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Effects of Detraining Followed by Aerobic Exercise on Cardiac Stem Cells in Aged Male Rats

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A B S T R A C T

Introduction: The regenerative potential of cardiac stem and progenitor cells is affected by aging and detraining, with the C-Kit cardiac stem cell expressing the Nkx2.5 transcription factor playing a crucial role. Exercise is known to enhance organ regeneration during aging, but the mechanisms involved in new cardiomyocyte formation during physiological cardiac remodeling remain unclear.

Material & Methods: Eighteen aged Wistar rats (~440g) were divided into three groups: Control (CO), aerobic training (AT) (5 days per week, 50-75% of maximum speed) for six weeks, and detraining (DT) for four weeks. RT-PCR analysis determined Nkx2.5 gene expression, while immunohistochemical staining identified C-kit-positive and Ki67-positive cardiac progenitor cells.

Results: In heart tissue, C-Kit and Ki67 values significantly differed between the control–training (P=0.001) and training-detraining (P=0.001) groups but not between the control and detraining groups for C-Kit (P=0.502) and Ki67 (P=0.475). Nkx2.5 exhibited a significant difference between control-training (P=0.001), training-detraining (P=0.001), and control-detraining (P=0.006).

Conclusion: Exercise increased the proliferation of heart stem cells, activating C-Kit differentiation and elevating Nkx2.5 expression, thereby delaying the effects of aging. However, detraining significantly impacted heart stem cell function, emphasizing the importance of sustained exercise for optimal cardiac health.

Keywords: Physical activity, Aging process, Physical Deconditioning, Myocardial Regeneration, Stem Cells

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Introduction

The aging process is a general biological characteristic of live organisms. The gradual loss of heart tissue function is one of the most prominent aspects of aging (1). Various mechanisms have been identified that play a role in increasing lifespan. Many of these mechanisms are active in stem cells, and their primary role is to repair and restore cells, reducing the cellular aging process (2). There is a reserve of cardiac stem cells (CSCs) in the human heart (4). A special cardiac stem cell is C-Kit+ or CD117 (5). Its regeneration ability allows it to repair and regenerate cells as well as restore the anatomical and physiological features to the tissue, thereby enhancing the healing process and repairing heart damage more efficiently (6). A heart attack can be successfully treated with CSCs, especially with C-Kit+ (7). Myocardial repair was reduced in c-kit-deficient mice compared with healthy mice after heart damage; therefore, the decline in heart function and aging were accelerated. Along with aging, the number of colonies and the repair power of cardiac stem cells transplanted into C-Kit+ heart tissue decreases significantly.

C-Kit+ also expresses the transcription factor Nkx2.5 (8). Nkx2-5 is one of the earliest markers of the cardiac lineage, as it is abundantly expressed in the cardiac progenitor cells that form the cardiac crescent (9). Nkx2.5 is expressed throughout cardiac development and persists in the adult myocardium. Embryos lacking Nkx2-5 are nonviable due to growth retardation and gross abnormalities of the heart, including failure in ventricular chamber development (10). Several recent studies have demonstrated that early Nkx2-5 expressing progenitor cells are multipotent, giving rise to cardiomyocytes, smooth muscle, and endothelial lineages (11), and likewise, Nkx2.5 knockout embryos have significant defects in these lineages (10-12). Heart tissue self-renewal is indicated by increased Nkx2.5 gene expression during differentiation and regeneration (10). Alternatively, the Ki67 antigen is one of the most well-known proteins related to the cell cycle that exists only in proliferating cells. A positive Ki67 result is the best test for measuring cell proliferation (13).

C-kit plays a key role in CSCs during cardiac hypertrophy. Physiologic cardiac hypertrophy occurs simultaneously with an increase in heart cells during exercise. The heart maintains its normal structure with physiological hypertrophy. Cell differentiation, however, depends on the presence of stimuli and is still very limited in the heart. Exercise increases the number of stem cells and the ability of cells to self-renew under the influence of growth factors and positive mitogens, which could promote CSC proliferation (14). Increasing the number of resident stem cells in organs and improving their function provides a higher potential for the maintenance and repair of cell ability in the elderly.

It is critical to maintain an active lifestyle with aging because it contributes to the health of the heart and body. However, withdrawal from regular exercise is a very common problem in this population. Sports withdrawal caused by injury, vacation, or any other reason is associated with a loss of adaptations acquired via training, which also appears to affect heart tissue (15).

However, functional adaptation and morphology can be reduced after a short period of detraining (16).

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Detraining can be considered a partial or complete interruption of an exercise program or a partial or complete loss of exercise benefits in response to an inadequate exercise stimulus (17). Some studies have shown that metabolic and functional adaptation of exercise programs can be reduced even after short periods of inactivity due to illness and vacations (18). Detraining causes the loss of various adaptations, including cardiovascular (17).

A previous study showed that heart tissue is affected by aging and detraining (19). Cardiac physiological hypertrophy due to aerobic exercise has also been reported; however, it seems that the physiological hypertrophy mechanism and the regeneration pathways of cardiac stem cells also decrease with aging (15, 19).

Cardiovascular adaptation seems to be proportionate to exercise characteristics such as intensity and duration of exercise; therefore, inactivity and detraining may weaken the heart and cardiovascular system. This problem becomes more obvious in older people. Therefore, considering the importance of aging and detraining as well as research limitations in this field, there is no information about how detraining affects the heart and cardiac stem cells during aging. In this study, we investigated the effect of four weeks of detraining after aerobic training on cardiac stem cells in aged male rats.

Materials and methods

Animal and Ethical Statement

A 65-year-old male patient was referred to the Department of Prosthodontics at Tehran Dental Branch, Islamic Azad University, with a complaint of a missing right ear due to a history of an accident. On examination, a small remnant of the ear was present, along with a normal ear on the left side with normal hearing. Informed consent was obtained from the patient to use the photos for publication in the Journal of Basic Research in Medical Science (Figure1).

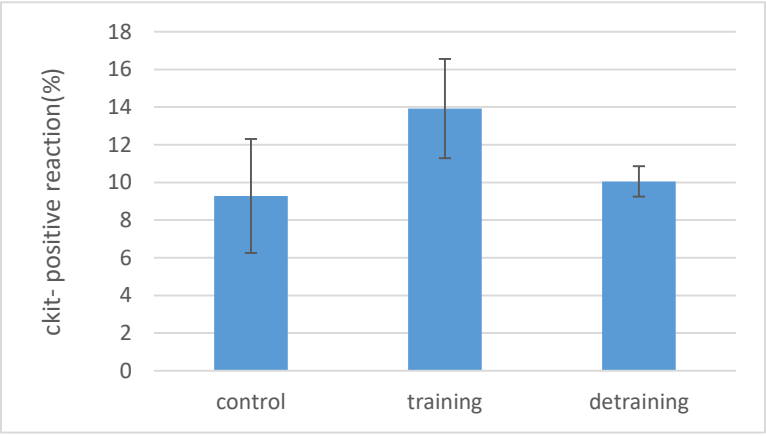


Figure 1. Data expressed as mean ± standard error for C-kit positive reaction among three groups (**CO**: Control, **AT**: Aerobic training, **DT**: Detraining). * $p<0.05$.

Immunohistochemistry

Heart tissue samples were fixed in 4% formaldehyde and rinsed with PBS. After dehydration with ethanol, samples were embedded in paraffin. Paraffin-embedded sections were placed on adhesive plates and dried before immunohistochemistry. Following deparaffinization in xylene, samples were dehydrated in

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decreasing alcohol concentrations. Antigen–antibody reactions were visualized using the EnVision System (Dako), following the manufacturer's instructions for antibody dilution. The presence of markers on the specimens was confirmed using an Olympus 400 mm fluorescence microscope. Images were captured using Cannon Power Shot cameras.

Real-Time PCR

Following sacrifice, total RNA was extracted from the heart tissue samples using QIAzol® Lysis Reagent (Qiagen). The RNA concentrations were determined by measuring the absorbance at 260 nm. RNA purity was assessed using ethidium bromide staining and the absorbance ratio at 260 and 280 nm, with a 260/280 nm absorbance ratio greater than 1.8 considered acceptable for purification. The extracted RNA was reverse transcribed into complementary DNA (cDNA) using a Revert Aid First Strand cDNA Synthesis Kit (Thermo Scientific, Fermentas K1622, United States). The reverse transcription process involved incubation at 25 °C for 5 min, reverse transcriptase incubation at 42 °C for 60 minutes, and refrigeration at 70 °C for 5 min, followed by storage at -20 °C.

Primers for real-time PCR were synthesized by Cinnagen Company (Iran) using NCBI and Gene Runner software. Gene expression was measured using a Step OneTM thermal cycler from Applied Biosystems with Master Mix and SYBR Green. The thermal cycle protocol included one cycle at 95°C for 10 min, followed by 40 cycles at 95°C for 15 seconds, 58°C for 30 seconds, and 72°C for 15 s. The PCR amplification reaction mixture had a total volume of 20 µL, comprising 3 µL of diluted template, 10 µL of SYBR Premix Ex TaqTM Kit (Perfect Real Time, Takara Code RR041A, Japan), and 2 µL of primers. Melting curve analysis was employed to monitor amplification specificity. The housekeeping gene, glyceraldehyde 3-phosphate dehydrogenase (Gapdh), was amplified to normalize relative expression. Data are presented as fold changes compared to the weight-bearing group

Statistical analysis

The data were analyzed using GraphPad Prism 6. All results are presented as mean and SD, unless otherwise stated, with a significance level of $P < 0.05$ (two-tailed). The normality of the distribution was evaluated using the Shapiro–Wilk test. Differences between the groups were assessed using one-way ANOVA, and the LSD post hoc method was employed to identify specific differences.

Results

All animals in the training group completed the 6-week aerobic training protocol. Due to the normal distribution of C-Kit ($P = 0.519$), Ki67 ($P = 0.625$), and Nkx2.5 ($P = 0.625$), one-way ANOVA was used with the LSD post hoc test. For C-Kit, there was a significant difference between the CO-AT groups ($P = 0.001$) and AT-DT groups ($P = 0.002$) (Figure 1). In the DT group, there was no significant difference for C-kit ($P = 0.502$). Similar results were also observed for Ki67, showing a significant difference between the CO–AT ($P = 0.001$) and AT–DT ($P = 0.001$) groups ($f = 17.784$, $P = 0.001$) (Figure 2). No significant difference was observed in Ki67 between the CO and DT groups ($P = 0.475$). There was a significant difference in Nkx2.5 between the CO-AT ($P = 0.001$), AT-DT ($P = 0.001$), and CO-DT ($P = 0.006$) groups (Figure 3).

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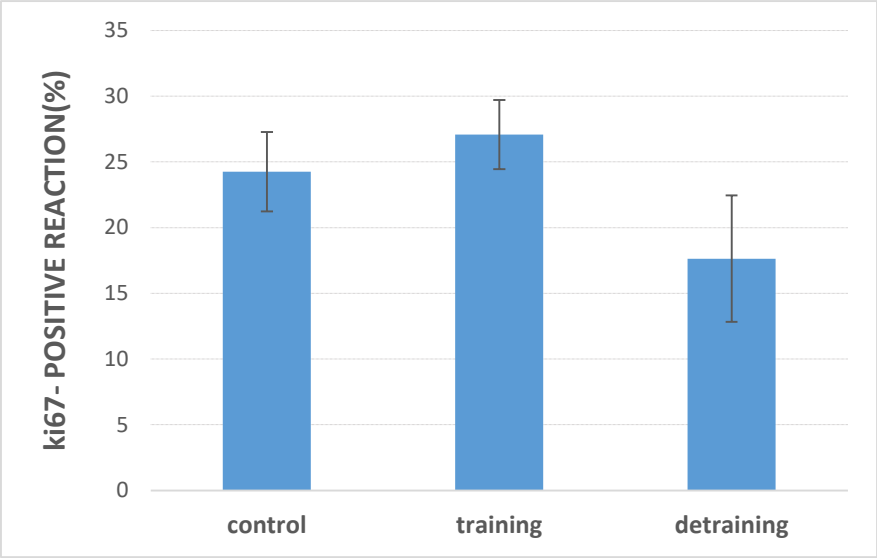


Figure 2. Data expressed as mean ± standard error for Ki67 positive reaction among three groups (**CO**: Control, **AT**: Aerobic training, **DT**: Detraining). * $p<0.05$.

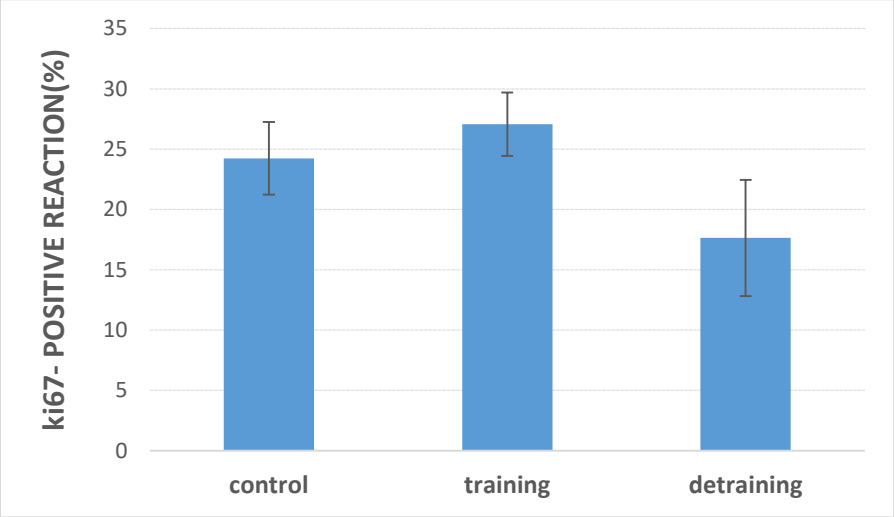


Figure 3. Data expressed as mean ± standard error for Nkx2.5 gene expression among three groups (**CO**: Control, **AT**: Aerobic training, **DT**: Detraining). * $p<0.05$.

Discussion

This study aimed to evaluate the effects of four weeks of detraining following aerobic exercise on cardiac stem cells in the elderly. The results showed that aerobic training significantly affected cardiac stem cells. Accordingly, the aerobic training group had high levels of KI67, NKX2.5, and C-Kit+. A positive effect of aerobic training on cardiac cell regeneration can be seen in these results.

In this regard, Xiao et al. (2014) showed that C-Kit and Sca-1 levels in rats after swimming for 21 days were significantly increased, where the physiological hypertrophy of mice caused by swimming is associated with the activation of cardiac progenitor stem cells (20).

Leite et al. (2015) reported that swimming for 4 weeks increases the number of resident CSCs (C-kit) in mice and is associated with physiological

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hypertrophy (21). Marino et al. (2019) also showed that exercise through the MAPK/AKT signaling cascade leads to physiological hypertrophy and improved cardiac function. By activating c-kit, this pathway increases CSC proliferation and differentiation. C-kit activation improves cardiac regeneration and repair after myocardial injury, but the level of C-kit decreases with age (22). A decrease in C-Kit levels was observed following four weeks of detraining in this study. The effects of detraining following exercise on cardiac stem cell function are still unknown, but some studies have shown that rat detention for two weeks reversed the bradycardic effect of chronic exercise. Marino found a decrease in myocardial repair after injury in C-kit-deficient mice compared with healthy mice (22). Accordingly, the significant reduction in C-Kit after a significant increase compared with the control group and old age are effective variables in reducing the number of CSCs.

Our results showed that aerobic training increases the expression of Nkx2.5. However, detraining after aerobic training reduces it. Nkx2.5 expression is affected by exercise and detraining, and a decrease in Nkx2.5 expression can be attributed to a reduction in shear stress (23). Shear stress up-regulated the phosphorylation of Akt and promoted growth factor in the heart (24). During exercise, shear stress increases (25). Accordingly, Bostrom (2015) showed that Akt regulates an exercise gene collection (26). Genes in this collection play a fundamental role in the hypertrophy and differentiation of cardiomyocytes. One of the most important genes is Nkx2.5, whose expression levels increase following endurance physical activity (19). It has been shown that increasing the expression of Nkx2.5 genes is associated with increased activation, proliferation, and differentiation of embryonic and adult stem cells into cardiomyocytes (capacity for cardiac regeneration) (27).

This study also suggests that aerobic training can increase the number and mass of myocytes (14). And exercise prevents aging-induced decline in function and the number of specialized stem cells of the heart by stimulating their function (15).

Aerobic training significantly increases Ki67 levels. The significant point is the decline in Ki67 levels after detraining. The monoclonal antibody Ki67 provides a means of measuring proliferative activity under various conditions (28). Therefore, aerobic training induces the formation of new cardiomyocytes. According to this hypothesis, cardiac self-renewal is stimulated by cardiomyocyte death caused by exercise stress (29). However, a warning reported that exercise did not result in cardiomyocyte death (14). Aerobic training increases the workload and tension in cardiomyocytes, resulting in an endocrine and autocrine response associated with the release of growth factors (30). In addition to protecting myocytes from apoptosis, necrosis, and hypertrophy, these responses can activate CSCs (30, 31). Because of, it can be assumed that stimulating the growth factors in the heart tissue and increasing their expression can repair damage and prevent disorders by stimulating cardiac self-renewal (32).

Current research is limited by the fact that animals cannot control how many calories they consume. We recommend that future research investigate the aging process at specific times, which is

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another limitation of the current research.

Conclusion

In summary, our study elucidates the dynamic impact of aerobic exercise, detraining, and aging on cardiac stem cells in aging male rats. Aerobic training enhances cardiac stem cell markers, including KI67, NKX2.5, and C-Kit+, highlighting its regenerative potential. However, detraining and aging introduce complexities, diminishing these markers and compromising cardiac stem cell function. The decline in Ki67 levels after detraining suggests a setback in proliferative activity induced by aerobic training. This underscores the importance of consistent exercise for sustaining cardiac regenerative benefits. Our findings emphasize the need for ongoing research to address limitations, such as calorie intake control in animal studies, and explore the nuanced interactions between exercise, detraining, and cardiac stem cells in the aging heart.

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Authors' contributions

AE contributed to scientific management, research concept, module development, final conclusions, drafting, methodology development, survey development, and follow-on text revision.

Conflict of interest

The authors declare that they have no conflict of interest.

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