

Swim Training Attenuates Myocardial Remodeling and the Pulmonary Congestion in Wistar Rats with Secondary Heart Failure to Myocardial Infarction

Leslie Andrews Portes and Paulo José Ferreira Tucci

Universidade Federal de São Paulo e Centro Universitário Adventista de São Paulo - São Paulo, SP - Brazil

OBJECTIVE

To evaluate the effects of swimming on pulmonary water content in animals with heart failure (HF) after myocardial infarction (MI).

METHODS

After coronary occlusion, MI size 20% < MI < 40% of the left ventricle (LV) were considered moderate and those $\geq 40\%$ of the LV large. The animals swam for 60 min/day, 5 days/week for 8 weeks. The wet weight of lung, liver, atriums, LV and right ventricle (RV) as well as the dry weight of the liver and lung were determined. ANOVA and Tukey test were used for statistical analysis.

RESULTS

An increase in the atrium/body weight ratio was noted in the sedentary animals with moderate (MImod-SED: n=8) and large (MIlg-SED: n=10) infarctions in comparison to the sedentary control (C-SED: n=14) and trained (C-TR: n=16) rats. An increase in the RV/body weight and LV/body weight ratios was noted in the MIlg-SED. The heart/body weight ratio was higher in MIlg-SED when compared to the other groups. The infarcted trained animals presented diminished hypertrophy. The pulmonary water content was higher in MIlg-SED animals ($81 \pm 0.4\%$) than in C-SED animals ($79 \pm 0.4\%$). No differences were found for the other comparisons (C-TR: $79 \pm 0.4\%$; MImod-SED: $80 \pm 0.3\%$; MImod-TR: $80 \pm 0.6\%$; MIlg-TR: $79 \pm 0.7\%$).

CONCLUSION

The increase of cardiac mass and pulmonary water content presented by MIlg-SED was diminished in the trained animals. The results suggest that the practice of physical exercise can diminish HF and contribute to favorable cardiac remodeling.

KEY WORDS

Rats, myocardial infarction, swimming pulmonary water content, myocardial hypertrophy.

The reduced exercise capacity of congestive heart failure (CHF) patients is directly related to the pulmonary alterations caused by pulmonary congestion: reduced vital capacity, lower air flow rates and compromised gas transfer¹⁻⁴. Generally speaking, pulmonary congestion related to cardiopathies is a result of an elevation in the pulmonary venous pressure caused by the increased left ventricle (LV) filling pressure which is a consequence of the chronic LV dysfunction. Similar to humans, the increased LV filling pressure in rats who develop CHF after suffering a myocardial infarction (MI) is secondary to the LV systolic and diastolic dysfunction⁵⁻⁸.

The classic indication of bed rest is one of the standard measures recommended to CHF patients⁹. The influence of physical exercise on myocardial remodeling is a common study topic^{5,10-14}. Nevertheless, few studies have investigated the effects of physical training on pulmonary congestion associated with CHF that develops after a MI. Bech and associates¹⁵ observed an improvement in physical exercise capacity and diminished pulmonary congestion in rats that had suffered an infarction and were treated with captopril and coenzyme Q10, but did not evaluate the isolated influence of physical exercise. Libonati¹⁶ confirmed that physical exercise on a treadmill improved the diastolic function of isolated hearts after a MI and considered that this influence could enable the prevention of pulmonary congestion. However, he did not evaluate the pulmonary water content.

The present study evaluated the effects of swim training on pulmonary water content in animals with CHF after suffering a MI. The pulmonary water content in the animals who had suffered moderate and large infarctions was analyzed during a prolonged period of swim training.

METHODS

Study animals - Healthy, ten week old Wistar-EPM rats (Current nomenclature of the "International Index of Laboratory Animals", 6th Ed., 1993. Lion Litho Ltd.: Carshalton, Surrey, U.K.) weighing between 170 and 190 grams were used in the study. The animals were kept in boxes supplied with water and Nuvital animal feed *ad libitum*, in a controlled ambient with a temperature of roughly 22°C, 54% humidity and a 12-hour light/dark cycle. The animals were distributed into six experimental groups: Sedentary controls (C-SED, n = 14), trained controls (C-TR, n = 16), sedentary with moderate infarction (MI_{mod}-SED, n = 8), sedentary with large infarction (MI_{lg}-SED, n = 10), trained with moderate infarction (MI_{mod}-TR, n = 9) and trained with large infarction (MI_{lg}-TR, n = 8). The control animals were submitted to all surgical procedures except the coronary occlusion. The sedentary animals did not exercise during the study timeframe.

Myocardial infarction induction - The method used

to induce the MI was based on the work of Johns & Olson¹⁷ with minor adaptations. The animals were anesthetized with the intraperitoneal administration of cetamine (50 mg/kg) and xylazine (10 mg/kg). After orotracheal intubation, the animal was submitted to mechanical ventilation using the Harvard model 683 ventilator (frequency: 90 m/min, tidal volume: 2.0 ml). After a trichotomy, a thoracotomy was performed on the left hemithorax in the intercostal space of the *ictus cordis* and the heart was quickly exposed. The anterior interventricular branch of the left coronary artery was identified and occluded between the edges of the left auricular appendage and pulmonary artery trunk with Prolene 6-0 sutures. Pulmonary hyperinflation was induced and the thoracic wall was closed with a purse string suture that had been made earlier.

Infarction size was determined by planimetry. After sacrificing the animals, the hearts were dissected. The atriums, left and right ventricles were weighed. The left ventricle musculature was isolated and an incision in the posterior wall enabled analysis in one plane. To this end, the muscle mass was stretched and placed between two flat, transparent, glass slides, appropriate for histological studies. Transillumination, accomplished by placing a light source behind the set of slides, clearly differentiated the regions of MI fibrous scarring from the remaining musculature. The MI fibrous scarring and the total ventricular mass including the fibrous scarring were traced onto the slide. Using the software SigmaScan Pro 5.0 (Systat Software Inc., Richmond, California, USA), the areas of MI fibrous scarring (FS) and total ventricular mass (TVM) were determined. The MI size, expressed as a percentage, was determined using the formula:

$$\% \text{ MI} = \text{FS} / \text{TVM} \times 100$$

After determining MI size, they were classified as small if the scarring was less than 20% of the total left ventricle area, moderate if the scarring was greater than 20% but less than 40% and large if the scarring was greater than or equal to 40%.

Physical training protocol - In accordance with the documented timeframe for completion of MI scarring^{18,19}, physical exercise was initiated three weeks after the MI. The exercise protocol used was swim training and included two phases: adaptation and training. The adaptation phase consisted of the first six days of training. On the first day, the animals exercised in the pool for ten minutes. The exercise period was extended by ten minutes each day until the rats were swimming for sixty minutes. The training phase consisted of five sixty minute sessions per week for eight weeks. All the animals were able to swim for the whole sixty minutes. The training pool consisted of a fiberglass water tank and the water temperature was maintained between 32 and 35°C. Each swimming group was comprised of a maximum of

eight rats per square meter of pool surface area. The rats that had suffered an infarction were separated from the control animals.

Variables analyzed - Body weights before and after the protocol were taken. Cardiac mass and the water content in the lungs and liver were determined. The water content of the organs was obtained based on wet and dry weights. After measuring the wet weight, the tissues were placed in an oven and maintained at a temperature of 80°C for 72 hours. After measuring the dry weight of the lung and liver of each animal, the water content (%H₂O) of each organ was determined using the following equation:

$$\%H_2O = (\text{wet weight} - \text{dry weight}) / \text{wet weight} \times 100$$

Statistical analysis - The results are presented as mean ± mean standard errors. One way variance analysis was used for data comparison between the six groups of animals. When significant statistical differences were found (p < 0.05) the Tukey post hoc test was used to identify which groups presented differences. All analysis was conducted using the statistical computer program SPSS 12.0 (Systat Software Inc., Richmond, California, USA).

RESULTS

Animals - Thirty rats underwent the false surgery and all survived (fourteen sedentary controls and sixteen trained controls). From the 85 animals who underwent surgery to induce a MI, eleven (10%) died either during or within 48 hours of the surgery. Thirty-four animals presented an infarction less than 20% and were eliminated from the study. From the remaining forty rats, 21 were included in the sedentary infarction group and nineteen in the training infarction group. Three of the sedentary infarction animals and two of the training infarction animals died during the training protocol. The three sedentary rats that died had infarctions between 46% and 57%. The two rats in training had infarctions in the range of 58% of the LV.

Body Weight - ANOVA did not identify significant differences between the initial weights of the rats in the six groups. After the twelve week protocol the C-TR animals (245 ± 4 grams) and MIIg-TR animals (244 ±

7 grams) were significantly heavier (p < 0.05) than the C-SED (224 ± 8 grams), MImod-SED (224 ± 8 grams) and MIIg-SED (223 ± 7 grams) animals. The weights for the MImod-TR animals (239 ± 9 grams) did not differ from the other groups.

Infarction size - After determining the MI size using planimetry, it was confirmed that the MI size was 31 ± 2% for the MImod-SED animals, 31 ± 1% for the MImod-TR animals, 47 ± 2% for the MIIg-SED animals and 49 ± 4% for the MIIg-TR animals. Only the differences between the moderate and large infarctions were statistically significant (p < 0.05). There were no significant differences between infarction size comparisons for MImod-SED vs MImod-TR and MIIg-SED vs MIIg-TR.

Cardiac Mass - The data of the above mentioned cardiac structure weights in relation to body weight are shown in table 1 and figure 1.

The myocardial mass data clearly demonstrate the functional repercussions of the coronary occlusion and the effects of physical exercise. The data demonstrate a clear tendency in relation to the increase of myocardial mass in the animals who suffered an infarction and the attenuation of the cardiac growth in rats that were submitted to training. This trend is particularly emphasized in the animals with large infarctions. A definite example is the cardiac weight/body weight ratio that was significantly higher in the MIIg-SED animals in comparison to the other groups (p < 0.05). This increase was prevented by the physical exercise. The modifications seen in the atrial musculature and right ventricle emphasize the secondary physiopathological repercussions of a myocardial infarction. The significant increase in myocardial mass identified in the MIIg-SED group which was not seen in the MIIg-TR group, indicates the presence of pulmonary hypertension in the sedentary animals, which was prevented or diminished in the rats that exercised.

Pulmonary and liver water content - The pulmonary and liver water content results are summarized in figure 2.

The pulmonary water content was higher in the MIIg-SED animals (81 ± 0.4%) than in the C-SED animals (79 ± 0.4%). No significant differences were found for the other group comparisons (C-TR: 79 ± 0.4%; MImod-

Table 1 - Data (mean ± standard errors) of the cardiac mass mentioned earlier in relation to the body weight of the animals from the groups: sedentary controls (C-SED), trained controls (C-TR), sedentary with moderate infarction (MImod-SED), trained with moderate infarction (MImod-TR), sedentary with large infarction (MIIg-SED) and trained with large infarction (MIIg-TR)

	C-SED	C-TR	MImod-SED	MImod-TR	MIIg-SED	MIIg-TR
Atrium/BW	0.30 ± 0.08 ^a	0.32 ± 0.05 ^a	0.54 ± 0.25 ^{bc}	0.41 ± 0.14 ^{ab}	0.91 ± 0.39 ^c	0.46 ± 0.25 ^{ab}
RV/BW	0.63 ± 0.09 ^a	0.70 ± 0.07 ^a	0.83 ± 0.35 ^a	0.85 ± 0.18 ^a	1.29 ± 0.45 ^b	0.91 ± 0.40 ^{ab}
LV/BW	2.14 ± 0.27 ^a	2.17 ± 0.13 ^a	2.43 ± 0.28 ^{ab}	2.26 ± 0.21 ^a	2.58 ± 0.22 ^b	2.29 ± 0.10 ^{ab}
HRT/BW	3.08 ± 0.38 ^a	3.19 ± 0.18 ^a	3.80 ± 0.85 ^a	3.53 ± 0.40 ^a	4.79 ± 0.87 ^b	3.66 ± 0.64 ^a

The different letters indicate the significant statistical differences between the mean values (p < 0.05). Data for the right ventricle (RV), left ventricle (LV), the heart (HRT) and body weight (BW) are expressed in milligrams per gram of body weight.

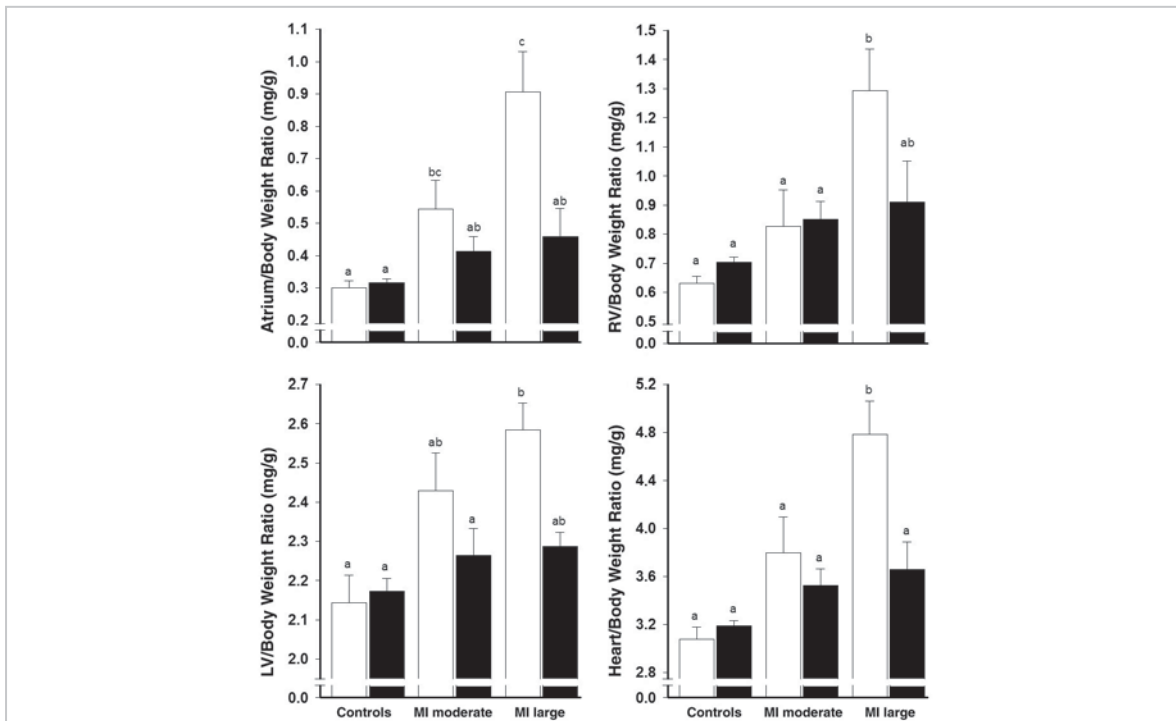


Fig. 1 - Ratio between cardiac mass (milligrams) and body weight (grams) after the experimental protocol. Significant statistical differences ($p < 0.05$) are indicated by the different letters. Data of the sedentary animals (white bars) and trained animals (colored bars) are expressed as mean \pm standard errors.

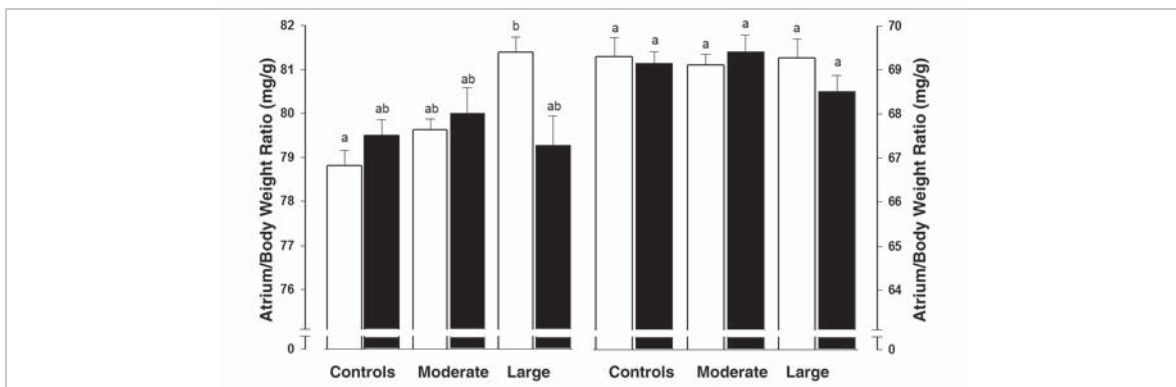


Fig. 2 - Percentage of liver and pulmonary water content after sacrificing the animals. Significant statistical differences ($p < 0.05$) are indicated by the different letters. Data of the sedentary animals (white bars) and trained animals (colored bars) are expressed as mean \pm standard errors.

SED: $80 \pm 0.3\%$; MI mod-TR: $80 \pm 0.6\%$; MI lg-TR: $79 \pm 0.7\%$). In summary, sedentary animals with large infarctions presented water accumulation – edema – in the lungs, while the animals that underwent physical training did not present elevated pulmonary water content.

DISCUSSION

Traditional cardiology therapy has established that it is beneficial or even necessary to recommend bed rest for patients with left ventricle dysfunction⁹. The basis of this proposal is intuitive: to diminish systemic demands that can overload the heart. This contradicts the concept that the regular practice of physical exercise is beneficial for the

heart. The results confirmed in this study indicate that non-observance of traditional therapy methods will not jeopardize and may even alleviate myocardial remodeling and heart failure, the most significant physiopathological outcome.

In our results, two indicators particularly emphasize the benefit of physical exercise in the animals that suffered an infarction: the pulmonary water content and the myocardial mass of the atriums and right ventricle.

Tissue water content in healthy organs is an extremely stable variable. Our results for the sedentary animals who did not suffer an infarction, demonstrate minimal pulmonary water content variations in a normal situation: $78.8 \pm 0.4\%$. It is logical to presume that the increased pulmonary water content, as seen in the sedentary



animals with large infarctions, is indicative of pulmonary congestion^{1,20-23}. Contrary to the condition of the MIIg-SED rats, the animals submitted to physical training – even those with large infarctions – did not present an increase in pulmonary water content, indicating the functional benefit of adequately programmed exercise routines. Similarly, the atrium and right ventricle myocardial mass indicators prove the beneficial action of exercise. The alterations in the mass of these structures emphasize the importance of left ventricle dysfunction and the consequent elevation of diastolic pressure in the LV, left atrium, pulmonary circulation and right chambers.

Only two literature references^{15,16} were found that considered the interactions between myocardial infarction in rats, pulmonary water content and physical exercise. Both considered that physical exercise could be beneficial in the prevention or attenuation of pulmonary congestion, however, they did not evaluate this parameter.

An inadvertent interpretation of the results, that indicate reduced myocardial remodeling and pulmonary congestion, could lead to the equivocal understanding that it is invalid to recommend that cardiopathy patients exercise caution in relation to physical training. The correct understanding of these data includes the recognition that the cardiac overload along with all its inconveniences and momentary risks still exist while practicing exercises due to the overload imposed on the heart and circulatory system. The benefits generated by regular and repeated exercise that is adequately intensified are a result of neurohumoral interferences that are activated during the physical exercise and are maintained either partially or for the entire interval between the training sessions.

There are supporting data indicating that the regular practice of physical exercise results in favorable modifications for the circulatory system related to: sympathetic activity²⁴⁻²⁸; parasympathetic activity²⁹; the renin-angiotensin system^{24,27,30}; reduced after load³¹; endothelial function^{32,33}; and plasmatic concentrations of aldosterone^{30,34}, endothelium³⁵⁻³⁸, arginine-vasopressin^{30,34}

and ANP³⁴. Additionally, heart activity caused by physical exercise promotes improved mechanical function in the myocardium and positively affects many functions such as the sarcolemma calcium channels³⁹, sarcoplasmic reticulum calcium content¹², sarcoplasmic reticulum calcium pump^{13,40}, phospholamban¹⁴, sodium-calcium exchange protein^{13,40}, and the responsiveness of myofilaments to calcium⁴¹.

Previous studies⁴² have documented neurohumoral data indicative of temporary deterioration of the congestive stage during physical exercise. The reported neurohumoral modifications are directly related to exercise intensity. Additionally, Braith and associates^{30,34} reported reduced neuroendocrine hyperreactivity in heart failure patients who were examined at rest following sixteen weeks of aerobic exercise. This information emphasizes the importance of two aspects related to the physical exercise/heart interaction: the intensity of the physical exercise and the importance of regular practice.

In summary, the increased cardiac mass and pulmonary water content, confirmed in the sedentary animals that had suffered large infarctions, were attenuated in animals submitted to swim training. These data suggest that regular physical activity routines after a myocardial infarction can diminish heart failure indicators and contribute to favorable cardiac remodeling.

Acknowledgments

This study was financed by Fapesp (Process 99/04533-4), CNPq (Process 300.692/80-3 NV), Unifesp and the Centro Universitário Adventista de São Paulo (Unasp). We would also like to express our thanks to Prof. Dr. Antônio Carlos Cicogna, for the lessons on papillary muscle methods and to Ednei L. Antônio, for the technical support.

Potencial Conflict of Interest

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

REFERENCES

- Hales CA, Kazemi H. Clinical significance of pulmonary function tests. Pulmonary function after uncomplicated myocardial infarction. *Chest*. 1977; 72 (3): 350-8.
- Moraes DL, Colucci WS, Givertz MM. Secondary Pulmonary Hypertension in Chronic Heart Failure: the Role of the Endothelium in Pathophysiology and Management. *Circulation*. 2000; 102: 1718-23.
- Mitani R, Haraguchi M, Takata S, et al. Excessive Ventilatory Response During Exercise in Patients With Non-Hypoxic Pulmonary Hypertension. *Circ J*. 2002; 66: 453-6.
- Gehlbach BK, Geppert E. The Pulmonary Manifestations of Left Heart Failure. *Chest*. 2004; 125: 669-82.
- Musch TI. Effects of sprint training on maximal stroke volume of rats with a chronic myocardial infarction. *J Appl Physiol*. 1992; 72 (4): 1437-44.
- Gheorghiadu M, Bonow RO. Chronic Heart Failure in the United States: a Manifestation of Coronary Artery Disease. *Circulation*. 1998; 97 (3): 282-9.
- La Vecchia L, Spadaro GL, Paccanaro M, et al. Predictors of right ventricular dysfunction in patients with coronary artery disease and reduced left ventricular ejection fraction. *Coron. Artery Dis*. 2002; 13: 319-22.
- Miyauchi T, Fujimori A, Maeda S, et al. Chronic Administration of an Endothelin-A Receptor Antagonist Improves Exercise Capacity in Rats with Myocardial Infarction-induced Congestive Heart Failure. *J Cardiovasc Pharmacol*. 2004; 44 (suppl 1): S64-S67.
- Smith TW, Braunwald E, Kelly RA. O Manejo da Insuficiência Cardíaca. In: Braunwald, E, editor. *Tratado de Medicina Cardiovascular*. 4ª ed. São Paulo: Roca; 1996: 497-501.
- Musch TI, Moore RL, Leathers DJ, Bruno A, Zelis R. Endurance training in rats with chronic heart failure induced by myocardial infarction. *Circulation*. 1986; 74: 431-41.

11. Musch TI, Moore RL, Smaldone PG, Riedy M, Zelis R. Cardiac adaptations to endurance training in rats with a chronic myocardial infarction. *J Appl Physiol*. 1989; 66 (2): 712-9.
12. Zhang XQ, NG YC, Musch TI, Moore RL, Zelis R, Cheung JY. Sprint training attenuates myocyte hypertrophy and improves Ca²⁺ homeostasis in postinfarction myocytes. *J Appl Physiol*. 1998; 84 (2): 544-52.
13. Zhang LQ, Zhang XQ, Musch TI, Moore RL, Cheung JY. Sprint training restores normal contractility in postinfarction rat myocytes. *J Appl Physiol*. 2000a; 89: 1099-105.
14. Zhang LQ, Zhang XQ, NG YC, et al. Sprint training normalizes Ca²⁺ transients and SR function in postinfarction rat myocytes. *J Appl Physiol*. 2000b; 89: 38-46.
15. Bech OM, Sorensen JD, Jensen MK, Diamant B, Steiness E. Effects of long-term coenzyme Q10 and captopril treatment on survival and functional capacity in rats with experimentally induced heart infarction. *J Pharmacol Exp Ther*. 1990; 255 (1): 346-50.
16. Libonati JR. Exercise and Diastolic Function after Myocardial Infarction. *Med Sci Sports Exerc*. 2003; 35 (9): 1471-6.
17. Johns TNP, Olson BJ. Experimental Myocardial Infarction. I. A Method of Coronary Occlusion in Small Animals. *Ann Surg*. 1954; 140: 675-82.
18. Pfeffer MA, Pfeffer JM, Fishbein MC, et al. Myocardial infarction size and ventricular function in rats. *Circulation Research*. 1979; 44: 503-12.
19. Fletcher PJ, Pfeffer JM, Pfeffer MA, Braunwald E. Left ventricular diastolic pressure-volume relations in rats with healed myocardial infarction. Effects on systolic function. *Circulation Research*. 1981; 49 (3): 618-26.
20. Dwyer Jr EM, Greenberg HM, Steinberg G. Clinical characteristics and natural history of survivors of pulmonary congestion during acute myocardial infarction. The Multicenter Postinfarction Research Group. *Am J Cardiol*. 1989; 63 (20): 1423-8.
21. Dwyer Jr EM, Greenberg H, Case RB. Association between transient pulmonary congestion during acute myocardial infarction and high incidence of death in six months. *Am J Cardiol*. 1986; 58 (10): 900-5.
22. Musch TI. Elevated diaphragmatic blood flow during submaximal exercise in rats with chronic heart failure. *Am J Physiol Heart Circ Physiol*. 1993; 265: H1721-6.
23. Jasmin JF, Mercier I, Hnasko R, et al. Lung remodeling and pulmonary hypertension after myocardial infarction: pathogenic role of reduced caveolin expression. *Cardiovascular Research*. 2004; 63 (4): 747-55.
24. Liu JL, Irvine S, Reid IA, Patel KP, Zucker IH. Chronic Exercise Reduces Sympathetic Nerve Activity in Rabbits With Pacing-Induced Heart Failure: a Role for Angiotensin II. *Circulation*. 2000; 102: 1854-62.
25. Negrão CE, Rondon MU, Tinucci T, et al. Abnormal neurovascular control during exercise is linked to heart failure severity. *Am J Physiol Heart Circ Physiol*. 2001; 280: H1286-92.
26. Roveda F, Middlekauff HR, Rondon MU, et al. The effects of exercise training on sympathetic neural activation in advanced heart failure: a randomized controlled trial. *J Am Coll Cardiol*. 2003; 42 (5): 854-60.
27. Zucker IH, Wang W, Pliquett RU, Liu JL, Patel KP. The Regulation of Sympathetic Outflow in Heart Failure: the Roles of Angiotensin II, Nitric Oxide, and Exercise Training. *Ann NY Acad Sci*. 2001; 940: 431-43.
28. Medeiros A, Oliveira EM, Gianolla R, Casarini DE, Negrão CE, Brum PC. Swimming training increases cardiac vagal activity and induces cardiac hypertrophy in rats. *Braz J Med Biol Res*. 2004; 37 (12): 1909-17.
29. Buch AN, Coote JH, Townend JN. Mortality, cardiac vagal control and physical training – what's the link? *Experimental Physiology*. 2002; 87 (4): 423-35.
30. Braith RW, Edwards DG. Neurohormonal Abnormalities in Heart Failure: Impact of Exercise Training. *Congest Heart Fail*. 2003; 9 (2): 70-6.
31. Hambrecht R, Gielen S, Linke A, et al. Effects of Exercise Training on Left Ventricular Function and Peripheral Resistance in Patients with Chronic Heart Failure: a Randomized Trial. *JAMA*. 2000; 283: 3095-101.
32. Symons JD, Rendig SV, Stebbins CL, Longhurst JC. Microvascular and myocardial contractile responses to ischemia: influence of exercise training. *J Appl Physiol*. 2000; 88: 433-42.
33. Kobayashi N, Tsuruya Y, Iwasawa T, et al. Exercise Training in Patients With Chronic Heart Failure Improves Endothelial Function Predominantly in the Trained Extremities. *Circ J*. 2003; 67: 505-10.
34. Braith RW, Welsch MA, Feigenbaum MS, Kluess HA, Pepine CJ. Neuroendocrine activation in heart failure is modified by endurance exercise training. *J Am Coll Cardiol*. 1999; 34 (4): 1170-5.
35. Bowles DK, Laughlin MH, Sturek M. Exercise training alters the Ca²⁺ and contractile responses of coronary arteries to endothelin. *J Appl Physiol*. 1995; 78 (3): 1079-87.
36. Maeda S, Miyauchi T, Iemitsu M, Sugawara J, Nagata Y, Goto K. Resistance Exercise Training Reduces Plasma Endothelin-1 Concentration in Healthy Young Humans. *J Cardiovasc Pharmacol*. 2004; 44: S443-6.
37. Maeda S, Tanabe T, Miyauchi T, et al. Aerobic exercise training reduces plasma endothelin-1 concentration in older women. *J Appl Physiol*. 2003; 95: 336-41.
38. Matsakas A, Mougios V. Opposite effect of acute aerobic exercise on plasma endothelin levels in trained and untrained men. *Med Sci Monit*. 2004; 10 (10): CR568-71.
39. Bowles DK, Hu Q, Laughlin MH, Sturek M. Exercise training increases L-type calcium current density in coronary smooth muscle. *Am J Physiol*. 275 (Heart Circ Physiol 44) 1998; H2159-69.
40. Lu L, Mei DF, Gu AG, et al. Exercise training normalizes altered calcium-handling proteins during development of heart failure. *J Appl Physiol*. 2002; 92: 1524-30.
41. Wisløff U, Loennechen JP, Currie S, Smith GL, Ellingsen Ø. Aerobic exercise reduces cardiomyocyte hypertrophy and increases contractility, Ca²⁺ sensitivity and SERCA-2 in rat after myocardial infarction. *Cardiovascular Research*. 2002; 54: 162-74.
42. Kjaer A, Appel J, Hildebrandt P, Petersen CL. Basal and exercise-induced neuroendocrine activation in patients with heart failure and in normal subjects. *Eur J Heart Fail*. 2004; 6 (1): 29-39.