Effect of Exercise Training on Aging-induced Changes in Rat Papillary Muscle

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Summary

Background: The effects of aging on papillary muscle have been widely demonstrated, but no data on the effects of exercise on the age-related changes are available.

Objective: To analyze the effects of aging on the morphological and quantitative properties of papillary muscle and investigate whether a long-term moderate exercise program would exert a protective effect against the effects of aging.

Methods: We used electron microscopy to study the density of myocytes, capillaries and connective tissue and the cross-sectional area of myocytes of the papillary muscle of the left ventricle of 6- and 13-month-old untrained and exercised Wistar rats.

Results: As expected, the volume density of myocytes declined significantly ($p<0.05$) with aging. The length density of myocardial capillaries also declined with aging, but not significantly. The interstitial volume fraction of the papillary muscle tissue increased significantly ($P<0.05$) with age. The number of myocyte profiles showed a reduction of 20% that was accompanied by myocyte hypertrophy in the aged rats ($P<0.05$). Animals submitted to a 60-minute daily session,, 5 days/wk at 1.8 km.h^{-1} of moderate running on a treadmill for 28 weeks showed a reversion of all the observed aging effects on papillary muscle.

Conclusion: The present study supports the concept that long–term exercise training restrains the aging-related deleterious changes in the papillary muscle. (Arq Bras Cardiol 2009;92(5):356-360)

Key words: Exercise; papillary muscles; aging; rats

Introduction

The papillary muscles are an integral part of the ventricles and play an important role in their geometry and systolic function[1-4] Previous studies of age-related variations in the papillary muscle[1-3,5] showed that the density of myocytes, connective tissue and capillaries as well as the cross-sectional area of myocytes and the physical and biochemical characteristics of the papillary muscle are dependent upon age[6-13]. The most evident age-related effects on the papillary muscle are the loss of muscle cells and an increase in connective tissue[9,10].

On the other hand, it has been demonstrated that aerobic exercise alters some of the age-associated changes in the wall of the left ventricular myocardium of rats[14-17]. However, no data are available on the effect of regular exercise on papillary muscle changes caused by aging. Therefore, in this study, we investigated the effects of aging on the morphology and the density of myocytes, capillaries and connective tissue and the cross-sectional area of myocytes of the papillary muscle of the left ventricle and hypothesized that a long-term moderate exercise program would have a protective role against the deleterious effects of aging.

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Material and methods

Animals and procedures

Fifteen 6-month-old male Wistar rats, weighing about 420 g each one, were housed in groups of five in a temperature- and light-controlled room and were fed ad lib. The animals were randomly divided into young control (YC), exercise-trained (E) and untrained (U) groups. The E group ran on a treadmill 5 days/wk. The program was progressively increased in intensity and duration of exercise until the 4th week, when the rats ran 1 hour daily at 1.8 km.h^{-1}. This level was maintained throughout the rest of the experimental period. The total training period lasted 28 weeks. The type of exercise used in this experiment can be considered a moderate exercise. The rats from the U group were placed daily for 10 min on the stationary treadmill

to provide an equivalent amount of handling. The animals´ handling was approved by our University Ethics Committee, in adherence to the "Guide for the Care and Use of Laboratory Animals" (Institute of Laboratory Animal Resources, National Academy of Sciences, Washington, D. C. 1996) and the Ethical Principles in Animal Experiments of the Brazilian College of Animals Experiments (COBEA).

Tissue sample preparation

At the end of the experiment, the animals from the U and E groups, at 13 months of age (considered to be "middle-aged"[4,18]), were anesthetized with Pentobarbital sodium (3 mg/100 g body weight, intraperitoneally) and then killed. The additional group of five animals (young control group, YC), was killed at 6 months of age. The hearts were arrested in diastole. The myocardium was perfused through the aorta at a constant pressure of 80 mmHg, using 0.1 M cacodylate buffer (3 min), followed by a 2.5% glutaraldehyde solution diluted in cacodylate buffer.

The animals were heparinized prior to fixation to optimize perfusion-fixation. The heart was then removed, the left ventricle was opened and the papillary muscle was dissected. Each papillary muscle was minced into approximately 3-mm wide and 5-mm long slices. These tissue slices were post-fixed in osmium tetroxide in sodium cacodylate buffer for 1 h. The tissue was dehydrated in graded alcohols, embedded in Epon resin and sectioned, so that the muscle cells of one half of the blocks were cut in cross-section and the other half in longitudinal section. Thin sections for transmission electron microscopy analysis were stained with uranyl acetate[19] and lead citrate[20]. The longitudinal sections were used to evaluate the fixation state of the myocardium by measuring the sarcomere length in the 3 groups.

Stereology

Two randomly chosen blocks from each papillary muscle, in which the myocytes were cut in cross-section were used for the quantitative analysis. The ultrathin sections were placed on a copper grid and 10 randomly chosen fields per block were selected for micrographs, which were taken with a Jeol transmission electron microscope. Low-power (x600) electron micrographs were used for a quantitative analysis of the tissue composition of the left ventricle papillary muscle. Each of the electron micrographs was analyzed using a digital image processing software for the biomedical area (Axio Vision, Zeiss). A total of 300 random electron micrographs were analyzed. A test-system with 72 sampling points was set upon the monitor screen and calibrated.

The myocardium was analyzed concerning the myocytes (my) and the cardiac interstitium (connective tissue [ct]). Volume density was estimated for myocyte (Vv[my]), and connective tissue (Vv[ct]): (V_V[structure]: = P_p[structure]/P_T) (P_P is the number of points that hit the structure; P_T is the total test points). Length density was estimated for myocardial capillaries, L_V[ca]: = $2Q_A$ mm/mm^3, and Q_A was also estimated for myocytes (Q_A [my]). Q_A is the numerical density per unit area. The numerical density of cell profiles or capillaries in a given area was estimated using the following formula: $Q_{A=}\sum$ profiles/given area.

Myocyte mean cross-sectional area (Area[my]) was also determined for each animal and in each group. Results are presented as means ± SEM, computed from the average measurements obtained from each animal.

Statistical analysis

Statistical significance was evaluated by ANOVA and Bonferroni's test, and *P* values lower than 0.05 were considered to be statistically significant.

Results

Effects of exercise

Figure 1 shows the ultramicroscopic appearance of the cross-sectional sections of papillary muscle of the left ventricle in the present groups of rats. Electron microscopic analysis of ultrathin sections revealed the presence of sparse focal areas of interstitial and replacement fibrosis across the myocardium of the papillary muscle of U group of rats at 13 months (Fig. 1C, D) when compared to YC rats (Fig 1A, B). In E rats, the program of continuous treadmill running used in this study reduced the interstitial fibrosis in the old trained papillary muscle (Fig. 1 E, F). The sarcomere length of all the tissue sections was of similar length (approximately 2.1 μm) (Fig. 2) and therefore, they could be compared. Otherwise, they could not have been compared, as different sarcomere lengths would affect the cell diameters and consequently, cell volumes, and thus, volume density.

General features of the three groups of rats and their left ventricles are shown in Table 1. Body weights of the E and U groups at the start of the training (at 6 months old) were 421±8 g and 427 ± 9 g, respectively (NS). At the end of the running program, body weight of the aged animals (at 13 months old) in the E group (461±10g) was significantly lower than in the aged (also 13 months old) U group (509±11g) ($P<0.05$). Left ventricular weight increased by 19.5 % in the E group ($P<0.05$). The LV weight to body weight ratio was significantly higher ($P<0.05$) in the aged animals from the E group compared to the animals in the U group.

The quantitative changes in the papillary muscle myocardium with age and the effects of exercise are shown

Table 1 – Body weights and heart weights of 3 groups of studied rats

Parameter/Groups	YC	U	E
n	5	5	5
Age, mo	6	13	13
Body wt, initial, g	421 ± 9	427 ± 9	421±8
Body wt, terminal, g	-	509±11	461±10[a]
LV, g	0.912± 8	0.920±32	1.100±46[b]
LV wt/body wt, x10^{-3}	2.1±0.05	1.8±0.07	2.3±0.15[a]

Groups: YC - Young Control; U - Untrained; E - Exercise-trained; Values are shown as means±SEM; n - number of rats; LV - Left Ventricle; [a] P<0.05 vs U; [b] P<0.05 vs YC and U.

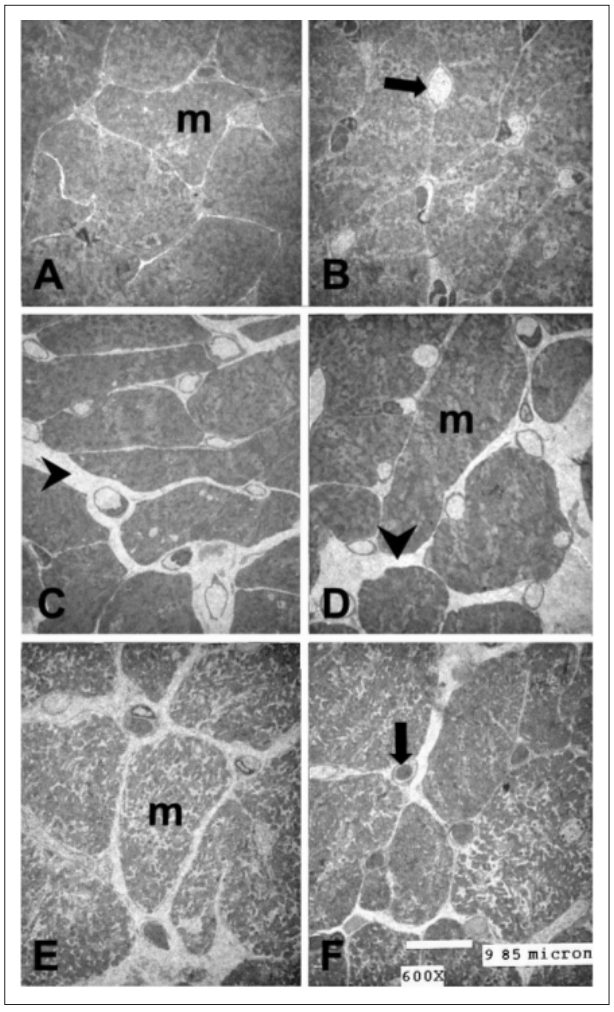

Figure 1 - Ultrathin sections of Epon-embedded tissue blocks of the papillary muscle of rats from the YC (panels A and B), U (panels C and D), and E (panels E and F) groups. Observe the interstitial and replacement fibrosis (arrowheads) in the U group (panels C and D), not observed in the other groups (YC, panels A and B and E group, panels E and F). Arrows - capillaries; m - myocytes. Capillaries profiles are easily identified and muscle cell boundaries can be definitely visualized. Bar - 9.85 µm.

in Table 2. The volume density of myocytes in the aged myocardium of the animals in the U group was 11% lower than that measured in the YC group ($P<0.05$). As shown in Table 2, alterations in the opposite direction from those seen for the myocyte compartment occurred in the interstitial volume fraction of the tissue. This quantitative constituent was higher in the aged U myocardium, compared with that of the YC group ($P<0.05$). The length density of the myocardial capillaries was slightly lower in aged U rats when compared with the YC rats (NS). Counts of myocyte profiles per square millimeter showed that a reduction of 20% in the number of myocytes occurred in the U group with age ($P<0.05$). Aged rats from the U group showed myocyte hypertrophy; the area [my] increased 50% in the aged U animals compared to those from the YC group ($P<0.05$). However, these age-related changes were not found in the aged rats submitted to the chronic exercise training (Table 2).

Table 2 – Stereological parameters (Mean±SEM)

Parameter /Groups	YC	U	E
Vv[my] (%)	83±4[a]	74±5	80±6[a]
Vv[ct] (%)	8±4[a]	14±5	12±1[a]
Cv[ca] (10^2 mm/mm^3)	17±9	16±8	20±2[b]
QA[my] /mm^2	5±1[a]	4±1	5±1[a]
Area [my] (µm^2)	180±74[a]	270±83	210±60[c]

Groups: YC - Young Control; U - Untrained; E - Exercise-trained; Vv - Volume Density; Lv - Length Density; QA - numerical density per unit area; my - myocytes; ct - connective tissue; ca - capillaries. [a] P<0.05 vs U, [b] vs YC and U and [c] vs YC

The physical exercise significantly increased the Vv[my], in the aged rats and it was also efficient in decreasing the myocardial fibrosis: Vv[ct] was more than 25% lower in the exercise-trained animals (Table 2). However, it maintained the increase in the Area[my] caused by aging. There was no difference between the U vs. E groups regarding the Area[my]. Physical exercise also apparently improved myocardial vascularization in the E group: L_V[ca] was higher in the aged E rats than in the U rats ($P<0.05$).

Discussion

The description of the changes in the structure and function of the heart and the factors that may contribute to reverse the changes that occur with aging is taking on greater importance, as more than 50% of the mortality and the majority of the hospitalizations of the elderly result from cardiovascular diseases[21]. Therefore, that is the importance of the isolation and identification of the effects of aging alone, from those related to pathological changes.

It is a general belief that as the heart ages, it undergoes a certain degree of hypertrophy[9,17,21]. However, based on the current results, left ventricular weight did not consistently increase with age, a pattern also observed by others[7,22,23], and the present results.

In the present study, it was observed that aging was accompanied by morphoquantitative alterations in the papillary muscle of the left ventricle, the majority of which were attenuated by physical exercise. The quantitative results demonstrate that a significant loss of myocytes occurred with age in the papillary muscle of the left ventricle in the U rats. In this group, it became apparent that a 20% reduction in the numerical density of myocytes was observed. These results are similar to the findings obtained by others, which have shown a reduction in the total number of myocytes in the ventricles of rats from 4 to 12 months[7] and from 4 to 15 months[9]. According to Anversa et al[24], a comparable phenomenon occurs in the human heart, as well[12,25,26]. It is possible that the myocyte hypertrophy that occurred in the aged heart was capable of compensating for the loss of these muscle cells.

Our findings confirm previous reports showing that the decrease in the number of myocytes with age is associated with an increase in interstitial tissue[9,10]. Collagen accumulation as a function of age was also observed in the human heart[11,12]. The mechanisms responsible for the loss of myocytes with areas of

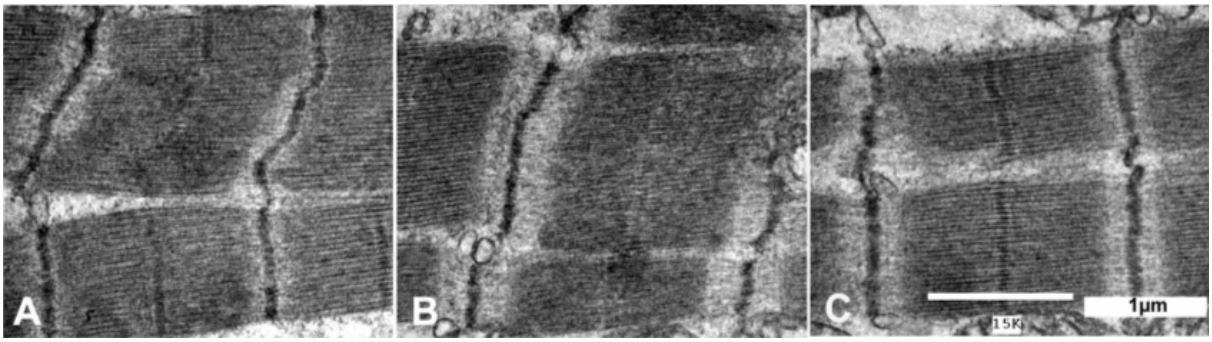

Figure 2 - Ultrathin sections of Epon-embedded tissue blocks of papillary muscle of rats from the YC (panels A), U (Panels B) and E (panels C) groups. Observe that muscle fibers show sarcomeres of approximately the same length in the three groups. Bar - 1 µm.

interstitial and replacement fibrosis in the myocardium are at present unknown[7]. Ischemia is a likely possibility[7,26]. However, in the present study, counts of capillary profiles did not show a decreased numerical density in the aged U rats. Under ischemia, the myocardial tissue responds by remodeling[27], by myocyte loss due to both apoptosis and necrosis[28-31] and by reparative fibrosis[32,33]. According to others[34], myocyte loss resulted mainly from apoptosis – genetically programmed cell death. It is possible that these age-related changes in the papillary muscle were responsible for the impairment of both papillary muscle stiffness and LV compliance in advanced aging[35].

Changes in the myocyte cross-sectional area[17] have been previously used to characterize aging–induced hypertrophy at the cellular level of organization. However, Anversa et al[7] demonstrate that myocyte cellular hyperplasia also participates in the hypertrophic response of the myocardium during senescence[7]. The continued loss of myocytes in the ventricles can be expected to generate a greater workload on the remaining myocytes, which may function as a chronic mechanical stimulus for cellular growth[7,11,12,24,36].

The aerobic exercise during aging improved the myocardial vascularization and normalized the myocardial fibrosis. The higher concentration of capillary profiles can be expected to produce an elevation in endothelial luminal surface accessible for oxygen exchange in the tissue and a smaller maximum diffusion distance for oxygen transport to the myocytes[7].

Conclusion

In conclusion, this study provided morphoquantitative information demonstrating that daily practice of moderate exercise during aging restrains some aging-related deleterious changes in the papillary muscle, such as loss of myocytes, increase in interstitial tissue, and a decreased numerical density of capillary profiles.

Potential Conflict of Interest

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

Sources of Funding

There were no external funding sources for this study.

Study Association

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