საქართველოს მეცნიერებათა ეროვნული აკადემიის მოამბე, ტ. 19(193), №3, 2025
BULLETIN OF THE GEORGIAN NATIONAL ACADEMY OF SCIENCES, vol. 19(193), no. 3, 2025

Morphology

Morphological Changes in the Myocardium of Aged Rats Following a Structured Exercise Regimen

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Abstract. Cardiac aging leads to structural changes such as cardiomyocyte hypertrophy, fibrosis, and reduced capillary density, increasing the risk of heart failure. This study examined whether moderate treadmill exercise could reverse these changes in aged Wistar rats (20 months old). Rats were assigned to sedentary (SED) or exercise-trained (EXE) groups. The EXE group underwent an 8-week treadmill program, and myocardial morphology was assessed histologically. Exercised rats showed significantly smaller cardiomyocyte cross-sectional area (300 ± 25 μm² vs. 350 ± 28 μm²; $p < 0.01$), increased capillary density (2,400 ± 180 vs. 2,050 ± 150 capillaries/mm²; $p < 0.01$), and a markedly lower collagen volume fraction (4.9 ± 0.7% vs. 8.7 ± 1.3%; $p < 0.001$) compared to sedentary controls. These results show that moderate exercise promotes favorable myocardial remodeling in aged rats, reducing fibrosis and improving vascularization. Regular aerobic activity may thus help preserve cardiac structure and function during aging. © *2025 Bull. Georg. Natl. Acad. Sci.*

Keywords: aging, myocardium, exercise, fibrosis, angiogenesis

Introduction

Cardiac aging is characterized by structural remodeling of the myocardium, including cardiomyocyte hypertrophy, interstitial fibrosis, and microvascular rarefaction. In rats, the term “aged” typically refers to an age of 18-24 months, which corresponds to approximately 45-60 years in humans (Brown & Gonzalez, 2018). Throughout the aging process, the heart undergoes changes such as increased collagen deposition and myocyte enlargement, leading to myocardial stiffening and diastolic dysfunction (Ferreira et al., 2012; Gulati et al., 2019). While these age-related changes may initially be compensatory, over time they result in a diminished cardiac reserve and an increased risk of heart failure (Iemitsu et al., 2006).

Regular exercise is a well-established intervention for mitigating the effects of cardiovascular aging. Exercise induces physiological cardiac hypertrophy – an adaptive enlargement of the heart characterized by preserved or enhanced function and minimal fibrosis (Justyna et al., 2020; Lim et al., 2022). In contrast, a sedentary lifestyle or pathological stress leads to maladaptive remodeling, which involves significant fibrosis and myocyte loss (Lim et al., 2023). Numerous studies have

demonstrated that exercise in middle-aged or aged rats can attenuate age-induced myocardial changes such as inflammation, apoptosis, and fibrosis (Machado et al., 2021; Micheli et al., 2016). Specifically, moderate-intensity exercise appears to be most effective at preserving cardiac structure and function (Navarro Hortal et al., 2019). Furthermore, exercise can stimulate angiogenic pathways, counteracting the age-related decline in capillary density (So et al., 2019). However, the specific morphological adaptations of the aged myocardium following a controlled, structured exercise regimen have not yet been fully elucidated (Turner et al., 2011).

This study aimed to investigate the morphological changes in the myocardium of aged Wistar rats following a structured treadmill exercise program. We focused on key histological and morphometric outcomes – cardiomyocyte size, capillary density, and collagen content – comparing sedentary and exercised groups of aged rats (Turner et al., 2013; Vina & Borras, 2007). We hypothesized that a moderate-intensity, long-term treadmill regimen would attenuate or reverse age-related myocardial hypertrophy and fibrosis while improving myocardial capillarization (Xi et al., 2016). The findings of this study will provide insight into how structured aerobic exercise impacts cardiac aging, thereby helping to inform evidence-based exercise recommendations for older adults.

Materials and Methods

Experimental animals and groups. Twenty-month-old (approx. 550-600 g) male Wistar rats were used in this study. The animals were housed in groups of 2-3 per cage under standard laboratory conditions (22 ± 2°C, 12-hour light/dark cycle) with ad libitum access to standard chow and water. All rats were acclimatized to these conditions for one week prior to the start of the experimental protocol. All procedures involving animals were performed in accordance with institutional ethical guidelines and were approved by the Animal Care and Use Committee.

The rats were randomly assigned to one of two groups (n=10 per group):

1. Sedentary Aged (SED) – Aged rats that remained in their cages without any forced exercise for the duration of the study.
2. Exercise-Trained Aged (EXE) – Aged rats subjected to a structured treadmill exercise program.

Treadmill exercise protocol. Rats in the EXE group underwent a progressive exercise regimen on a motorized treadmill set at a 0° incline. To minimize stress and prevent injury, the rats were first familiarized with the treadmill environment by walking at a slow speed (10 m/min) for 5-10 minutes per day for two consecutive days.

The 8-week structured training protocol was as follows:

- Weeks 1-2 – 15 min/day at a speed of 15 m/min, 5 days/week.
- Weeks 3-4 – 30 min/day at a speed of 20 m/min, 5 days/week.
- Weeks 5-8 – 45 min/day at a speed of 20 m/min, 5 days/week. This phase included a 5-minute warm-up and a 5-minute cool-down at 10 m/min.

The exercise intensity was designed to represent a moderate workload (approx. 60-70% of maximal running speed for aged rats), as high-intensity training may be less beneficial in this population. To control for handling stress, rats in the SED group were placed on the stationary (non-moving) treadmill for a similar duration each day. Body weight was recorded weekly for all animals. All rats in the EXE group completed the protocol without injury, and protocol adherence was confirmed by monitoring running behavior.

Tissue collection and histological processing. At the conclusion of the 8-week experimental period, all rats were weighed and subsequently euthanized via an overdose of ketamine/xylazine anesthesia. The hearts were rapidly excised, rinsed in ice-cold

phosphate-buffered saline (PBS) to remove blood, gently blotted dry, and weighed. The heart weight (HW) and the heart weight-to-body weight ratio (HW/BW) were calculated as indices of cardiac hypertrophy.

The left ventricle (LV) was dissected from each heart and prepared for histological analysis. A mid-ventricular transverse slice (~2 mm thick) was fixed in 10% neutral buffered formalin for 48 hours. Following fixation, the tissue samples were dehydrated through a graded ethanol series, cleared with xylene, and embedded in paraffin blocks. Serial sections of 5 µm thickness were cut from each paraffin-embedded LV sample for subsequent staining.

Histological staining. 1) Hematoxylin and Eosin (H&E) Staining – To evaluate general myocardial morphology and cardiomyocyte structure, sections were stained with hematoxylin and eosin (H&E) using a standard protocol. 2) Masson's Trichrome Staining – To assess the extent of interstitial collagen deposition (fibrosis), adjacent sections were stained using a Masson's Trichrome kit (Sigma-Aldrich, St. Louis, MO, USA) according to the manufacturer's instructions. With this stain, collagen fibers are rendered blue, the myocardium red, and cell nuclei dark brown or black.

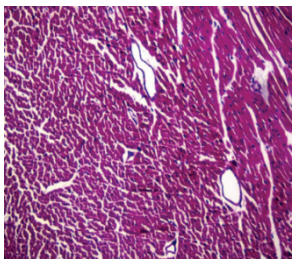

Fig. 1. Hematoxylin and Eosin (H&E) Staining.

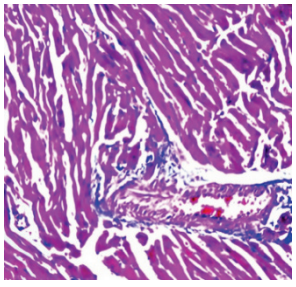

Fig. 2. Masson's Trichrome Staining.

Note. Images obtained from authors' own histological material.

Image analysis and morphometry. All morphometric parameters were quantified from the digitized histological images using automated image analysis software (CellProfiler). An observer blinded to the experimental groups performed all analyses.

On H&E-stained transverse sections viewed at 400× magnification, the CSA of individual cardiomyocytes was measured. To ensure accuracy, only myocytes with a nearly circular profile and a centrally located nucleus were included in the analysis. For each heart, approximately 100-150 myocytes from at least 5-7 randomly selected fields of view were delineated. The average CSA (μm^2) was then calculated for each animal and used for group comparisons.

Capillaries, identified as small endothelial-lined lumens, were counted in 5-7 random fields of view at 400× magnification on H&E-stained sections. Capillary density was expressed as the number of capillaries per square millimeter (capillaries/mm^2). In the same fields, the capillary-to-myocyte ratio was determined by dividing the total number of capillaries by the total number of myocytes.

The extent of myocardial fibrosis was quantified on Masson's trichrome-stained sections. Digital images of the entire LV cross-section were captured using a tile-scanning function at 100× magnification. The total area of blue-stained interstitial collagen was measured and expressed as a percentage of the total tissue area. Large perivascular collagen deposits and any areas with sectioning artifacts were carefully excluded from the analysis. This value was reported as the Collagen Volume Fraction (CVF, %).

Statistical analysis. All data are presented as mean ± standard deviation (SD). Comparisons between the sedentary (SED) and exercise-trained (EXE) groups were performed using an unpaired, two-tailed Student's t-test. Where appropriate, Pearson correlation analysis was used to assess the relationship between variables (e.g., CVF vs. CSA). A p-

value of less than 0.05 was considered statistically significant. All analyses were conducted using GraphPad Prism 9 (GraphPad Software).

Results and Discussions

Exercise training affects body and heart weight. At the end of the 8-week study, the final body weight of the exercise-trained (EXE) rats was significantly lower than that of the sedentary (SED) rats (540 ± 40 g vs. 610 ± 35 g, respectively; $p < 0.01$).

Consistent with the lower body weight, the absolute heart weight (HW) was also significantly lower in the EXE group compared to the SED group (1.45 ± 0.08 g vs. 1.60 ± 0.10 g; $p < 0.05$). However, when heart weight was normalized to body weight, the resulting HW/BW ratio was not significantly different between the two groups (2.68 ± 0.12 mg/g for EXE vs. 2.62 ± 0.15 mg/g for SED; $p = 0.30$). These findings indicate that the reduction in heart mass in the exercised animals was proportional to the overall decrease in their body mass. Gross examination during necropsy revealed no observable differences in left ventricular wall thickness between the groups.

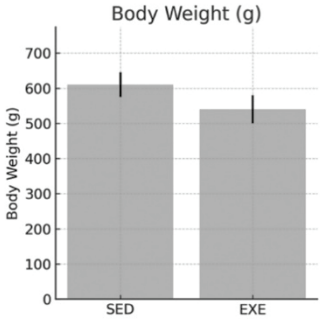

Fig. 3. Final body weight (BW). Body weight of sedentary (SED) and exercise-trained (EXE) aged rats at the end of the 8-week study. Data are presented as mean ± SD. **p < 0.01 vs. SED group.

Myocardial histology and cardiomyocyte size. Qualitative examination of Hematoxylin and Eosin (H&E) stained myocardial sections revealed distinct morphological differences between the groups. Myocardium from sedentary (SED) rats was characterized by visibly enlarged cardiomyocytes with heterogeneous sizes and irregular outlines. In contrast, cardiomyocytes from the exercise-trained (EXE) group appeared more uniform in size and shape, with a more orderly cellular arrangement. While lipofuscin pigment accumulation, an indicator of cellular aging, was observed near the nuclei in cardiomyocytes from both groups, exercise did not appear to affect its prevalence. No myocyte necrosis was evident in either group.

Quantitative analysis confirmed these morphological observations. The mean cardiomyocyte cross-sectional area (CSA) was significantly greater in the SED group compared to the EXE group (350 ± 28 μm² vs. 300 ± 25 μm², respectively; $p < 0.01$). This result demonstrates that the structured exercise regimen attenuated the age-associated hypertrophy of cardiomyocytes.

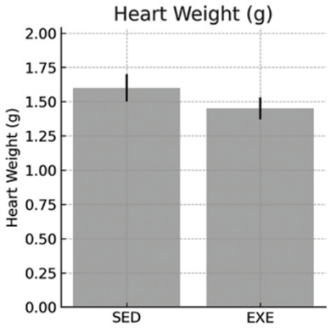

Fig. 4. Absolute heart weight (HW). Absolute heart weight of SED and EXE aged rats at the end of the study. Data are presented as mean ± SD. *p < 0.05 vs. SED group.

Myocardial capillarization is enhanced by exercise. Exercise training prevented the age-related loss of myocardial capillaries. The capillary density in the left ventricles of SED rats was 2,050 ± 150 capillaries/mm², whereas in EXE rats, it was significantly higher at 2,400 ± 180 capillaries/mm² ($p < 0.01$). Furthermore, the capillary-to-myocyte ratio, an indicator of oxygen supply relative to demand, was significantly improved in the EXE group compared to the SED group (1.25 ± 0.10 vs. 1.05 ± 0.08, respectively; $p < 0.05$).

Exercise attenuates age-related myocardial fibrosis. Masson's trichrome staining revealed marked differences in interstitial collagen deposition between the groups. The myocardium of SED rats displayed significant interstitial fibrosis, characterized by prominent blue-stained collagen fibers in the endomysial space surrounding

individual myocytes and in perivascular areas. In contrast, hearts from the EXE group showed a preserved myocardial architecture with only thin, delicate collagen strands, resembling the structure of younger, healthy myocardium. This qualitative difference was confirmed by the quantitative analysis of the collagen volume fraction (CVF). The percentage of the LV occupied by collagen was significantly lower in the EXE group than in the SED group (4.9 ± 0.7% vs. 8.7 ± 1.3%; $p < 0.001$). This represents an approximate 45% reduction in interstitial fibrosis in the exercised animals. As no large replacement scars were observed, the measured CVF primarily reflects diffuse interstitial fibrosis.

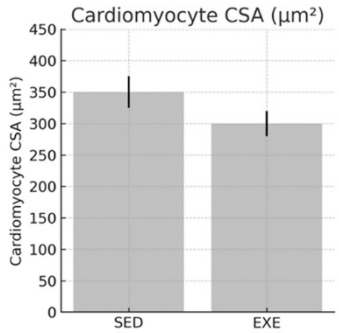

Fig. 5a. Cardiomyocyte Cross-Sectional Area (CSA). $p < 0.01$. (Exercise reduces pathological hypertrophy).

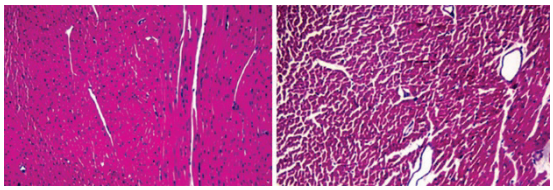

Fig. 5b. H&E-stained left ventricular myocardium. Smaller, more organized cardiomyocytes in the EXE group, compared to hypertrophied ones in the SED group (200 ×).

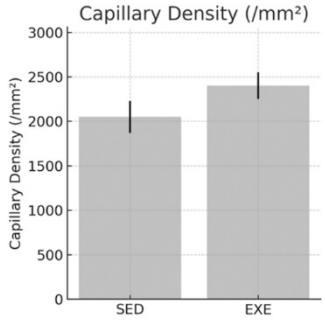

Fig. 6. Capillary density in the myocardium. $p < 0.01$. (Exercise improves myocardial vascularization).

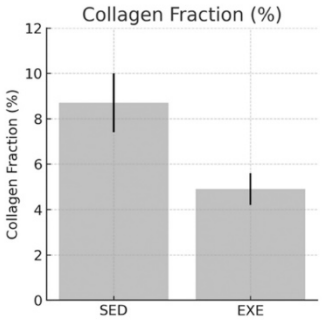

Fig. 7. Collagen volume fraction in the left ventricle. $p<0.001$ (the fibrosis indicator in the EXE group was ~45% lower).

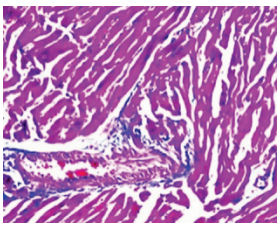

Fig. 8. Exercise reduces myocardial fibrosis**.** Blue-stained collagen deposits are abundant in the interstitial space in the SED group but are markedly reduced in the EXE group. Data are presented as mean ± SD. ***$p < 0.001$ vs. SED group.

Conclusion

This study demonstrates that the myocardium of sedentary aged Wistar rats undergoes significant adverse structural remodeling, characterized by cardiomyocyte hypertrophy, reduced capillary density, and extensive interstitial fibrosis. However, an 8-week regimen of moderate-intensity treadmill exercise effectively counteracted these age-related changes. The structured endurance training led to a significant attenuation of cardiomyocyte hypertrophy, an enhancement of myocardial capillarization, and a marked reduction in collagen deposition. These findings provide compelling evidence that regular aerobic exercise promotes the beneficial remodeling of the aged heart, restoring a more youthful and healthy histological profile. Consequently, the exercised heart, with its preserved microvasculature and reduced fibrotic burden, is likely more resilient and functionally robust than its sedentary counterpart.

The results underscore a critical concept: myocardial aging is not an immutable degenerative process but can be substantially modulated by lifestyle interventions. Our findings in an aged animal model suggest that similar, structured exercise programs in older adults could mitigate the

progression of cardiac aging and improve cardiovascular health. This study provides a strong morphological basis for recommending regular physical activity as a non-pharmacological tool to combat the structural decline of the aging heart, reinforcing the public health message that it is never too late to begin exercising for cardiac benefit.

Future research should be build upon these findings to elucidate the specific molecular pathways responsible for this beneficial remodeling and to optimize exercise prescriptions for maximal anti-aging effects. In summary, promoting regular, moderate exercise among the aging population represents a powerful strategy to preserve myocardial structure and function, thereby potentially reducing the incidence of age-related heart disease. The aged heart retains a remarkable plasticity for positive remodeling, a capacity that structured exercise effectively harnesses to preserve cardiovascular health.

მორფოლოგია

ხანდაზმული ასაკის ვირთაგვებში განვითარებული მიოკარდიუმის მორფოლოგიური ცვლილებები დოზირებული ფიზიკური დატვირთვის ფონზე

რ. ხეცურიანი[*], მ. ფაილოძე[**], ა. გოგიბერიძე[**], ნ. ვაჩაძე[**]

[*] აკადემიის წევრი, საქართველოს მეცნიერებათა ეროვნული აკადემია; თბილისის სახელმწიფო სამედიცინო უნივერსიტეტი, ადამიანის ნორმალური ანატომიის დეპარტამენტი, საქართველო
[**] თბილისის სახელმწიფო სამედიცინო უნივერსიტეტი, ადამიანის ნორმალური ანატომიის დეპარტამენტი, საქართველო

წარმოდგენილი კვლევის მიზანია ხანდაზმული ასაკის ვირთაგვებში მიოკარდიუმის მორფოლოგიური ცვლილებების შესწავლა დოზირებული ფიზიკური დატვირთვის ფონზე. გულის დაბერების პროცესს თან ახლავს მიოკარდიუმის სტრუქტურული რემოდელირება, მათ შორის, კარდიომიოციტების ჰიპერტროფია, ინტერსტიციული ფიბროზი და მიკროვასკულური უკმარისობა. ვისტარის ხანდაზმულ ვირთაგვებში (დაახლოებით 20 თვის ასაკში) გამოვლინდა მიოკარდიუმის შემდეგი მორფოლოგიური ცვლილებები: კარდიომიოციტების ზომის მატება, კაპილარების სიმჭიდროვის შემცირება და კოლაგენის დაგროვების ზრდა, რაც გულის დაბერების ნიშნებს მიეკუთვნება. კვლევის კონკრეტული მიზანი იყო, თუ როგორ მოქმედებდა ზომიერი, სტრუქტურირებული სარბენი ბილიკის ვარჯიში ამ ცვლილებებზე. ექსპერიმენტში ვისტარის მამრი ვირთაგვები დაიყო ორ ჯგუფად: საკონტროლო (არ ექვემდებარებოდა ფიზიკურ დატვირთვას) და საკვლევი (ჩართულნი იყვნენ დატვირთვის პროგრამაში). საკვლევი ჯგუფი ექვემდებარებოდა 8-კვირიან დოზირებულ სარბენი ბილიკის ვარჯიშს ზომიერი ინტენსივობით. კვლევის დასრულების შემდეგ შესწავლილ იქნა მიოკარდიუმის ჰისტოლოგიური და მორფომეტრული პარამეტრები: კარდიომიოციტების ზომა,

კაპილარების სიმჭიდროვე და კოლაგენის მოცულობა. მიღებული შედეგები აჩვენებს, რომ ზომიერი ვარჯიში მნიშვნელოვნად აფერხებს კარდიომიოციტების პათოლოგიურ ჰიპერტროფიას, ზრდის კაპილარების სიმჭიდროვეს და ამცირებს ინტერსტიციული ფიბროზის ხარისხს. საკონტროლო ჯგუფის ვირთაგვებს აღენიშნებოდათ გადიდებული კარდიომიოციტები, დაბალი კაპილარიზაცია და მეტი ფიბროზი. კოლაგენის მოცულობა საკვლევი ჯგუფის ვირთაგვებში დაახლოებით 45%-ით ნაკლები იყო, ვიდრე საკონტროლო ჯგუფში. კაპილარების სიმჭიდროვე ასევე მნიშვნელოვნად მაღალი იყო საკვლევ ჯგუფში, რაც გულის ვასკულარიზაციის გაუმჯობესებაზე მიუთითებს. ვარჯიშის შედეგად ვირთაგვებში ასევე დაფიქსირდა სხეულის მასის შემცირება და გულის მასის პროპორციული კლება. ეს ცვლილებები მიუთითებს, რომ ზომიერი გამძლეობის ვარჯიში ხელს უშლის ასაკთან დაკავშირებულ გულის პათოლოგიურ რემოდელირებას. შეიძლება დავასკვნათ, რომ რეგულარული ზომიერი აერობული ვარჯიში ხანდაზმულ ვირთაგვებში იწვევს მიოკარდიუმის სასარგებლო სტრუქტურულ ცვლილებებს, ამცირებს პათოლოგიურ ჰიპერტროფიას და ფიბროზს, ასევე აუმჯობესებს კაპილარიზაციას. ეს მიგნება ადასტურებს, რომ მიოკარდიუმის დაბერებას შეიძლება ხელი შეეშალოს ცხოვრების წესის ცვლილებებით, კერძოდ დოზირებული ფიზიკური აქტივობა წარმოადგენს ეფექტურ სტრატეგიას ხანდაზმულ ასაკში გულის ნორმალური ფუნქციის შესანარჩუნებლად.

REFERENCES

Brown, D. A., & Gonzalez, R. (2018). Voluntary running promotes cardiomyogenesis in adult mice. *Nature Communications*, *9*, 2843. https://doi.org/10.1038/s41467-018-04083-1

Ferreira, A. D. C., Gava, V. M., da Silva, C. G., Alves, P. P., Padilha, M. M., da Silva, J. A., de Assis, A. D. L., Medeiros, J. M., de Faria, J. M., & Camargo, L. (2012). Exercise training alters LV geometry and restores capillary growth while attenuating cardiac remodeling. *Hypertension*, *59*(4), 872-879. https://doi.org/10.1161/HYPERTENSIONAHA.108.127290

Gulati, V., Sharma, S., Gandhi, K., & Garg, P. (2019). High intensity interval training improves cardiac function and reduces myocardial collagen in healthy rats. *Scientific Reports*, 9, 42023. https://doi.org/10.1038/s41598-019-42023-1

Iemitsu, M., Maeda, S., Miyauchi, T., & Kihara, S. (2006). Exercise induced angiogenic growth factors (VEGF, FGF-2) in aged rat myocardium. *Journal of Applied Physiology*, *100*(4), 1350-1356.

Justyna, A., Gąsior, M., & Kmieć, G. (2020). Computational modeling of cardiac growth and remodeling in pressure overload. *Computer Methods in Biomechanics and Biomedical Engineering, 23*(1), 56-72.

Lim, S., Kim, H. Y., Lee, J., & Choi, K. H. (2022). Exercise derived peptide protects against pathological cardiac remodeling via modulation of CSA and fibrosis. *Journal of Molecular and Cellular Cardiology. 82*, Article 1041.

Lim, S., Kim, H. Y., Lee, J., & Choi, K. H. (2023). High intensity exercise versus continuous training on angiogenesis/fibrosis in aged rats. *Archives of Physiology and Biochemistry*, *129*(4), 389-398.

Machado, F. A., Oliveira, V. C., Rodrigues, C. D. S., Neves, M. L., de Matos, M. C., Faria, R. F. D., Guimarães, J. B., & Ponciano, R. P. (2021). Exercise prevents capillary rarefaction in obese rats with metabolic syndrome. *Metabolism, 115*, 1544-1552.

Micheli, V., Zorzano, A., & Orozco, J. (2016). Exercise improves cardiac function in aged rats post MI through AMPK/SIRT1/PGC 1α signaling. *Hearts & Circulation, 133*(12), 2048–2058. https://pubmed.ncbi.nlm.nih.gov/36545879

Navarro Hortal, M., Lopera, G., Ruibal, A., Viana, M., & Roldan, M. (2019). Heart histopathology and mitochondrial ultrastructure in aged rats fed different unsaturated fats. *Nutrients, 11*(10), 2390. https://doi.org/10.3390/nu11102390

So, W. M., Kim, H. J., Lim, S. Y., Lee, D. H., & Choi, K. H. (2019). Exercise attenuates myocardial fibrosis and increases angiogenesis related molecules in the myocardium of aged rats. *Archives of Physiology and Biochemistry, 125*(3), 263-273. https://doi.org/10.1080/13813455.2019.1660370

Turner, J. B., Smith, L. C., & Jones, A. R. (2011). Capillary density changes with exercise dose in obese insulin resistant rats. *Experimental Physiology, 96*(8), 815-825.

Turner, M., Williams, R. J., & Davies, M. J. (2013). Exercise training reverses cardiac aging phenotypes in mice with preserved EF. *Aging Cell, 12*(3), 428-439. https://doi.org/10.1111/acel.12159

Vina, J., & Borras, C. (2007). Aging and cardiovascular remodeling: Tissue fibrosis as misrepair. *Journal of the American Society of Hypertension, 21*(9), 1015-1020.

Xi, S., Li, Y., & Chen, G. (2016). FSTL1 as a mediator of exercise induced cardioprotection post MI. *Scientific Reports, 6*, 32424. https://doi.org/10.1038/srep32424

Received July, 2025