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Original Article

# The effect of a period of detraining followed by resistance training on fibrosis, angiogenesis, and cardiac dimensions of male Wistar rats

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**Abstract**

**Background and aims:** Resistance training is associated with certain cardiovascular adaptations. However, a lack of exercise may affect it. Thus, the aim of this study was to investigate the effect of a period of detraining followed by resistance training on the fibrosis, angiogenesis, and cardiac dimensions of male Wistar rats.

**Methods:** In this experimental study, 20 male Wistar rats at the age of five weeks were randomly divided into control, training, control + detraining, and training + detraining groups. The control (8 weeks) and control + detraining (11 weeks) groups were placed in their respective cages with 24-hour free access to food and water and did not exercise for eight weeks. The resistance training + detraining and resistance training groups performed eight weeks of the resistance training protocol. The resistance training + detraining group did not do any type of training after the training period of 3 weeks. The results were analyzed by SPSS version 18.

**Results:** In terms of fibrosis, there was a significant difference between the control and control + detraining groups ($P=0.001$), between control and training groups ($P=0.001$), and between control + detraining and training + detraining groups ($P=0.001$). In addition, a significant difference was found between the training and training + detraining groups in terms of fibrosis ($P=0.001$). In terms of angiogenesis, a significant difference was observed between the control and training groups ($P=0.001$) and between the training and training + detraining groups ($P=0.007$).

**Conclusion:** Resistance training could reduce fibrosis and increase cardiac angiogenesis in rats. Based on the findings, fibrosis increased significantly in the detraining period compared to the active period, and angiogenesis was also reduced due to detraining.

**Keywords:** Detraining, Heart, Training, Fibrosis, Angiogenesis

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## Introduction

Exercise training, especially resistance training, is associated with different adaptations in body organs. Some of these adaptations are in the cardiovascular system, and in addition to their positive effect on a person's health, they also increase performance (1). In response to repeated exercise, the formation of new capillaries from existing capillaries begins, which is a highly dynamic and tightly controlled process called angiogenesis (2). Exercise causes angiogenesis by increasing the proliferation of endothelial cells, which are directly responsible for the formation of blood vessels (3). Research shows a reduction in cardiac fibrosis due to exercise. Reducing cardiac fibrosis is an important process to maintain optimal heart function and improve the prognosis of patients with heart failure. Clinical evidence indicates the beneficial effects of exercise, such as reducing cardiac fibrosis and improving ventricular compliance (4).

However, functional and morphological adaptations can decrease after a short period of non-training (5). Non-exercise means a person moving away from the consistency and regularity of daily and appropriate sports exercises, and it may occur due to factors such as injury, disease, or cessation of exercise. In this case, the person loses the effects and benefits of exercise (6). Inactivity is an independent risk factor for the development of stroke, coronary artery diseases, and peripheral vascular diseases (7).

Lack of physical activity or a significant reduction in physical activity is associated with numerous physiological changes that may affect the function and structure of the heart (8). The lack of volume loading and pressure that occurs during exercise, along with the significant reduction in serum, leads to a significant reduction in heart wall stress (8). Several studies have examined healthy individuals to evaluate the effects of long-term bed rest on heart function (8-10) and find ways to prevent cardiac atrophy in patients exposed to long-term bed rest or spaceflight. These studies have shown that bed rest for as little as 2 weeks can cause a measurable reduction in left ventricular (LV) mass and LV chamber size (11). In a study, it was also reported that 7 days of de-training

after regular aerobic training causes vascular endothelial growth factor (VEGF) (12).

Longitudinal studies attempting to define the temporal nature of wall thinning have produced mixed results. Among marathon runners who participated in a detraining study at 4 and 8 weeks after completing the Boston Marathon, Pedlar et al noted right atrial (RA) remodeling associated with a decrease in the RA area ($cm^2$) (13). It should be noted that most of the reduction in the right atrium area for 4 weeks occurred in parallel with the reduction of plasma volume, LV wall thickness, and LV mass (13). However, the extent of adaptation of the left atrium, including fibrosis and angiogenesis, to exercise and subsequent detraining among strength athletes remains unknown. It seems that the changes that occur in the heart and blood vessels following non-exercise begin in the second to fourth weeks. However, the effects of non-training on ventricular wall thickness, fibrosis, and angiogenesis following resistance training have not been determined. In general, considering the importance of non-training after sports training and the limited results in this field, this research aimed to investigate the effect of a non-training period following resistance training on fibrosis, angiogenesis, and cardiac dimensions of male Wistar rats.

## Materials and Methods

Overall, 20 male Wistar rats at the age of five weeks were purchased from the Pasteur-Amel Institute and transferred to the laboratory animals of Mazandaran University Sports Physiology Department. Then, they were randomly divided into control (n = 5), control + detraining (n = 5), resistance training + detraining (n = 5), and resistance training (n = 5) groups (14). Considering that the transfer and relocation of the subjects caused them stress, they adapted to the environment with the way the power devices worked for one week after the transfer (Figure 1).

The device used for squat movement, designed and manufactured has a screen with a voltage of 3 Hz and a force-applying lever on the subject's back . The animal wears a special vest for rodents and is placed on the screen, and the intensity of the exercise is applied according to the weight on the lever. To prevent tissue damage. Further, during the familiarization period, the rats were covered with the vest three times a day and performed the squat exercise without applying force to get familiar with the vest and the movement device (15). In this research, the handgrip device is also utilized to strengthen the muscles of the shoulder girdle and hands by performing the barfix movement using weights attached to the subjects' tails in a barfix manner with the support of the researcher from the end of the tail without any help and applying force from the researcher's side.

During the research, the control and control groups without exercise were placed in their respective cages with free access to water and food and did not exercise. The duration of the sedentary group in the control group without exercise lasted 3 weeks longer than the control group. The resistance training group and the resistance training group without training performed the resistance training protocol according to Table 1 during the research. Resistance training group — no training — did not have any type of training for 3 weeks after finishing the training period. At the end of the period, first, the rats were anesthetized, and then their heart tissue was removed using a surgical tool and immediately placed in formalin liquid. Animals were anesthetized with ketamine (60 mg/kg) and xylazine (10 mg/kg) and euthanized. Subsequently, LV heart tissues were collected, weighed, and stored either at -80°C for immunoblotting or in formaldehyde 10% for histological examination (16).

- Control Group: To determine the values of the research variables in the basic stage, the animals of this group were sacrificed at the beginning of the research.
- Resistance Training Group: The animals participated in eight weeks of resistance training, and then they were sacrificed.
- Resistance Training Group + Detraining: The rats participated in eight weeks of resistance training, three weeks without training, and then they were sacrificed.
- Control Group + Detraining: To determine 11 weeks of absolute detraining, the animals of this group were sacrificed after 11 weeks.

### *Resistance training program*

In the squat strength exercise, a familiarization program to prepare the rats, the animals wore the vest every day for 20 minutes with the help of the researcher, and in the first week, they did 3 sets of 10 with the help of the researcher. In the second week of familiarization, the rats were placed on the designed squat machine and performed 3 sets of 10 squats without weights every day. To stimulate movement execution, a mild electric shock was applied to the bottom of the device and the soles of the subject's feet.

**Table 1.** ANOVA results for fibrosis, thickness, and angiogenesis

| Markers | Groups | Mean ± SD | SE | *P* value |
|---|---|---|---|---|
| Fibrosis | Control | 35.1 ± 3.2 | 1.5 | 0.001# |
| | Control-De | 28.3 ± 2 | 1 | |
| | Training | 13.5 ± 2.4 | 1.2 | |
| | Training-De | 24.3 ± 3.1 | 1.5 | |
| Thickness | Control | 1 ± 0.4 | 0.2 | 0.2 |
| | Control-De | 1.25 ± 0.2 | 0.1 | |
| | Training | 2.25 ± 0.64 | 0.32 | |
| | Training-De | 1.5 ± 0.4 | 0.2 | |
| Angiogenesis | Control | 0.5 ± 0.57 | 0.28 | 0.003# |
| | Control-De | 0.75 ± 0.95 | 0.47 | |
| | Training | 5.5 ± 1 | 0.5 | |
| | Training-De | 2.25 ± 0.5 | 0.23 | |

*Note*. # Significant difference; ANOVA: Analysis of variance; SD: Standard deviation; SE: Standard error; De: Detraining.

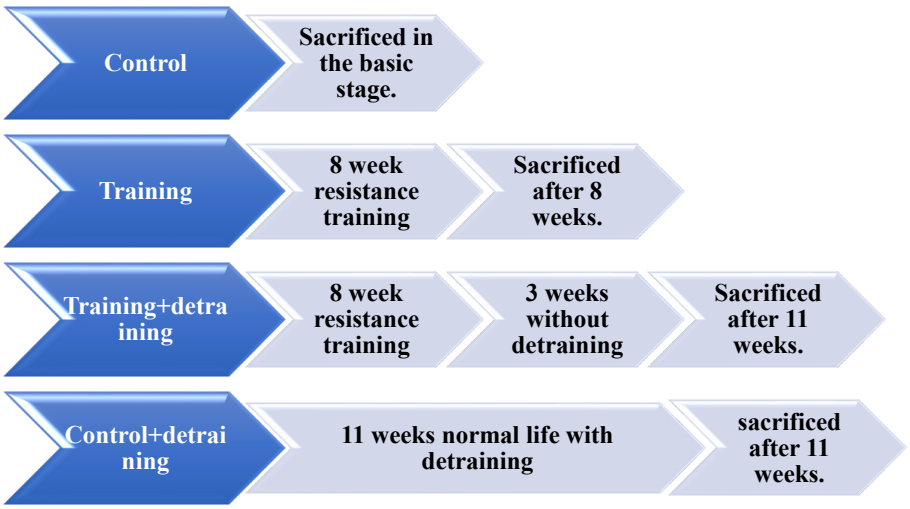


**Figure 1.** Study Design

To prevent the possible effect of electric shock on research findings, during the animal familiarization phase with the device, the rats were taught to refrain from standing and resting on the device by conditioning them with the device. The training period for the mice lasted for 8 weeks and was performed in 10 sets. The training intensity at the beginning, the end of the fourth week, and the end of the eighth week was taken from the training protocol. Then, the protocol was implemented over two periods of 4 weeks (Table 1). The weight moved by the subject was determined by considering the weight of the vest and the lever of the measuring device, as well as the strength and intensity of the exercise. Apart from the main activity time, 5 minutes for warm-up and 5 minutes for cool-down were considered as well. In such a way that, by covering the vest, the squat movement was done with the help of the researcher.

Hand strength training was also performed according to the squat exercise and with the hand grip device, with the difference that in the hand grip training, the rats hung from the device without weights in 3 sets of 10 repetitions in the first two weeks of the training. Then, the training intensity was determined by measuring 1RM. The hand grip exercise was conducted using weights attached to the rat's tail as a barfix, with the researcher supporting the end of the tail without any assistance or force. The rats were separated from the machine and had active rest between exercise sets. Apart from the main activity time, the rats exercised to warm up (5 minutes) and cool down (5 minutes) with the assistance of the researcher. The training was performed with 80% maximum repetition.

Moreover, 1RM was calculated according to the moved weight and the number of repetitions according to the following formula:

Formula A: 100 - (number of repetitions × 2)
1RM = A / 100 × moved weight

### *Histopathology*

After harvesting the desired tissue, fixation was performed using a 10% formalin solution. Using this fixative during tissue preparation leads to better staining results. Then, to dehydrate the tissue, the sample was placed in 70%, 80%, and 90% absolute alcohol, respectively, and with this action, the tissue water was absorbed by the alcohol and replaced by alcohol. Next, the sample was placed inside a solution called hexylol, which also replaces alcohol (70% alcohol for 50 minutes, 80% alcohol for 50 minutes, 96% alcohol for 50 minutes, and 100% alcohol for 50 minutes). Subsequently, the tissue was placed in Xylel-1 for 40 minutes and then in Xylel-2 for 40 minutes. Then, the sample was placed inside the melted paraffin to penetrate the tissue. For this stage, the tissue was placed in paraffin-1 for 50 minutes and paraffin-2 for 50 minutes. In the molding stage, the sample impregnated with paraffin was placed inside the mold filled with melted paraffin. While the paraffin was frozen, the sample remained inside and was ready to be cut. The sample was cut with a paraffin mold by a device called a microtome with a thickness of 5 to 10 microns.

### *Statistical Method*

In this research, data were analyzed by SPSS (version 18), and the Shapiro–Wilk test was used to check the normality of the data distribution. In addition, the analysis of variance and the Bonferroni post hoc test were employed to investigate the research variables. All the tests were performed using Prism software (version 5) at the level of $P \leq 0.05$.

## Results

The research results demonstrated a significant difference between the groups in terms of fibrosis of heart tissue in Wistar rats ($P = 0.001$). Based on the results of the follow-up test, a significant difference was found between the control and control-detraining groups ($35.1 \pm 3.2$ and $28.3 \pm 2$, $P = 0.001$), the control and training groups ($35.1 \pm 3.2$ and $13.5 \pm 2.4$, $P = 0.001$), and the control and training + detraining groups ($35.1 \pm 3.2$ and $24.3 \pm 3.1$, $P = 0.001$). Further, a significant difference

was observed between the control+detraining and training groups (24.3±3.1 and 13.5±2.4, $P=0.001$), and the control+detraining and training+detraining groups (28.3±2 and 24.3±3.1, $P=0.001$). In addition, there was a significant difference between the training and training+detraining groups (13.5±2.4 and 24.3±3.1, $P=0.001$; Tables 1 and 2, Figure 2).

In terms of LV wall thickness, no significant difference was found between the groups ($P=0.2$, Table 1).

Regarding the amount of angiogenesis, there was a significant difference between the groups ($P=0.003$). No significant difference was observed between the control (0.5±0.57) and control detraining (0.75±0.95) groups ($P=0.21$). However, a significant difference was found between the control (0.5±0.57) and training (5.5±1) groups ($P=0.001$), as well as the control-detraining (0.75±0.95) and training (5.5±1) groups ($P=0.001$). The difference between the training (5.5±1) and training+detraining (2.25±0.5) groups ($P=0.007$) was also significant (Tables 1 and 2).

## Discussion

This research was conducted to investigate the effect of a period of non-training followed by resistance training on the fibrosis, angiogenesis, and cardiac dimensions of male Wistar rats. The results revealed that resistance training for eight weeks could reduce fibrosis in the heart tissue of Wistar rats. However, a subsequent lack of exercise increased the amount of fibrosis. Feng et al reported that aerobic exercise reduces cardiac fibrosis in rats (4). Based on their results, exercise reduces Ang II-induced cardiac fibrosis by reducing POU2F1. Exercise inhibits POU2F1

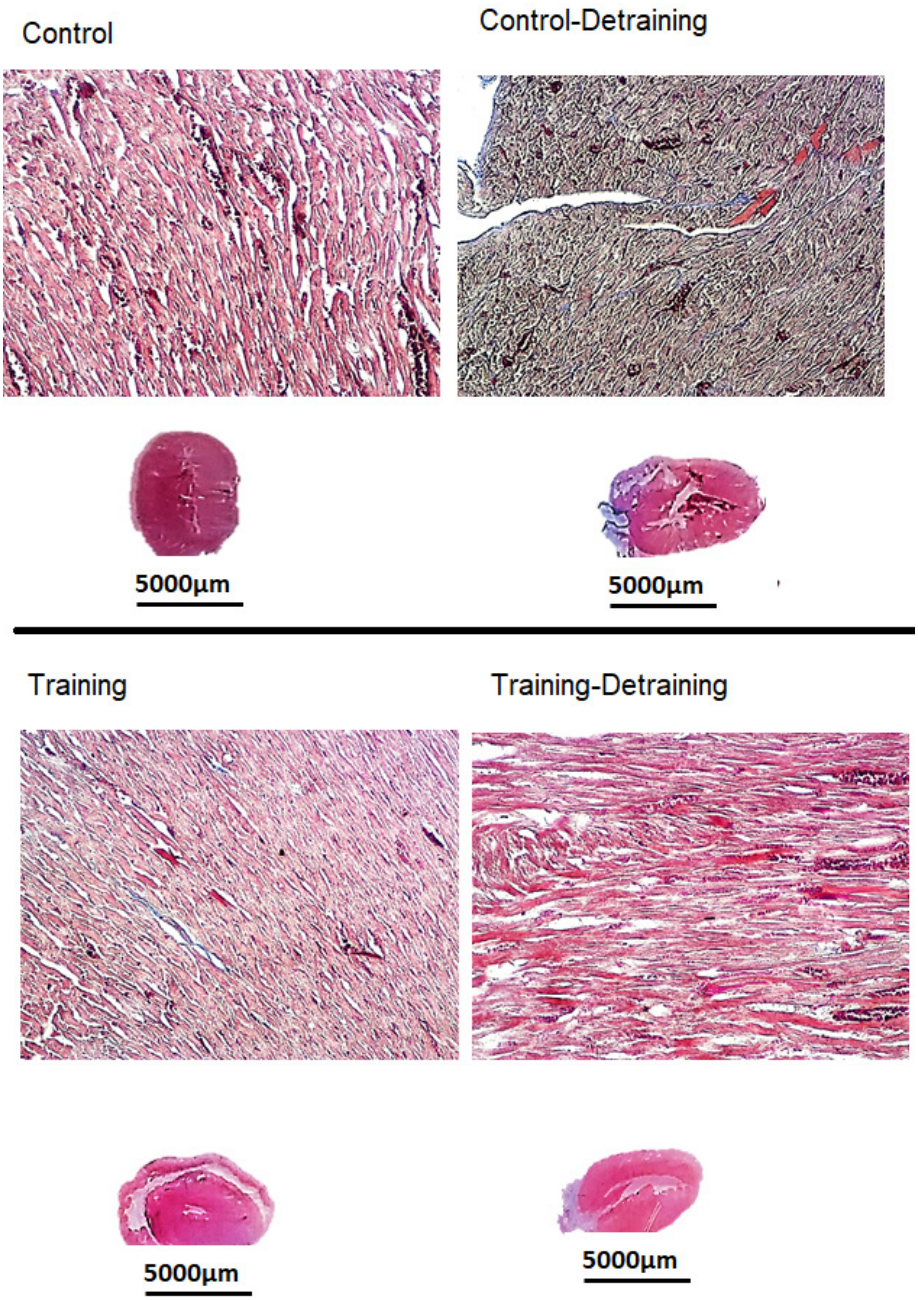


**Figure 2.** Fibrosis in Studied Groups

**Table 2.** Post-hoc results for fibrosis and angiogenesis

| Markers | Group | | Mean Difference | SE | *P* value |
|---|---|---|---|---|---|
| | I | II | | | |
| Fibrosis | Control | Control-De | -5.780 | 1.2 | 0.001# |
| | | Training | -26.21 | 1.2 | 0.001# |
| | | Training-De | -14.15 | 1.2 | 0.001# |
| | Control-De | Training | -20.43 | 1.2 | 0.001# |
| | | Training-De | -8.365 | 1.2 | 0.001# |
| | Training | Training-De | -12.32 | 1.2 | 0.001# |
| Angiogenesis | Control | Control-De | -0.2500 | 0.1 | 0.21 |
| | | Training | -5.000 | 0.1 | 0.001# |
| | | Training-De | -1.750 | 0.1 | 0.2 |
| | Control-De | Training | -4.50 | 0.1 | 0.001# |
| | | Training-De | -1.500 | 0.1 | 0.11 |
| | Training | Training-De | 3.250 | 0.1 | 0.007# |

*Note*. # Significant difference; De: Detraining; SE: Standard error.

by activating active adenosine monophosphate-activated protein kinase, which is followed by the downregulation of C/EBPβ, a POU2F1 transcription factor (4). Ma et al reported that both aerobic exercise and resistance training significantly reduced cardiac dysfunction and fibrosis, upregulated fibroblast growth factor 21 protein expression, and activated the fibroblast growth factor-β1-suppressor of mothers against the decapentaplegic2/3-matrix metalloproteinase signaling pathway. 9/ and inhibited collagen production. Meanwhile, antioxidant capacity is increased and cell apoptosis is decreased in the infarcted heart (17).

Despite the above explanations, we did not want to achieve congruent studies regarding the effect of non-exercise following resistance exercise on cardiac tissue fibrosis. However, our results showed that tissue fibrosis increased during the non-training period after eight weeks of resistance training. In this regard, Lee found that cardiac regression occurs after 21 days of exercise cessation and may be partially mediated by protein kinase B inactivation (18).

In addition, our results demonstrated that resistance training had no significant effect on LV wall thickness. Aerobic exercises seem to have a better effect on the thickness of the ventricular wall (16,19,20). On the other hand, our results confirmed that resistance training increased cardiac angiogenesis in Wistar rats. However, a lack of training led to a decrease in cardiac angiogenesis. However, there was a significant difference between the exercise-detraining and detraining control groups, and this group had more angiogenesis compared to the detraining control group. Yeo et al also reported that resistance training and aerobic training are effective on angiogenesis, although the effect of aerobic training was more noticeable (21). Exercise training increases cardiac angiogenesis through the increase of endothelial nitric oxide synthase and VEGF (14). In a study 28 days after non-exercise, Olenich et al also found that angiogenesis was reduced in skeletal muscle. Based on their results, *thrombospondin*-1 may be a mediator of capillary regression with no exercise, even in the face of high VEGF, suggesting that pro-angiogenic regulators may not be able to prevent skeletal muscle capillary regression under physiological conditions. The response of matrix metalloproteinase, endostatin, and nucleolin was weakly correlated with capillary regression caused by detraining (22).

## Conclusion

The results of this research revealed that resistance training reduces fibrosis and increases cardiac angiogenesis in Wistar rats. However, it did not affect the thickness of the ventricular wall. Based on our results, fibrosis increased significantly in the non-training period compared to the active period. However, it was still lower than the control-detraining group. In addition, angiogenesis was also reduced due to a lack of exercise. However, there was no significant difference compared to the control-detraining group, indicating a significant decrease in angiogenesis due to detraining.

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### Authors' Contribution

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### Competing Interests

The authors have no conflict of interests.

### Ethical Approval

This article was approved by the Ethics Committee at Ardabil University of Medical Science with the code IR.ARUMS.REC.1398.555.

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