

## Impact of Aerobic Exercise on Restoration of Soleus and Gastrocnemius Muscles Microcirculations in Wistar Rats with Chronic Heart Failure

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**ABSTRACT**

**Background and Objective:** Scientific evidence reveals that the density of skeletal muscle microcirculations decreases in the patients with chronic heart failure. Therefore, this study aimed to determine the impact of submaximal aerobic exercise training on restoration of fast/slow-twitch muscle fibers microcirculation in rats with myocardial infarction.

**Methods:** In this experimental study, 30 male infarcted Wistar rats were randomly divided into three groups of sham (N=10), control (N=10) and exercise (N=10), four weeks after surgery (blockage of anterior descending artery). Daily aerobic exercise included running on a treadmill for 10 weeks (5 times a week) at a speed of 17 m/min within 10 to 50 minutes. The animals were anesthetized and then sacrificed with chloroform, 48 hours after the last training session. The microcirculation density of soleus (SOL) and gastrocnemius (Gw) muscles was evaluated by immunohistochemistry assay.

**Results:** Capillary density and capillary to SOL muscle fiber ratio significantly increased in response to exercise training. However, capillary density, capillary to Gw muscle fiber ratio and Gw muscle arteriolar density of Wistar rats with chronic heart failure were not significantly affected by the exercise program. Moreover, arteriolar density of Gw muscle increased significantly after the exercise program.

**Conclusion:** The ten-week aerobic exercise might non-uniformly improve the SOL and Gw muscles microcirculation in rats with chronic heart failure by increasing capillary density and capillary to fiber ratio in the SOL muscle and increasing the arteriolar density of the Gw muscle.

**Keywords:** Heart Failure, Angiogenesis, Exercise.

## INTRODUCTION

Scientific evidence suggests that heart failure not only affects myocardial function, it reduces the local blood flow and leads to loss of skeletal muscle function. The fatigue caused by physical activity and reduced functional capacity that are common in patients with heart failure could be due to blood flow deficiency and impaired skeletal muscle physiology (1, 2). Studies reveal that the structural and functional characteristics of skeletal muscles change dramatically in response to myocardial infarction (MI). In this regard, laboratory studies indicate muscle mass loss (3), increased number of fast-twitch fibers, decreased slow-twitch fibers (4), and restriction of skeletal muscle blood flow in humans (5) and animals (6, 7) after MI. In addition, systemic blood flow in the skeletal muscle of mice with heart failure decreases at rest and during physical activity (8). Experimental studies at the capillary level show that capillary density parameters (9) and capillary/fiber ratio (CF ratio) decrease following MI. On the other hand, at the arteriole level, the arteriole tonic sympathetic vasoconstriction activity increases that decreases the arteriolar diameter and elasticity capacity (10). However, the hypoperfusion phenomenon also further accelerates catabolism, reduces oxidative stress in muscles, decreases intracellular pH, increases production of free radicals and inflammatory cytokines, and leads to cell apoptosis. Meanwhile, chronic hypoxia caused by low systemic blood flow also cause damage to the muscle fibers and eventually leads to atrophy (11). In contrast, endurance exercise training contribute in revascularization of the damaged skeletal muscle through the processes of angiogenesis and arteriolar genesis. Scientific evidence reveals that exercise training significantly increases the number of capillaries in active skeletal muscles. In addition, long-term aerobic exercise mainly influences the endings of the vascular network (capillaries).

When performing physical activity, a set of factors take place including the skeletal muscle ischemic and hypoxic conditions, increase in blood flow or shear stress, vasodilation caused by increased shear stress, mechanical stretch in tissue, skeletal muscle contraction and the resulting metabolites that provide the conditions for vascularization of skeletal muscle tissue. In this regard, experimental studies indicate changes in the skeletal muscle capillary network that depends on the contraction pattern (isotonic or isometric), the type of exercise (endurance, strength or concurrent), work intensity or duration of activity (12). Although the mechanism of vascular repair in the skeletal muscle is not clear, recent studies show that vascularization of the target tissue depends on the myofibrils' type and pattern of using skeletal muscle fibers involved in physical activity (13). Based on the available scientific evidence, this study aimed to determine whether long-term aerobic exercise intervention program affect capillary density and arteriolar density of selected soleus (SOL) and gastrocnemius muscles of rats after MI and in laboratory conditions. Moreover, we aimed to investigate the possible rehabilitating role of aerobic exercise pattern on microcirculation density of slow/fast-twitch SOL and gastrocnemius muscle fibers in infarcted rats.

## MATERIAL AND METHODS

This experimental-laboratory study was performed on 30 male Wistar rats aged 6-8 weeks with a weight range of 180-210 g. Day/night light cycle regulation was done in standard laboratory environment (12-hour light/12-hour dark that began at 8 am). The animals had free access to food and water. Laboratory temperature was stabilized at  $24 \pm 2$  °C with 46-51% humidity. The subjects were divided into groups of four, and kept in standard polypropylene cages. The subjects with MI were randomly divided into a control (N=10, Sed-MI) and an exercise intervention

group (N=10, Ex-MI), four weeks after surgery. In addition, sham group (N=10) included opened-chest rats with no MI. Characteristics of the subjects are presented in Table 1.

First, rats were anesthetized with intraperitoneal injection of thiopental sodium (50 mg/kg ip). They were placed supine, their chest area was shaved and then they were placed on the operating table. The rats were prepared for intubation after complete anesthetization. Subject were connected to a ventilator (Model 683 Small Animal Ventilator, Harvard-USA) specific for rodents. Thoracic cavity was opened gently to expose the heart. Then, left anterior descending artery (LAD) was blocked with 0.6 silk threads. Lead II ECG (Powerlab) was recorded to ensure induction of MI. For the sham group, silk threads were passed underneath the LAD. After open surgery, the animals were exposed to pure oxygen to gradually recover from anesthesia.

Four weeks after surgery, rats in the exercise group were subjected to an exercise program consisting of running on treadmill for

rodents. This group performed long-term aerobic activity continuously with an increasing intensity for 10 weeks. The subjects performed running exercise at 10 m/min for 10 min during the first week. In the second week, they were forced to run at 14 m/min for 20 minutes. In the third week, the subjects performed the activities at 17 m/min (1 km/h) for 30 minutes. This group run at a constant speed of 17 m/min [equivalent to 50-60 % of maximal oxygen consumption ( $VO_2$  max)], with increase in the duration of the activity in each workout from 40 to 50 minutes. However, the intensity and duration of aerobic exercise remained constant from the fifth week until the tenth week. The rodent treadmill's incline was set at zero during the first three weeks, and then increased to 5% in the fourth week. However, the incline remained stable at 10% during weeks 5 to 10 (Table 2) (14). The rats performed treadmill exercise voluntarily. When the animal was feeling fatigued, treadmill was stopped for a short time (less than a minute). Meanwhile, the control (infarcted) and sham groups performed no exercise during the aerobic exercise program.

Table 1- General characteristics of subjects in the experimental groups

Group	Sham	Sed-MI	Ex-MI
Number	10	10	10
Age (Weeks)	6-8	6-8	6-8
Heart Rate (bpm)	375±48	370±35	364±41
Wight of Body (g)	336±23	356±41	321±19
Wight of Soleus (mg)	117±18	121±23	125±32
Wight of Gastrocnemius (mg)	1232±230	1200±176	1364±241
Heart Wight/Body weight Ratio (g/g) ×10 <sup>-3</sup>	2.74±0.21	2.97±0.12	3.72±0.17

Data are shown as Mean±SEM.

Table 2-Exercise training program

Weeks	1	2	3	4	5	6	7	8	9	10
Intensity (m/min)	10	14	17	17	17	17	17	17	17	17
Time (min)	10	20	30	40	50	50	50	50	50	50
Incline (%)	0	0	0	5	10	10	10	10	10	10

The subjects were sacrificed 72 hours after the last session of aerobic exercise, in desiccator containing a cotton wool soaked in chloroform. Then, middle section of the soleus (SOL) muscle and superficial, white part of the gastrocnemius (Gw) muscle of the animal's right leg were removed. Tissue samples were kept in paraffin at  $-20^{\circ}\text{C}$  for immunohistochemistry testing. After cutting 5-micron thick sections and preparation of slides for determination of capillary density and CF ratio, hematoxylin and eosin staining was performed. Arteriolar density was evaluated using alpha-actin antibodies (SantaCruz Biotechnology, Santa Cruz, CA, USA). Then, the slides were observed and studied using an Olympus microscope (BX53, Shinjuku, Tokyo, Japan) and the ImageJ software. In order to measure the desired indices (capillary density, CF ratio and arteriolar density), arterioles, muscle fibers and arteries were counted on 10 random microscope fields per section ( $200\times$  magnification) and expressed per square millimeter. The normality of data distribution was confirmed by the Kolmogorov-Smirnov test. One-way analysis of variance (ANOVA) and Tukey's test were used at significance level of  $P<0.05$ . Data were reported as mean  $\pm$  SEM.

## RESULTS

There was significant difference in the capillary density of SOL muscle between the

groups ( $P=0.003$ ,  $F=9.42$ ). In contrast, the capillary density of the control group reduced significantly compared to that of sham group ( $P=0.03$ ). The 10-week aerobic exercise increased the capillary density of SOL muscle compared to control group ( $P=0.025$ ), but that is not the case for sham group ( $P=0.69$ ). The capillary density of the Gw muscle differed significantly between the groups ( $P=0.04$ ,  $F=6.01$ ). Gw muscle capillary density in the infarcted groups reduced significantly compared to that of the sham group ( $P<0.05$ ). However, endurance training increased the Gw muscle capillary density by 8% in comparison with control group ( $p=0.056$ ). The capillary density of Gw muscle in the exercise group was significantly lower than that of in the sham group ( $P=0.01$ ). Moreover, the CF ratio in SOL muscle increased significantly in response to aerobic exercise compared to control group ( $P=0.01$ ). However, such difference was not observed in the case of SOL muscle from the sham group and CF ratio of the Gw muscle. On the other hand, the immunohistochemical evaluation revealed that the arteriolar density of SOL muscle in different groups had no significant difference ( $P=0.7$ ,  $F=2.04$ ). Arteriolar density of the Gw muscle in the control and sham groups had no significant difference. However, 10 weeks of exercise significantly increased the Gw muscle's arteriolar density in comparison with the sham and control groups (Table 3).

Table 3- Revascularization of SOL and Gwmuscles

Group	SOLmuscle			Gw muscle		
	Capillary density	CF ratio	Arteriolar density	Capillary density	CF ratio	Arteriolar density
Sham	813 $\pm$ 23	2.2 $\pm$ 0.6	65 $\pm$ 5	567 $\pm$ 29	1.2 $\pm$ 0.1	21 $\pm$ 5
Sed-MI	600 $\pm$ 17*	1.6 $\pm$ 0.3*	69 $\pm$ 10	465 $\pm$ *30	1.1 $\pm$ 0.1	17 $\pm$ 4
Ex-MI	772 $\pm$ 36&	2.0 $\pm$ 0.5&	77 $\pm$ 18	504 $\pm$ 16	1.5 $\pm$ 0.3	28 $\pm$ 11&*

Data are represented as mean $\pm$ SEM; \*  $P<0.05$  vs. sham group; & $P<0.05$  vs. sed group

## DISCUSSION

Based on the findings, the capillary density of SOL and Gw muscles in four-week infarcted rats reduced significantly by 26% and 17%, respectively. However, the arteriolar density of the muscles did not change significantly after MI. This is consistent with the results of Ogoh et al. study that reported a significant reduction in the capillary density of slow-twitch SOL muscle fibers and fast-twitch long toe extensor muscle fibers four weeks after MI, by 18.5% and 18.2%, respectively (15). Unlike the capillary density, 4-week post-MI arteriolar density of the SOL and Gw muscles did not change significantly.

No scientific evidence was found on arteriolar changes of the skeletal muscles after heart failure. Our study also showed that 10 weeks of submaximal aerobic training at 50-60%  $\text{VO}_2$  max four week after MI, helped restore capillary density and capillary to SOL muscle fiber ratio, but did not affect the arteriolar density of this muscle. This is consistent with the findings of Suzuki et al. that showed SOL muscle capillary density in healthy rats does not change significantly in response to endurance treadmill running at a speed of 20 m/min (16). This inconsistency could be due to the differences in parameters related to health and sickness of animals (MI) or physiological adaptation of the target tissue in response to aerobic exercise training. In this regard, study of White et al. suggested that the changes in blood vessel density depend on length of the exercise program (13). On the other hand, long-term aerobic exercise in the present study significantly increased the arteriolar density of gastrocnemius muscle compared to sham and control groups. Consistent with the findings of the present study, Laughlin et al. noted that 10 weeks of aerobic exercise with intensity of 30 m/min significantly increases the gastrocnemius muscle arteriolar density of healthy rats, but does not affect their SOL muscle arteriolar density (17).

Considering these findings, this study seems to be the first to evaluate the effect of submaximal aerobic exercise (running) on

reconstruction of blood vessels in skeletal muscles of rats with heart failure. Given the limited number of available studies, the mechanism of changes in blood vessels of fast/slow-twitch muscle fibers after heart failure is unclear. On the other hand, the results of this study show that regeneration of blood vessels in the fast/slow-twitch skeletal muscle fibers following chronic aerobic exercise does not follow the same pattern. It seems that this type of activity mostly affects the capillary density, capillary to SOL muscle fibers ratio and gastrocnemius muscle arteriolar density. In general, characteristics of skeletal muscle vascularization depend on muscle fibers recruitment pattern, composition of muscle fibers and secretion of factors involved in angiogenesis. Study of Gute et al. also showed that the highest rate of capillary growth in response to exercise was seen in muscles with the greatest relative increase in contractile activity (18). On the other hand, angiogenesis and arteriolarogenesis were triggered in response to exercise training by local metabolic factors (such as adenosine) induced by muscle fiber recruitment and shear stress, which led to secretion of angiogenic factors (19). Difference in CF ratio of various muscle fibers (20) or dependence of skeletal muscle angiogenic response to exercise on the type of muscle fiber (18) indicated that the signals required to initiate the angiogenesis process, originated in the contacted muscle fibers. In this regard, study of Brutsaert et al. demonstrated that secretion of vascular endothelial growth factor (VEGF) was the most important mitogen for endothelial cells that played a key role in angiogenesis, so that the role of oxidative fibers (slow-twitch) was more than the fast-twitch glycolytic fibers, following a session of treadmill exercise (21). Since submaximal aerobic exercise mostly recruits slow-twitch muscle fibers, it is likely that the increased VEGF secretion and presence of angiogenic metabolites such as adenosine lead to more increase in the capillary density of slow-

twitch muscle fibers compared to fast-twitch muscle fibers. Thus, local differences in the skeletal muscle capillary density between humans and animals with chronic heart failure in response to exercise training could be due to the unique phenotypic characteristics of muscle fibers. In the present study, submaximal endurance exercise increased the SOL and gastrocnemius muscles arteriole densities by 10% and 39%, respectively. Increased arteriolar density and unaltered capillary density of the gastrocnemius muscle in the exercise group indicate the hypothesis that the fast-twitch muscle fibers experience arteriole argenesis more than the slow-twitch muscle fibers. In this regard, the previous studies showed increased arteriolar density following exercise, induced by increase in density of arterioles with diameter of 8 to 20 micrometers (22). In fact, the formation of new arteries caused by the migration of smooth muscle cells to the capillary wall ultimately leads to generation of arterioles from capillaries (arteriolargenesis). In this regard, reports indicate the role of exercise training in stimulation of the arteriolargenesis process with an unknown mechanism (13). However, the balance between angiogenic factors such as tumor growth factor that leads to the proliferation and migration of smooth muscle endothelial cells (23) and recruitment of pericytes (24) is controlled by angiostatic factors such as angiostatin that inhibits the mentioned processes (25,26). Thus, it is necessary to evaluate the contribution of each of these factors in various muscle fibers in

response to exercise training to gain a better understanding of changes in the arteriolargenesis process in different types of muscle fibers. Muscle tissue's partial pressure of oxygen ( $PO_2$ ) is another factor affecting the arteriolargenesis process during exercise (27). The oxidative slow-twitch muscle fibers have more capillary density. Therefore, they might experience less  $PO_2$  decline during endurance training compared to fast-twitch muscle fibers. These changes will eventually lead to stimulation of arteriolargenesis in the fast-twitch muscle fibers.

### CONCLUSION

The results reveal that long-term submaximal aerobic exercise intervention at 50-60%  $VO_2$  max in infarcted rats might be effective in restoring SOL muscle capillary density and increasing the gastrocnemius muscle arteriolar density. In other words, aerobic exercise at intensities below the lactate threshold could act as an agent for cardiac rehabilitation of rats with chronic MI.

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### CONFLICT OF INTEREST

The authors declare no conflicts of interest regarding this manuscript.

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