57 - EXERCISE REVERSES AGE-ASSOCIATED MORPHOMETRIC CHANGES IN THE RAT HEART

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INTRODUCTION

Myocites contribute 80% to the ventricular mass while the remained 20% are represented by the extracellular matrix, which contains fibroblasts, capillaries, collagen , elastin and other structural proteins (Roffe, 1998, Debessa et al, 2001; De Souza, 2002). With physiological ageing, the myocardium of the left ventricle undergoes structural change that includes a reduction in the number of myocites, the development of cardiac fibrosis and a reduction in the capillary density (Anversa et al, 1990; Aguila et al, 1998; Roffe, 1998; Debessa et al, 2001; De Souza, 2002). These alterations are associated with a reduction in function of the heart in the elderly (Wei e Gersh, 1987; Lakatta, 2000; 2002). In a recent publication, Lakatta & Levy (2003) emphasized the importance of the age-associated changes to the heart, as they provide an important precursor to cardiovascular disease. Other processes in the ageing heart that are likely to be detrimental to function are the reduction in calcium transport across membranes and decreased sensitivity of the cardiovascular system to â-adrenergic stimulation (Roffe, 1998).

Several investigations have shown that some of the age-associated changes in the heart can be reversed by exercise, thus improving the cardiac function (Blomqvist e Saltin, 1983; Moore & Korsick, 1995; Woolf-May et al, 1997), However, Gates et al (2003) reported that a programme of habitual aerobic exercise does not consistently modulate the changes in the left ventricle structure and diastolic function that occur with physiological ageing in men.

To provide insight into the possible modulation of left ventricle structure by habitual aerobic status with advancing age, we studied groups of young and middle-aged sedentary rats and middle-aged exercised trained rats.

MATERIALS AND METHODS

Five 6-month-old male Wistar rats weighing about 420 g each were trained to run 60 min/day 5 days/ wk at 30 m/min on a treadmil . The treadmill was maintained throughout the experiment on a 0 % slope. In this exercise program it has been shown that at the faster speed the rats were performing, at approximately 60% of their maximum oxygen consumption capacity (Lambert & Noakes, 1990). There was an initial training period of 10 days during which the speed and duration of the exercise was progressively increased. The total training period lasted for 24 weeks. An additional group of 5 animals was used as controls. These rats were placed daily for 10 min on the stationary treadmill to provide an equivalent amount of handling. All animals were killed at 12 months of age (middle-aged). Another group of 5 animals (initial group) were killed at 6 months.

After anesthesia with Nembutal (pentobarbital sodium), 3 mg/100 g body wt, intraperitoneally) the hearts were arrested in diastole. The myocardium was perfused through the aorta at a constant pressure of 80 mmHg, using 0.1 M cacodilate buffer (3 min) followed by 2,5% glutaraldehyde solution diluted in cacodilate buffer. The heart was then removed and a section was performed oriented at the middle of the ventricles. Measurements of the wall thickness were made on these sections, using a camera lucida attached to the microscope. Each piece was then embedded in Epon resin.

A total of 30 blocks with myocites oriented transversely were employed for the quantitative electron microscopic analysis of the components of the myocardium. A total of 300 random low-power electron micrographs, 10 from each tissue block , were printed at a calibrated magnification x1,800 and analysed morphometrically using a computerised program (Axion-Plus, Zeiss). The numbers of capillary and myocite profiles in the sampled area were counted to estimate their numerical densities per unit area. The transverse luminal area of capillary profiles and the transverse cross-sectional area of myocites were also determined using the same program. Results are presented as means ± SEM obtained from the average measurements from each animal. Statistical analysis significance for comparisons between two measurements was determined using the paired Student's *t* test, and *P* values of less than 0.05 were considered to be significant.

RESULTS

Qualitative observations. Electron microscopic analysis of ultrathin sections revealed the presence of focal areas of interstitial fibrosis across the ventricular wall of sedentary rats at 12 months. These areas were not observed in hearts of trained rats. The impact of these modifications resulted in an accumulation of collagen within the myocardium (figure 1)

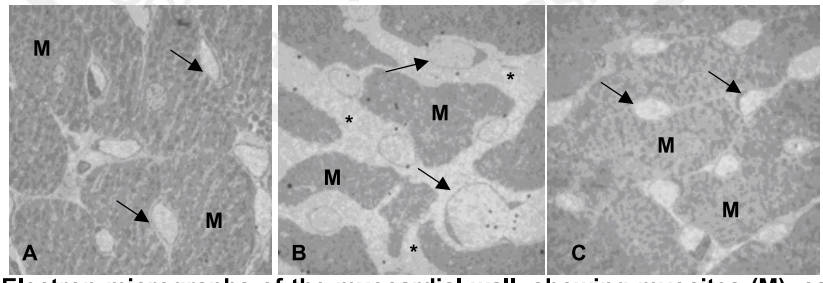

Figure 1- Electron micrographs of the myocardial wall, showing myocites (M), capillaries (arrows) and interstitium (*) from 6 months (A), sedentary (B) and trained (C) rats. Notice the increase of the interstitium in (B). (x 600)

Quantitative observations. The changes in capillary bed and myocites are illustrated in Table 1.

Table 1 Changes in the morphometric characteristics in the myocardium of Wistar rats as a function of age and exercise

	Age (mo)		
(exercised)	6	12 (sedentary)	12
¹ . of myocite profiles/mm² 2,990 ± 27 of myocardium	2,918 ± 27	2,653 ± 29	
Transverse cross seccional area of 287 ± 7.6 myocites ìm²	281 ± 9.5	88	± 8.3?
¹ . of capillary profiles/mm²of 3,826 ± 28.9 myocardium	3,900 ± 31	3,666 ± 28.7	
Transverse luminal area of capillary ± 1.8 profiles, ìm²	21 ± 1.6	28 ± 2.5?	20

Values are means ± SEM; ?Significantly different from those measured at 6 months of age (p<0.05).

The results showed no intergroup differences in the ventricular wall thickness. During the interval from 6 to 12 months, the number of myocites profiles per square millimeter of myocardium decreased by 9% (p 0.05), in the sedentary rats. However, in the 12 months exercised rats, the number of myocytes per square millimeter was similar to that of the 6 months rats.

In the sedentary rats, the transverse cross-sectional area of myocites decreased by 33% in the myocardium during the interval from 6 to 12 months (P<0.05). However, in the exercised rats, this parameter showed practically no variation.

On the whole tissue level, counts of capillary profiles showed a reduced numerical density of 6% in the myocardium of the sedentary rats (P > 0.05) but the mean area of capillary lumen increased by 33% in this group (P< 0.05*)*.

DISCUSSION

The morphometric results of this investigation domonstrate that a significant loss of myocites occurred with age in the left ventricular myocardium of sedentary rats. In this group, it became apparent during the period from 6 to 12 months, when a 9% reduction in the number of myocites was counted. These results confirm the findings of other research which have shown a reduction in the total number of myocites in the ventricles of rats from 4 to 12 months (Anversa et all, 1990). According to Anversa et al (1986), muscle cell loss that occur in the aging process of the myocardium precedes ventricular dysfunction (Anversa et al, 1986). These observations provide evidence that a comparable phenomenon occur in the human heart as well (Unverferth et al, 1986; Roffe, 1998). The decrease in the number of cardiac myocites with age is associated with an increase in interstitial tissue (Roffe, 1998; Águila et al, 1998 and present results).

The mechanisms responsible for the loss of myocites and the interstitial fibrosis in the myocardium with age are at present unknown. It is possible that ischemia is a likely possibility, since the capillary bed presents similar decrease in number (Rakusan & Poupa, 1964; Tomanek, 1970; Yin et al, 1982; Hachamovitch et al, 1989 and present paper). This decrease produces a reduction in the endothelial surface accessible for oxygen exchange in the tissue and a greater average diffusion distance for oxygen transport to the myocites. This phenomenon in association with impairment in coronary blood flow hemodynamics may constitute the principal factors implicated in cell loss and conjunctive tissue accumulation in the ventricles with aging (Anversa et al, 1990). On the other hand, we observed an increase in the transverse luminal area of capillary profiles in the sedentary group of rats. It is possible that this phenomenon occurred as a mechanism of compensating for the loss of capillaries with aging.

The aging associated changes observed in the sedentary rats were not present in the exercised rats. These results provide some evidence to indicate that a 24 week running programme, in rats, can alter some cardiac morphometric parameters of ageing from the left ventricle. These findings also provide evidence of the effect of moderate intensity upon ageing of cardiac myocites and capillaries, and while they cannot automatically be considered a health benefit, if viewed in the context of other findings it could be postulated that this is a positive change since myocites and capillaries becomes more like that of younger rats.

In conclusion, the results of this study showed that the morphology and morphometric characteristics of myocites and capillaries from rat myocardium are influenced by chronic exercise and that adaptations to cardiac structure may be possible using a moderate exercise intensity with realistic exercise volumes. Admitting that these results can be applied to humans, they provide a further indication of the potential health benefits of moderate exercise, which are of a nature that can be performed by a large proportion of the adult population (Woolf-May et al, 1997). Additional advantages of advocating this form of exercise are a reduced risk of injury.

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REFERENCES

ÁGUILA, M.B.; MANDARIN-DE-LACERDA, C. A.; APFEL, M. I. R. Estereologia do miocárdio de ratos jovens e idosos. **Arq Bras Cardiol**, 70(2):105-109, 1998 .

ANVERSA, P.; HILER, B.; RICCI, R.; GUIDERI, G.; OLIVETTI, G. Myocyte cell loss myocyte hypertrophy in the aging rat heart. **J Am Coll Cardiol** , 8: 1441-1448, 1986.

ANVERSA, P.; PALACKAL, T.; SONNENBLICK, E.H.; OLIVETTI, G.; MEGGS, L. G.; CAPASSO, J. M. Myocyte cell loss and myocute cellular hyperplasia in hypertrophied aging rat heart, **Circulation Research** 67:871-885, 1990.

BLOMQVIST, C. C.; SALTIN, B. Cardiovascular adaptations to physical training. **Ann Rev Physiol**, 45:169-189, 1983.

DEBESSA, C.R.G.; MAIFRINO L.B.M.; DE SOUZA, R.R. Age related changes of collagen network of the human

heart. **Mech Ageing Dev**., 122:1049-1058, 2001
DE SOUZA, R.R. Aging of myocardial collagen., **Biogerontology,** 3:325-335, 2002
GATES, P. E.; TANAKA, H.; GRAVES, J.; SEALS, D.R. Left ventricular structure and diastolic function with human ageing. **Eur Heart J**, 24:2213-2220, 2003.
HACHAMOVITCH, R., WICKER, P.; CAPASSO, J. M.; ANVERSA, P., Alterations of coronary blood flow and reserve with aging in Fischer 344 rats. **Am J Physiol,** 256: H66-H73, 1989.
LAKATTA, E. G. Cardiovascular aging in health. **Clin Geriatr Med,** 16(3):419-444, 2000.
LAKATTA, E. G. Age-associeted cardiovascular changes in health: impact on cardiovascular disease in older persons. **Heart Fail Rev** , 7(1):29-49 2002).
LAKATTA, E. G.; LEVY, D. Arterial and cardiac aging: major shareholders in cardiovascular disease enterprises: Part II: the aging heart in health: links to heart disease. **Circulation,** 107(2): 346-354, 2003.
LAMBERT, M. I.; NOAKES, T. D. Spontaneous running increases VO2 max and running performance in rats. **J Appl Physiol**, 68:400-403, 1990.
MOORE, R. L. & KORSICK, D. H. Cellular adaptations of the myocardium to chronic exercise. **Prog cardiovasc Dis,** 37:371-396, 1995.
RAKUSAN, K.; POUPA, O. Capillaries and muscle fibers in the heart of old rats. **Gerontology,** 9:107-112, 1964.
ROFFE,C. Ageing of heart. **Br J Biom Sci** 55:136-148, 1998.
TOMANEK, R.J. Effects of age and exercise on the extent of the myocardial cappilary bed. **Anat Rec**, 167:55-62, 1970.
UNVERFERTH ET, D.V.; BAKER, P. B.; ARN, A.R.; MAGORIEN, R. D., FETTERS, J.; LEIER, C. V. Aging of the human myocardium: A histologic study based upon endomyocardial biopsy. **Gerontology,** 32:241-251, 1986.
YIN, F. C. P.; SPPURGEON, H. A. ; RAKUSAN. K.; WEISFELDT, M. T.; LAKATTA, E. G. Use of tibial lenght to quantify cardiac hypertrophy: Application in the aging rat. **Am J Physiol**, 243:H941-H947, 1982.
WEI. J. Y.; GERSH, B. J. Heart disease in the elderly. **Curr Prob Cardiol**, 12:1-65, 1987.
WOOLF-MAY, K.; BIRD, S.; OWEN, A. Effects of an 18 week walking programme on cardiac function in previously sedentary or relatively inactive adults. **Br J Sports Med**, 31:48-53, 1997.

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EXERCISE REVERSES AGE-ASSOCIATED MORPHOMETRIC CHANGES IN THE RAT HEART

Abstract. Aging of the heart is associated with a reduction in the number of myocites, a lower capillary density and accumulation of collagen within the myocardium. The aim of this investigation was to determine if these changes in the heart can be reversed by moderate aerobic-exercise. Fifteen Wistar rats were studied: 5 rats aged 6 months (initial group) and 10 other rats aging 12 months, 5 of which were sedentary and 5, runner rats (60 min/day, 5 days/week at 30 m/min on a tredmill). Several ultrathin sections obtained from the left ventricle were examined in the electron microscope and electronmicrographs were used for morphometric studies of the myocardium. The density of myocites and capillaries and the transverse cross-sectional area of myocites decreased in the sedentary rats, whereas in the trained rats, they remained unchanged. The interstitial tissue also increased in hearts of sedentary rats but not in the trained rats. The cross-sectional areas of capillaries increased significantly in the sedentary rats, possibly as a compensatory mechanism, due to the loss of capillaries with aging. These findings provide evidence of the effect of a moderate intensity exercise upon aging of cardiac myocites and capillaries. It could be postulated that this is a positive change since myocites and capillaries becomes more like that of younger rats.

Key-Words. Myocardial aging , exercise, morphometry

EXERCICE DÉLAIE CHANGEMENTS MORPHOLOGIQUES DUS AU VIEILLISSMENT DU COEUR DU RAT

Sommaire : Au cours du vieillissement du myocarde a lieu une perte de myocites, de capillaires et un accroissement du tissu interstitiel, surtout du collagène. Ce travail à pour but vérifier si ces altérations peuvent être délayés par un programme d'exercices modérés. Pour cette étude ont été utilisés quinze rats Wistar: 5 rats âgés de six mois (group initial) et les autres 10 rats ayant 12 mois, 5 entre eux sédentaires et 5 exercés (course sur le tapis roulant, 60 min/jour, 5 jours/semaine à vitesse de 30 m/min.) Une série d'échantillons ultraminces ont été faits à partir de blocs contenant des fragments du myocarde des animaux. Les échantillons furent examinés au microscope électronique de transmission et usés pour préparer des electronmicrographies qui ont servi pour des études morphométriques des myocites et capillaires. La densité des myocites et des capillaires et les surfaces des myocites en coupe transversale, réduisirent le cœur de rats sédentaires, tandis qu'ils ne produisirent pas d'altérations dans les rats exercés. La surface de la coupe transversale des capillaires a augmenté dans les rats sédentaires, peut-être par un mécanisme de compensation contre la perte de capillaires. Les résultats de ce travail, mettent en relief que la pratique d'exercices modérés peut décaler les changements provoqués par le vieillissement des myocites et capillaires du myocarde. Ainsi, peut-on considérer ce résultat comme positif, puisque les myocites et capillaires ont gardé des caractéristiques similaires a celles du rat jeune.

Mots clés. Vieillissment du myocarde, exercices, morphométrie.

EJERCICIO REVERTE ALTERACIONES MORFOMÉTRICAS DEBIDAS AL ENVEJECIMIENTO EN EL CORAZÓN DE LA RATA

Resumen. Durante el envejecimiento del miocardio ocurre pierda de miocitos, de capilares y aumento del tejido intersticial, especialmente del colágeno. El objetivo de este trabajo fue verificar si estas alteraciones pueden ser revertidas por un programa de ejercicios moderados. Quince ratas Wistar fueron utilizadas para este estudio: 5 ratas con 6 meses de edad (grupo inicial), y 10 otras ratas, con 12 meses de edad, siendo 5 ratas sedentarias y 5, entrenadas (corrida en rueda de andar, 60 mins/día, 5 días/semana, con velocidad de 30 m/mins). Fueron hechos varios cortes ultrafinos a partir de bloques conteniendo fragmentos del miocardio de los animales. Los cortes fueron examinados en el microscopio electrónico de transmisión y utilizados para hacer electronmicrografías, las cuales sirvieron para estudios morfométricos de los miocitos y capilares. La densidad de miocitos y de capilares y las áreas de los miocitos en corte transversal disminuyeron en el corazón de las ratas sedentarias mientras que no se alteraron en las ratas entrenadas. El área de corte transversal de los capilares aumentó en las ratas sedentarias, tal vez por mecanismo de compensación por la pérdida de capilares. El tejido intersticial aumentó en el grupo sedentario, pero no en el entrenado. Los resultados de este trabajo colocan en evidencia que la práctica de ejercicios moderados puede revertir alteraciones promovidas por el envejecimiento de los miocitos y capilares en el miocardio. Se puede considerar que éste es un resultado positivo porque los miocitos y capilares mantuvieron características semejantes a las de la rata joven.

Palabras llave: Envejecimiento del miocardio, ejercicio, morfometría

EXERCÍCIO REVERTE ALTERAÇÕES MORFOMÉTRICAS DEVIDAS AO ENVELHECIMENTO NO CORAÇÃO DO RATO

Resumo. Durante o envelhecimento do miocárdio ocorre perda de miócitos, de capilares e aumento do tecido intersticial, especialmente do colágeno. O objetivo deste trabalho foi verificar se estas alterações podem ser revertidas por um programa de exercícios moderados. Quinze ratos Wistar foram utilizados para este estudo: 5 ratos com 6 meses de idade (grupo inicial), e 10 outros ratos, com idade de 12 meses, sendo 5 ratos sedentários e 5, treinados (corrida em esteira, 60 min/dia, 5 dias/semana, com velocidade de 30 m/min). Vários cortes ultrafinos foram feitos a partir de blocos contendo fragmentos do miocárdio dos animais. Os cortes foram examinados ao microscópio eletrônico de transmissão e utilizados para fazer electronmicrografias, as quais serviram para estudos morfométricos dos miócitos e capilares. A densidade de miócitos e de capilares e as áreas dos miócitos em corte transversal diminuíram no coração dos ratos sedentários ao passo que não se alteraram nos ratos treinados. A área de corte transversal dos capilares aumentou nos ratos sedentários, talvez por mecanismo de compensação pela perda de capilares. O tecido intersticial aumentou no grupo sedentário, mas não no treinado. Os resultados do presente trabalho colocam em evidência que a prática de exercícios moderados pode reverter alterações promovidas pelo envelhecimento dos miócitos e capilares no miocárdio. Pode-se considerar que este é um resultado positivo, pois os miócitos e capilares mantiveram características semelhantes às do rato jovem.

Palavras-chaves. Envelhecimento do miocárdio, exercício, morfometria.