]. The cardiomyocytes were placed in an experimental chamber with glass base and bathed in buffer containing the following composition (in mM): 136.9 NaCl; 5.4 KCl; NaH2PO4 0.37; 0.57 MgCl2; 5 Hepes; 5.6 Glucose; 1.0 CaCl2 (pH 7.4). The cardiomyocytes were viewed on a monitor via a camera (Myocam, Ionoptix, frequency of 240 Hz) attached to the microscope using an image detection program (Ionwizard, Ionoptix). The cardiomyocytes were stimulated at a frequency of 1 and 3.0 Hz (10 Volts, lasting 5 min) using a pair of steel electrodes and an electric field stimulator (Myopacer, Ionoptix). The movements of the longitudinal edges of the cardiomyocytes were captured by the edge detection system and stored for later analysis. Contraction measurements employed only those cardiomyocytes that were in good condition, with the edges and well-defined sarcomeric striations, at rest, with no voluntary contractions. Contractions were measured using a home software application developed on the Matlab® platform.

Dimensions of cardiomyocytes

The cell length and width of cardiomyocytes were measured using an edge detection system. The images of the cells were viewed and captured as described previously[14]. The length was defined by measuring the image generated on the monitor through a μm rule, from the right edge to left edge of the cells. The width was determined by measuring the image generated on the monitor, through a μm rule, from the top edge to bottom edge at the midpoint of the cells. The cell volume was calculated using the formula: [Volume (pl) = length (mm) x width (mm) x (7.59 x 10^{-3}pL/mm^2)][16]. The sarcomere length was measured using an image capture

system (Ionoptix, USA). This system lets you see the streaks of cardiomyocytes, and through the contrast of the image generated, it measures the distance between them.

Statistical analysis

The final data analysis was done using unpaired t-test. Data are presented as mean ± standard error of mean. The level of significance adopted was 5%.

Results

Based on the selection criteria adopted[12], the LP and SP groups were composed of animals with TET of 13.67 ± 2.11 min and 37.26 ± 6.61 min, respectively ($p < 0.05$).

There were no significant differences between the LP and SP groups in body mass, heart mass, relative weight of the heart and ventricular mass (Table 1).

The results of cardiomyocyte morphology of LP and SP animal are shown in Table 2. The length of left ventricular cardiomyocytes was significantly lower in the LP group compared to the SP group. However, the cell width was not statistically different between groups. The cell volume was lower in LP animals compared to SP animals ($p < 0.05$). There were no significant differences between the sarcomere length of LP and SP groups.

Table 3 presents the results of contractile variable among LP and SP groups at the frequencies of 1 Hz and 3 Hz. At 1 Hz no significant differences were found for the variables observed. When cardiomyocytes were stimulated at 3 Hz, the results showed that there were differences for the parameters time to peak contraction (T_{peak}) and time of peak contraction to total relaxation (T_{relax}). Higher values were found in the cardiomyocytes of the LP group compared to the SP group ($p < 0.05$). There were no significant differences in contraction amplitude and time to peak contraction to 50% of total relaxation ($T_{50\%}$).

Discussion

This study aimed to compare the morphological and contractile properties of isolated rat cardiomyocytes with low performance and standard performance for exercise. Confirming our hypothesis, we demonstrated that the selection of rats with low intrinsic aerobic capacity was associated with a loss in morphology and contractile capacity. It was also observed that the cardiomyocytes of low performance rats had lower length and cell volume, and a higher time to peak contraction and cell relaxation.

For the selection of groups in this study, the LP animals were selected according to the TET and then compared to SP rats. Previous studies with LIAC/HIAC animals showed that in the 11th generation, the two lineages diverged 347% in the distance walked to fatigue, which corresponded to a TET of 14.3 and 41.6 min, respectively[9,17]. In this study, the LP and SP animals obtained TET values of 13.67 min and 37.26 min, respectively. These values are similar to those obtained in previous studies. The main differences for the selection of animals in this study, compared to the original model, were the use of rats with standard performance instead of rats with high performance, the non-use of any crossover to select the animals and the use of a lineage of rats that is not a disease model.

Data from this study showed that the cardiomyocytes of LP animals presented loss in morphology and contractile capacity. LP animals had lower cell length associated with a smaller volume. Such differences may reflect a smaller ventricular cavity and hence a smaller cardiac output and ejection volume[4,18]. Moreover, gains in morphology may reflect changes in muscle size and ventricular chamber volume. The physiologic hypertrophy of the heart muscle is a response of cardiomyocytes to mechanical and neurohormonal stimuli, allowing an improvement of cardiac pump function[19]. The physiological hypertrophy occurs through cardiac muscle mass increase caused primarily by increased cardiomyocite dimensions[20,21]. Furthermore, increased internal dimension of ventricular chambers is especially linked to increased elasticity of the cardiac muscle tissue and increased length of cardiomyocytes[22,23].

In studies with LIAC/HIAC animals, LIAC animals had lower aerobic capacity, cardiac output and ejection volume[4] compared to HIAC animals. Previous studies also analyzed the morphology of left ventricular cardiomyocytes of these animals. It was observed that LIAC animals have a smaller cell length and volume compared to HIAC animals[9,24]. Similar to previous studies, our results show that the cardiomyocytes of LP animals have shorter cell length and volume compared to SP animals.

Associated with differences in morphology, we found that cardiomyocytes of LP animals presented changes in contractile capacity. T_{peak} and T_{relax} were higher in the cardiomyocytes of the LP group. In cardiomyocytes, the velocity of contraction is directly related to two factors. Firstly, the release of Ca^{2+} stored in the sarcoplasmic reticulum (SR) into the cytoplasm upon ryanodine receptors (RyR2), generating a transient of $[Ca^{2+}]_i$.[11] Secondly, the ability of myosin ATPase to hydrolyze ATP[10]. Previous data from our research group showed that the transient

Table 1 - Body mass, heart mass, relative heart mass and ventricular mass of LP and SP rats

Variables	LP (n = 10)	SP (n = 10)
Body mass (g)	351.5 ± 11.0	365.0 ± 10.0
Heart mass (mg)	1.6 ± 0.1	1.7 ± 0.1
Relative heart mass (mg/g)	4.5 ± 0.1	4.6 ± 0.2
Ventricular mass (mg)	1.5 ± 0.1	1.4 ± 0.1

Data are expressed as mean ± standard error of mean; n - number of animals; LP - low performance; SP - standard performance.

$[Ca^{2+}]_i$ is directly related to physical performance in this selection model[12]. The same results were also observed in LIAC animals compared to HIAC animals[25]. Furthermore, a relation between intrinsic aerobic capacity and RyR2[12] expression was also found.

In addition to losses in the velocity of contraction, a slower velocity of relaxation of LP cardiomyocytes was also observed. The ability of relaxation of the cardiac cells is related to the removal of Ca^{2+} from the citosol[11]. In rat cardiomyocytes, removal of Ca^{2+} from the cytosol occurs by four processes involving Ca^{2+}-ATPase from the SR (SERCA2a) and sarcolemma, the sodium-calcium exchanger (NCX) and mitocondrial reuptake[10]. The activity of SERCA2a is the leading mechanism[26], accounting for approximately 92% of the total Ca^{2+} reuptake in the cardiomyocytes of rats[27]. The faster relaxation may have a positive impact on cell contraction, because it accelerates the reuptake of Ca^{2+} to the SR[11]. Accordingly, an increased expression of SERCA2a in rats with high capacity for aerobic exercise may reflect an improvement in the contractile function[12].

This study aimed to compare the morphological and contractile properties of isolated rat cardiomyocytes with low performance and standard performance for exercise. Confirming our hypothesis, we demonstrated that the selection of rats with low intrinsic aerobic capacity was associated with a loss in morphology and contractile capacity. It was also observed that the cardiomyocytes of low performance rats had lower length and cell volume, and a higher time to peak contraction and cell relaxation.

Conclusion

The cardiomyocytes of low performance animals had decreases in cell length and volume. Furthermore, increased time to peak contraction and peak contraction to total relaxation were also observed in the cardiomyocytes of these animals.

Acknowledgments

We thank the Foundation for Research Support of Minas Gerais (FAPEMIG) and the National Council for Scientific and Technological Development (CNPq)

Potential Conflict of Interest

No potential conflict of interest relevant to this article was reported.

Sources of Funding

This study was funded by CNPq (Process 479509/2011-5) and partially funded by FAPEMIG.

Study Association

This paper is part of the doctoral thesis in Biological Sciences (Physiology) of TN Prímola-Gomes, Universidade Federal de Minas Gerais and the final paper of the undergraduate course (Physical Education) of JF Quintão-Júnior, Unidade Federal de Viçosa. AJ Natali and JS Cruz are CNPq research fellows under productivity Level II and ID, respectively.

Table 2 - Morphological variables of isolated left ventricular cardiomyocytes of LP and SP rats

Group	LP (n = 323)	SP (n = 395)
Length (μm)	157.2 ± 1.3	161.4 ± 1.3*
Width (μm)	21.4 ± 0.3	21.9 ± 0.3
Volume (pL)	25.5 ± 0.4	26.8 ± 0.4*
Sarcomere length (μm)	1.7 ± 0.1	1.7 ± 0.1

Data are expressed as mean ± standard error of mean; The asterisk (*) indicates significant difference between groups (p <0.05); n - number of cells; LP - low performance; SP - standard performance.

Table 3 – Contractile properties of left ventricular isolated cardiomyocytes of LP and SP rats

Variables	LP		SP	
	1Hz (n = 110)	3Hz (n = 101)	1Hz (n = 111)	3Hz (n = 99)
Systolic function				
Amplitude (% r.c.l)	6.2 ± 0.2	8.5 ± 0.2	6.0 ± 0.2	8.2 ± 0.3
T_{peak} (ms)	153 ± 4	116 ± 1*	151 ± 3	111 ± 2
Diastolic function				
T_{relax} (ms)	237± 9	232 ± 3*	235 ± 8	143 ± 3
$T_{50\%}$ (ms)	79 ± 3	60 ± 2	80 ± 3	60 ± 2

Data are expressed as mean ± standard error of mean; The asterisk (*) indicates difference (p < 0.05) compared to the LP group; n - number of cells. LP = low performance; SP - standard performance. Amplitude (% r.c.l) - amplitude of contraction expressed as a percentage of resting cell length; T_{peak} - Time to peak contraction; T_{relax} - time of peak contraction to total relaxation; $T_{50\%}$ - time of peak contraction to 50% total relaxation.

References

1. Blair SN, Kampert JB, Kohl HW 3rd, Barlow CE, Macera CA, Paffenbarger RS Jr, et al. Influences of cardiorespiratory fitness and other precursors on cardiovascular disease and all-cause mortality in men and women. JAMA. 1996;276(3):205-10.
2. Lee IM, Sesso HD, Oguma Y, Paffenbarger RS Jr. Relative intensity of physical activity and risk of coronary heart disease. Circulation. 2003;107(8):1110-6.
3. Henderson KK, Wagner H, Favret F, Britton SL, Koch LG, Wagner PD, et al. Determinants of maximal O(2) uptake in rats selectively bred for endurance running capacity. J Appl Physiol. 2002;93(4):1265-74.
4. Hussain SO, Barbato JC, Koch LG, Metting PJ, Britton SL. Cardiac function in rats selectively bred for low- and high-capacity running. Am J Physiol Regul Integr Comp Physiol. 2001;281(6):R1787-91.
5. Troxell ML, Britton SL, Koch LG. Selected contribution: variation and heritability for the adaptational response to exercise in genetically heterogeneous rats. J Appl Physiol. 2003;94(4):1674-81.
6. Bouchard C, Lesage R, Lortie G, Simoneau JA, Hamel P, Boulay MR, et al. Aerobic performance in brothers, dizygotic and monozygotic twins. Med Sci Sports Exerc. 1986;18(6):639-46.
7. Klissouras V. Heritability of adaptive variation. J Appl Physiol. 1971;31(3):338-44.
8. Bouchard C, Rankinen T, Chagnon YC, Rice T, Perusse L, Gagnon J, et al. Genomic scan for maximal oxygen uptake and its response to training in the HERITAGE Family Study. J Appl Physiol. 2000;88(2):551-9.
9. Wisloff U, Najjar SM, Ellingsen O, Haram PM, Swoap S, Al-Share Q, et al. Cardiovascular risk factors emerge after artificial selection for low aerobic capacity. Science. 2005;307(5708):418-20.
10. Bers DM. Cardiac excitation-contraction coupling. Nature. 2002;415(6868):198-205.
11. Bers DM. Calcium cycling and signaling in cardiac myocytes. Annu Rev Physiol. 2008;70:23-49.
12. Primola-Gomes TN, Campos LA, Lauton-Santos S, Balthazar CH, Guatimosim S, Capettini LS, et al. Exercise capacity is related to calcium transients in ventricular cardiomyocytes. J Appl Physiol. 2009;107(2):593-8.
13. Soares DD, Coimbra CC, Marubayashi U. Tryptophan-induced central fatigue in exercising rats is related to serotonin content in preoptic area. Neurosci Lett. 2007;415(3):274-8.
14. Natali AJ, Turner DL, Harrison SM, White E. Regional effects of voluntary exercise on cell size and contraction-frequency responses in rat cardiac myocytes. J Exp Biol. 2001;204(Pt 6):1191-9.
15. Silva MF, Pelúzio MCG, Amorim PRS, Lavorato VN, Santos NP, Bozi LH, et al. Treinamento em natação atenua a disfunção contrátil de cardiomiócitos de ratos diabéticos. Arq Bras Cardiol. 2011;97(1):33-9.
16. Satoh H, Delbridge LM, Blatter LA, Bers DM. Surface: volume relationship in cardiac myocytes studied with confocal microscopy and membrane capacitance measurements: species-dependence and developmental effects. Biophys J. 1996;70(3):1494-504.
17. Koch LG, Britton SL. Artificial selection for intrinsic aerobic endurance running capacity in rats. Physiol Genomics. 2001;5(1):45-52.
18. Gledhill N, Cox D, Jamnik R. Endurance athletes' stroke volume does not plateau: major advantage is diastolic function. Med Sci Sports Exerc. 1994;26(9):1116-21.
19. Rohini A, Agrawal N, Koyani CN, Singh R. Molecular targets and regulators of cardiac hypertrophy. Pharmacol Res. 2010;61(4):269-80.
20. Hart G. Cellular electrophysiology in cardiac hypertrophy and failure. Cardiovasc Res. 1994;28(7):933-46.
21. Richey PA, Brown SP. Pathological versus physiological left ventricular hypertrophy: a review. J Sports Sci. 1998;16(2):129-41.
22. Levine BD, Lane LD, Buckey JC, Friedman DB, Blomqvist CG. Left ventricular pressure-volume and Frank-Starling relations in endurance athletes: implications for orthostatic tolerance and exercise performance. Circulation. 1991;84(3):1016-23.
23. Moore RL, Musch TI, Yelamarty RV, Scaduto RC Jr, Semanchick AM, Elensky M, et al. Chronic exercise alters contractility and morphology of isolated rat cardiac myocytes. Am J Physiol. 1993;264(5 Pt 1):C1180-9.
24. Kemi OJ, Hoydal MA, Macquaide N, Haram PM, Koch LG, Britton SL, et al. The effect of exercise training on transverse tubules in normal, remodeled, and reverse remodeled hearts. J Cell Physiol. 2011;226(9):2235-43.
25. Hoydal MA, Wisloff U, Kemi OJ, Britton SL, Koch LG, Smith GL, et al. Nitric oxide synthase type-1 modulates cardiomyocyte contractility and calcium handling: association with low intrinsic aerobic capacity. Eur J Cardiovasc Prev Rehabil. 2007;14(2):319-25.
26. Tada M, Katz AM. Phosphorylation of the sarcoplasmic reticulum and sarcolemma. Annu Rev Physiol. 1982;44:401-23.
27. Bassani JW, Bassani RA, Bers DM. Relaxation in rabbit and rat cardiac cells: species-dependent differences in cellular mechanisms. J Physiol. 1994;476(2):279-93.