Original article

Influence of resistance exercise on the effects of aging upon the rat myocardium

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Abstract

Maintaining the proportions of the myocytes, interstitium and collagen, is fundamental for myocardial function, This study examined the changes produced by aging in the densities of myocites, interstitium and collagen of the left ventricular myocardium male Wistar rats and to see whether resistance exercise may influence these changes. Three groups of animals were used: Control Group, sacrificed at 13 months of age, Sedentary Group, sacrificed at 16 months of age, and Trained Group, sacrificed at 16 months of age. The volume densities (Vv) of cardiac myocytes, interstitium, and collagen were determined in histological sections of the left ventricular wall. It was concluded that aging decreased the area occupied by myocytes with an increase in the area occupied by the interstitium and collagen. Resistance exercise promoted the reversal of collagen accumulation, not influencing the effects of aging in the space occupied by myocytes and interstitium in the myocardium.

Keywords: aging, left ventricle, resistance exercise, stereology.

1 Introduction

In a normal adult male, the proportion of components of the ventricular myocardium is such that the myocytes occupy about 80% of the space, and the extracellular matrix occupies 20%, where collagen predominates (ÁGUILA, MANDARIM-DE-LACERDA and APFEL, 1998). With aging, these proportions change, with a decrease in the density of myocytes, and an increase of the matrix and collagen (JUNQUEIRA and CARNEIRO, 1999). These changes reduce the functional reserve of the heart (CARVALHO FILHO, SOUZA and FIGUEIRA, 1998), predisposing to ventricular dysfunction and heart failure in the elderly (NÓBREGA, FREITAS, OLIVEIRA et al., 1999; BENEDICTO and BOMBONATO, 2003).

Numerous studies have shown the benefits of exercising regularly for the cardiovascular system during aging (MAIOR, 2003). It helps regulate blood pressure and decrease heart rate changes (SANTARÉM, 2000), reducing risk factors for ventricular dysfunction (WEINECK, 2005). However it is not known whether resistance exercise can influence the changes of proportions in the components of the myocardium due to aging.

In this study we used Wistar rat, and stereological methods to assess whether anaerobic exercise can reverse the changes produced by aging in the densities of myocytes, interstitial space, and myocardial collagen components in the left ventricle.

2 Material and methods

Fifteen thirteen-month-old male *Wistar* rat, which were divided into three groups with five animals in each group, were used: The Control group (CG), whose animals were sacrificed at 13 months of age; the Sedentary group (SG), whose animals were sacrificed at 16 months of age; and the Trained group (TG), with animals subjected to a resistance training program from 13 to 16 months of age, when they were sacrificed.

2.1 *Training protocol*

All animals underwent a pre-adaptation to the training protocol and equipment for five days. The equipment used to carry out the program of strength training with the animals was a vertical ladder made of wood with iron steps. The height of the equipment (ladder) is 110 cm with an inclination angle of 80°. The top of the equipment has a plastic box lined with newspaper for the accommodation of the animals in the interval between sets (DUNCAN, DAVID and GORDON, 1998; HORNBERGER and FARRAR, 2004).

For the training of the animals, a burden on their tails was added every week, with lead weights. The rat climbed the ladder in order to reach a resting area at the top. This procedure was repeated six times during five days of the week, when we completed the process of adaptation.

The SG group animals did the climbing of the ladder exercises only once a day, five times a week, without overload until their sacrifice in order to cause a stress similar to that of the trained group. The training of the TG group animals was carried out with six continuous repetitions per day, five times a week for sixteen weeks with an interval of 45s between reps in order to rest the animals. The overcharge was established from Heyward's proposal (1998). As the increased overload was related to the weight of animals, every week all animals were weighed and their loads adjusted.

After anesthesia, the animals were sacrificed, the hearts were removed, sectioning the great vessels near the heart, and weighed in a digital analytical scale with accuracy of 0.001g using the Scherle method (1970) (immersion in saline physiological solution in a Becker, suspended by a wire without touching the walls of the container). Then the atria were removed and the left ventricle was isolated and weighed separately.

2.2 Preparation for stereological analysis

Histological sections 6 μm² thick were stained with the Picrosirius red technique. The volume densities of myocytes ($Vv_{[myoc]}$), of cardiac interstitium ($Vv_{[int]}$) and of collagen fibers ($Vv_{[col]}$) were evaluated in these sections. The collagen fibers were analyzed with polarized light.

For stereological analysis, five randomly selected fields in each histological section were used, totaling 25 fields per animal. A rectangular test system model M42 with 408 points (multipurpose test system), which was superimposed on images displayed on the computer screen, was used. The Vvs were determined by counting the points that touched the myocytes, the cardiac interstitium and the collagen (MANDARIM-DE-LACERDA, 1995; ÁGUILA, MANDARIM-DE-LACERDA and APFEL, 1998). The following formula was used:

$$Vv_{[element]} = \Sigma P_{[element]}/Tp \quad (1)$$

where: $Vv_{[element]}$ = Volume density of each element; $\Sigma P_{[element]}$ = Sum of points on the element; and Tp = total number of points (408).

2.3 Statistical analysis

The data are presented as average standard deviation, and to detect differences among groups, it was used the Analysis of Variance (ANOVA) followed by Tukey post-test with a significance level of 5%. The data were analyzed with the statistical software SPSS, version 12.0.

3 Results

Figure 1 shows the appearance of myocardial components, in histological sections.

The area occupied by myocytes in CG (91%) decreased significantly to 88% ($P < 0.05$) in animals of SG and

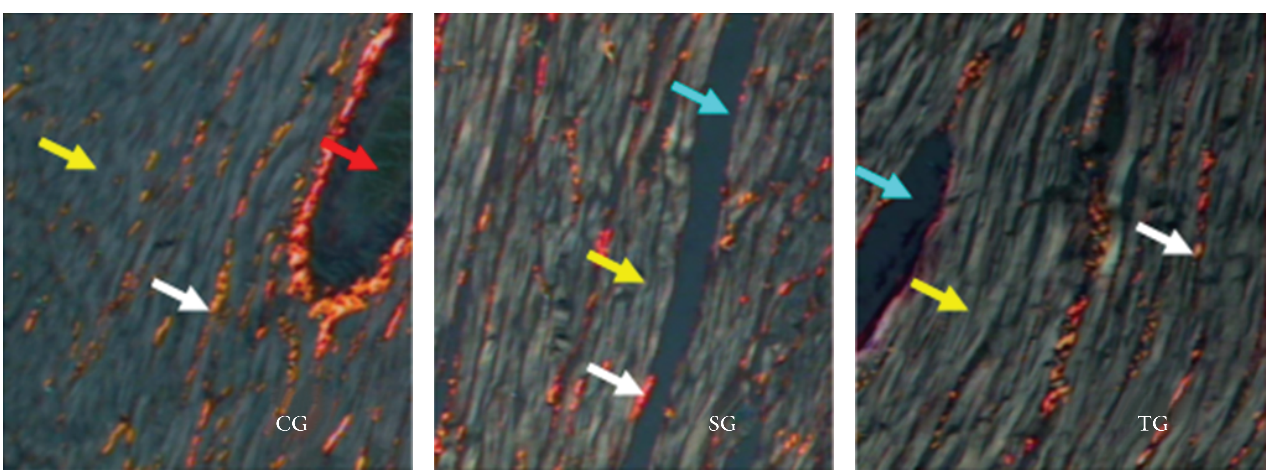

Figure 1. Histological sections of the wall of the left ventricle of the three groups of animals, stained with picrosirius and observed under polarized light. The arrows show: myocytes (yellow), collagen (white), interstitial space (blue), and blood vessel (red).

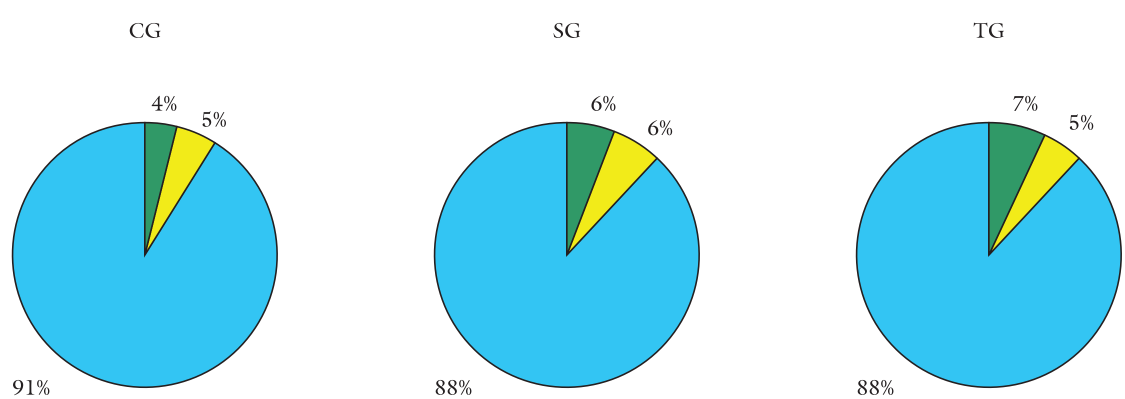

Figure 2. Volume density (Vv) of each of the components of the groups of animals' LV. (yellow: Collagen, blue: myocytes, green: interstitium).

TG groups. The SG and TG groups did not differ among themselves on this parameter (Figure 2).

The area occupied by interstitial space increased significantly in the SG (6%) and TG (7%) groups compared to the CG group (4%) ($p < 0.05$). The TG group animals showed a significant increase of interstitial space in relation to the SG group animals ($p < 0.05$) (Figure 2).

The area occupied by collagen increased significantly in the SG group (6%) compared to the CG group (5%) ($p < 0.05$). The trained group (TG) of animals showed no difference compared to the CG group (Figure 2).

4 Discussion

The present results were obtained using the rat as an animal model. The laboratory rat have been used by many investigators to study the structural adaptations of aging and exercises on the myocardium (THOMAS, 2000; DIFFE, SEVERSEN and TITUS, 2001; KEMI, CECI, WISLOFF et al., 2008; KWAK, SONG and LAWLER, 2006), and much useful information has emerged from these studies.

There are two major findings in this investigation. First, it was verified that aging altered the proportions of the three components of the myocardium, myocytes, interstitium and collagen in relation to the young group. Second, exercises did not reverse all these alterations, but only that of collagen tissue.

The present study showed that there was a reduction in the area occupied by myocytes promoted by aging and an increase of the areas occupied by interstitium and collagen. The reduction of the area occupied by myocytes in the myocardium of the elderly is due to cell death. The authors suggest the possibility of two mechanisms to explain the reduction of the number of cardiomyocytes in aging: necrosis (accidental and passive process) and apoptosis (programmed and active process).

Apoptosis, or programmed cell death, is a phenomenon actively regulated by proper cells destined for destruction (ÁGUILA, MANDARIM-DE-LACERDA and APFEL, 1998). This process typically occurs during cardiac development, postnatal maturation, hypoxia, ischemia, overload, and heart failure (ALBERTS, 2004). However, necrosis is cell death due to an acute injury. In this case, the cell swells and bursts spewing its contents over neighboring cells, causing an inflammatory response (ZAZYCKI and GOMES, 2009). Nowadays, it is believed that there are many factors that determine the aging of cardiomyocytes: oxidative process, inflammation, telomere integrity, metabolism, and altered expression of genes (BERNHARD, 2008).

The cardiac interstitium is composed primarily by collagen and a liquid component derived from capillaries by filtration (SULLIVAN, MARTINEZ, GENIS et al., 1998). With the loss of myocytes promoted by aging, there will be an overload of the remaining myocytes, which will require a greater supply of water, oxygen, nutrients, and electrolytes molecules, besides an increase of cellular excreta, carbon dioxide, and others. These related factors favor the increase in interstitial volume that we observed in the elderly group (GUYTON and HALL, 1997). In the present study, the area occupied by collagen increased significantly in the SG group (6%) compared to the CG group (5%) ($p < 0.05$). There was an increase in proportion of collagen in the myocardium with aging. The increase in the area occupied by myocardial collagen in aging is due to the death of cardiomyocytes (ANVERSA, PALACKAL, SONNEMBLICK et al., 1990). The increase in myocardial collagen which occurs with aging contributes to the decrease in ventricular elasticity in the elderly (BENEDICTO and BOMBONATO, 2003). Debessa, Maifrino and Souza (2001), studying the aging of human hearts and, in particular, collagen fibers, suggest a possible mechanism to the increase of cardiac collagen with age that could be an inhibition of the mechanisms of collagen degradation.

In the present work, resistance exercise did not reverse the reduction of the area occupied by myocytes promoted by aging. Maybe because young tissues respond better to forces than aged tissues (LANE and BUKWALTER, 1993). It was demonstrated an increase in the area occupied by myocardial interstitium in aging. The exercise not only failed to reverse this process, but also promoted a significant increase of interstitial space in the exercised group of rats.

The animals that performed physical exercise showed the same levels of collagen concentration of the control group, i.e.,resistance exercise reversed the accumulation of collagen promoted by aging. It is known that the collagen fibers, which are the major constituents of the interstitial space of the LV myocardium are influenced by exercises (THOMAS, 2000). Brilla, Matsubara and Weber (1996) states that in response to physical exercise, there is an accentuated remodeling of heart tissue, with beneficial changes that improve cardiac performance, increasing ventricular compliance, coronary reserve potential of oxygen, and nutrition to the myocyte, which reduces the risk of ventricular dysfunctions.

5 Conclusion

In conclusion, aging of the heart promotes the reduction of the area occupied by myocytes, with an increase in the area occupied by the interstitium and collagen. Resistance exercise promoted the reversal only of collagen accumulation, not influencing the effects of aging in the space occupied by myocytes and interstitium in the myocardium.

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