Journal of Sports Physiology and Athletic Conditioning (J.S.P.A.C) 2023; 3 (10): 26-34

Research Article

Effects of eight weeks resistance training on cardiac fibrosis in elderly rats

Fateme Guderzi[1], Hossein Abed Natanzi[2]*, Marzieh Mazrae Khatiri[1]

1. Ph.D. in exercise physiology, Department of Physical Education and Sports Sciences, Faculty of Humanities, Science and Research Unit, Islamic Azad University, Tehran, Iran.
2. Assistant Professor, Department of Physical Education and Sports Sciences, Faculty of Humanities, Science and Research Unit, Islamic Azad University, Tehran, Iran.

Received: 15 September 2023
Revised: 29 September 2023
Accepted: 25 October 2023

Abstract

Background: The purpose of this study was to investigate the effect of eight weeks of resistance training on the improvement or prevention of cardiac fibrosis in elderly rats. Main Topic this study was to investigate the effect of eight weeks of resistance training on the rehabilitation or prevention of cardiac fibrosis in elderly rats.

Materials and Methods: In this experimental study, 18 Wistar rats with mean age of 24 months were randomly divided into control and endurance training groups (9 rats in each group). After a week of familiarization and adaptation, the experimental group performed their training program on a rats' resistance training ladder for 8 weeks and 5 days per week. The control group did not perform any exercise during this time. Research variables were measured by ELISA method and histological tests by trichrome-staining. For inferential analysis of data from independent t-test was used.

Results: The results showed that eight weeks of aerobic training had a significant effect on SOD (P = 0.001), CAT (P = 0.006), GPX (P = 0.012), TGF (P = 0.001) and Tissue collagen in cardiac tissue of elderly rats. It has.

Conclusion: The results of the present study confirm the positive role of resistance training in improving cardiac fibrosis due to collagen depletion due to TGF-β inhibition and its signaling pathway due to the improvement of cardiac tissue antioxidant enzymes. These exercises can be used to rehabilitate or prevent cardiac fibrosis.

Keywords:
Resistance training, Cardiac fibrosis, Aging

***Corresponding author:** Hossein Abed Natanzi
Address: Department of Physical Education and Sports Sciences, Faculty of Humanities, Science and Research Unit, Islamic Azad University, Tehran, Iran. **Email:** mailto:abednazari@gmail.com **Tell**: +989126107064
H N: 0000-0001-6638-1131

1. Introduction

Aging is known as an independent and important risk factor for cardiovascular diseases and mortality. Compared to the young heart, the elderly heart shows different biological characteristics and cellular-molecular processes, which include: increased apoptosis, chronic inflammation, hemodynamic changes, and tissue fibrosis (1). Fibrosis is characterized by the excessive increase of collagen in the extracellular matrix (ECM) in the heart tissue, which occurs due to the stimulation of factors involved in the increase of collagen synthesis and causes the creation of fibrotic tissue in the heart. Cardiac fibrosis in old age may involve activation of fibro genic signaling and inhibition of antifibrotic signaling, which leads to an imbalance of ECM circulation, which are involved in age-related fibrotic cardiac remodeling. Fibrotic tissue is hard and has little elasticity, which leads to dysfunction of the heart (2, 3).

This excessive accumulation of collagen hardens the ventricles, which disrupts contraction, expansion (relaxation) and double electrical stimulation of cardiac myocytes and reduces capillary density. Tissue fibrosis and reduction of capillary density increases the oxygen diffusion distance and causes myocardial ischemia, which ends in heart failure (4). Fibrosis is a common feature of many heart diseases, including myocardial infarction, ischemia, hypertrophic cardiomyopathies and heart failure (5). Fibrosis is multifactorial and can be caused by processes and factors such as ischemia, aging, and inflammation. Two different types of cardiac fibrosis have been proposed: compensatory fibrosis and reactive fibrosis. Compensatory fibrosis refers to ECM deposition that occurs during scar formation due to heart tissue damage or cell death, and is actually a process that begins during ischemia or aging. Reactive fibrosis indicates an increase in ECM due to direct stimulation of cardiac fibroblasts without cell damage that occurs in high blood pressure (6). Previous studies have reported the positive role of cytokine transforming growth factor-beta (TGF-β) in the induction of fibrosis and the expression of genes involved in it both in vitro and in vivo (7). TGF-β and its signaling pathway is known to be the most important regulator of the fibrosis process, which shows increased expression in fibrotic tissues as well as with age (8). In response to activated TGF-β, 1) fibroblast cells, which make up 60-70% of heart cells, differentiate into myofibroblasts. Myofibroblast cells, which are responsible for the production of extracellular matrix components, cause the production of large amounts of matrix components and excessive deposition of matrix proteins. 2) Activated TGF-β binds to its cell surface receptor, transmits its message to the nucleus and up-regulates the expression of collagen genes. β does not only participate in fibrosis by strengthening ECM collagen synthesis, but also by inhibiting ECM decomposition through reducing the expression of matrix degrading enzymes or increasing the expression of MMPs inhibitors, and on the other hand, it increases oxidative stress and self-activity. It is found in tissue (9,10,11). In fact, cardiac fibrosis is an important process in the reconstruction of heart structures, and aging plays an important role in its formation. TGF- Today, exercise is considered as a main non-pharmacological strategy as a kind of rehabilitation and auxiliary treatment or prevention of cardiovascular diseases. Many studies have shown the beneficial effects of exercise on the primary and secondary causes of heart diseases and cellular-molecular changes in heart tissue and have reported its benefits (12).

Therefore, research on the effectiveness of appropriate sports activities in order to improve or slow down the process of creating fibrotic tissue in the heart, which is the basis of many cardiovascular diseases in the elderly, independent of the underlying diseases due to aging, from the point of view Cellular-molecular is required. Many experts in the field of health recommend aerobic sports to improve the performance and cardiovascular structure, but the College of Sports Medicine and the American Heart Association stated that in addition to aerobic activities, the elderly should take care of the reduction of muscle mass and the occurrence of sarcopenia in the aging process. also benefit from resistance activities (13). The question that arises is whether doing resistance exercises can affect the process of heart fibrosis in the elderly in the direction of improvement. Therefore, he reviewed the researches, but in relation to the effect of resistance training on cardiac fibrosis in elderly rats, only one study was found, in which Gazzouni et al. (2017) found the effect of high-intensity resistance training on collagen accumulation in the left ventricle They investigated the aged Wistar rats and reported that high-intensity resistance training has a protective effect on the accumulation of collagen in the heart tissue and maintaining myocardial function (14). Therefore, considering the few available and non-reliable information regarding the effect of resistance training on cardiac fibrosis caused by old age, it is necessary and necessary to carry out research in this field, to investigate and present the most effective method of resistance training program. The opinion of the intensity and duration of its implementation on the structural changes of the heart tissue in the elderly. Therefore, in order to find the most suitable exercise program for rehabilitation or prevention of cardiac fibrosis in the elderly, the effect of eight weeks of low-intensity resistance training on TGF-β and its signaling pathway, which is the most important cause of cardiac fibrosis associated with old age, in the left ventricular tissue We examined the hearts of elderly rats.

2. Materials and Methods

The current research, which is registered with the code of ethics in the Physical Education Research Institute, is of an experimental and fundamental type with a post-test design with a control group. The statistical population was Wistar male laboratory rats, from which a statistical sample including 18 Wistar laboratory rats with an average age of 24 months was prepared from Royan Research Institute in Tehran and according to the instructions for the care and use of laboratory animals in scientific affairs approved by the Vice The research and technology of the Ministry of Health, Treatment and Medical Education were transferred to the Animal Laboratory Animal House of the Laboratory Complex of Tehran University of Science and Research. They were randomly divided into two groups of control and resistance training in equal numbers (9 heads in each group) (15). All three rats were placed in transparent polyethylene cages that were coded. All groups were fed with the same type of food for rodents (standard pellet) so that they had free access to food and water. All groups were kept in the same place with average temperature conditions of 23±3, average humidity of 50±10 and light-dark cycle of 12-12. The animals were kept for two weeks after being transferred to the laboratory to adapt to the environment. After familiarizing the training group with how to

climb the ladder for rodent resistance training, the training protocol began. Each training session consisted of eight repetitions with 60-second rest intervals for each repetition. At the beginning of each week, the weight of the animal was measured and based on the body weight, the training weight was applied, so that the amount of training weight in the first week was 5% of the body weight, and after that, 5% was added to it at the beginning of each training week. In the eighth week, it reached 45% of the animal's body weight. (16). During the research period, the control group did not do any exercises. 48 hours after the last training session at 8 am, both groups were sacrificed after induction of complete anesthesia (by intraperitoneal injection of a combination of xylazine and ketamine). After making sure that the chest was completely anesthetized, the heart was removed and washed in physiological serum, then the heart was divided into two halves by means of a transverse cut, and half of it was transferred to a microtube and immediately frozen in liquid nitrogen for further measurements. They were transferred to 80-degree freezer and the other half was fixed in formalin for histological studies.

Measuring the activity of antioxidant enzymes (SOD, CAT and GPX) and measuring the amount To observe the tissue changes in the amount of collagen in the extracellular matrix of the heart tissue, a specialized Trichrome-Masson staining method was used. In this staining method, purple nuclei, red muscle cytoplasm and red erythrocytes, and blue collagen strands are observed (17).

Statistical method

SPSS version 21 statistical analysis software was used to extract the results. The Shapiro-Wilk test was used to determine the normality of the data distribution, and the independent t-test was used to check the research hypotheses. The significance level was considered to be $\alpha \geq 0.05$ in all tests.

3. Results

The results of the independent t test showed that eight weeks of aerobic training in the resistance training group increased the tissue amount of SOD (P=0.02) and GPX (P=0.046), which was statistically significant. The amount of CAT (P=0.124) and TGF-β (P=0.1) increased in the heart tissue of aged rats compared to the control group, but this increase did not reach a statistically significant level (Table 1).

Table 1. Independent t-test results for changes in research variables in two control and experimental groups

P-value	t-value	Degree of Freedom	Standard Deviation	*Mean*	Statistical index / Group	
.1	-1.748	16	7.78	44.08	Control (n=9)	TGF-β
			11.25	52.05	Experimental (n=9)	
.02 *	-2.584	16	9.16	60.92	Control (n=9)	SOD
			22.88	82.15	Experimental (n=9)	
.124	-1.624	16	4.99	6.68	Control (n=9)	CAT
			9.48	12.49	Experimental (n=9)	
.046*	-2.271	۱۶	151.02	787.2	Control (n=9)	GPX
			401.55	1111.92	Experimental (n=9)	

*It indicates significance at the level (P≤0.05). Superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPX)

Regarding collagen changes, as seen in Masson's trichrome staining (Figure 1), the results indicate a decrease in tissue collagen in the training group compared to the control group.

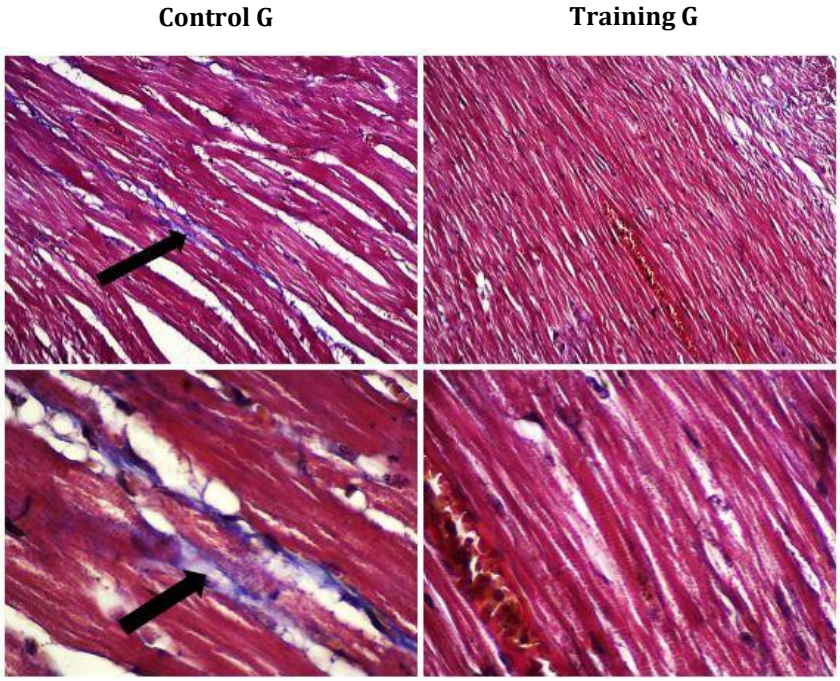

Figure 1. Masson's trichrome staining, collagen fibers can be seen in the tissue in blue color and are marked with an arrow in the picture. The images were prepared with 100 mμ and 20 mμ magnification.

After eight weeks of resistance training in the training group compared to the control group, the percentage of collagen in the heart tissue of elderly rats decreased so that the amount of collagen in the heart tissue of the training group reached (22.92) percent after eight weeks of training. Compared to the control group (40.18) percent (Figure 2).

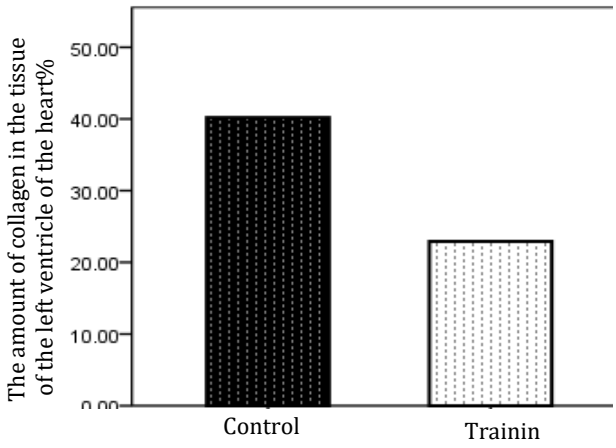

Figure 2. Comparison of tissue collagen percentage between two groups

4. Discussion

In the present study, the effect of physical activity on the signaling pathway that causes cardiac fibrosis in the elderly without underlying disease was investigated and the beneficial results of performing eight weeks of low-intensity resistance training in reducing collagen and protecting the heart against fibrosis due to aging showed The results of Masson's trichrome staining showed that performing eight weeks of exercise reduces the accumulation of collagen in the extracellular matrix of the heart, which is in line with the results of Gazzoni et al., despite the difference in the type and intensity of exercise (14). As mentioned, TGF-β is a strong stimulus for collagen synthesis in the extracellular matrix of the heart, which causes the positive regulation of collagen expression in the ECM of the heart. Therefore, reducing the expression of TGF-β and inhibiting its signaling pathway can reduce the expression of genes involved in the production of extracellular matrix collagen (8,18). Gazzoni et al.'s studies also confirmed this and stated that resistance exercise decreases TGF-β gene expression and subsequently decreases the amount of tissue collagen in the extracellular matrix of the heart, which can be a protective mechanism against cardiac fibrosis due to Age increase. However, the results of our research were contrary to the results of Gazzoni and his colleagues and showed that eight weeks of low-intensity resistance training in the training group compared to the control group increased the tissue amount of TGF-β. On the other hand, despite this increase, we saw a decrease in tissue collagen percentage, which is in line with the results of the only research. In relation to this contradiction, it can probably be said that since TGF-β has a high affinity for its receptor, it needs to be in an inactive form.

By binding to two polypeptides, TGF-β forms a large multiple protein complex (LLC), which is called latent TGF-β complex. Secreted TGF-β is secreted from the cell to the extracellular matrix and stored there. LLC binds with other components of the extracellular matrix such as fibronectin, fibrolin-1 and provides a pool of TGF-β hidden in the matrix (19,20). Recently, oxygen free radicals have been shown to play a major and upstream role in the activation of inactive TGF-β. After activation, TGF-β binds to its receptors on the surface of cells and sends its message into the cell and increases collagen gene expression (21,22). In our research, it was shown that TGF-β increased after exercise, but this increase did not increase collagen expression, which may be due to its inactivity. Because based on the results of the research and examination of the amount of antioxidant enzymes in the heart tissue, it was shown that resistance training has caused a significant increase in the activity level of the antioxidant enzymes SOD, CAT and GPX in the heart tissue that this has caused the removal of oxygen free radicals in the heart tissue, and subsequently, the reduction of reactive oxygen species (ROS) can prevent the activation of TGF-β and its signaling pathway, despite the increase in tissue amounts inhibit that this will reduce the expression of target genes of this signaling pathway (collagen). Therefore, the increasing effect on the activity of the body's internal antioxidant defense can be recognized as a valuable non-pharmacological treatment solution for inhibiting or modulating the process of heart tissue fibrosis due to the reduction of collagen gene expression by not activating TGF-β and inhibiting its signaling pathway in the elderly. The results of the present study showed that low-intensity resistance exercise can reduce ROS by increasing the activity of heart tissue antioxidant enzymes, which plays an important

role in the formation of fibrotic tissue due to the activation of the TGF-β signaling cascade. It plays a role in old age, and the increase in tissue amount of TGF-β, which has been introduced in previous researches as a key factor in increasing collagen in fibrotic tissue, cannot play a role in increasing collagen expression.

Conclusion

Because the results of our research showed that contrary to the increase in the amount of tissue TGF-β, the amount of tissue collagen decreased, which indicates that other important and necessary upstream and downstream factors are involved in the fibrotic process, which has a regulatory role on the activity of this key factor and messenger. It has, but due to the lack of sufficient research in this field, there is a need for more cellular-molecular research on this signaling pathway, as well as the effect of resistance training with different intensities and training times, in order to identify upstream and downstream factors affecting the path of heart tissue fibrosis. As a result of old age.

Acknowledgements

The researchers hereby express their gratitude and thanks to the research subjects.

Funding

This study did not have any funds.

Compliance with ethical standards

Conflict of interest None declared.

Ethical approval the research was conducted with regard to the ethical principles.

Informed consent Informed consent was obtained from all participants.

Author contributions

Conceptualization: F.G, H.N, M.M.K; Methodology: F.G, H.N; Software: F.G, H.N, M.M.K; Validation: F.G, H.N, M.M.K; Formal analysis: F.G, H.N, M.M.K; Investigation: F.G, H.N, M.M.K; Resources: F.G, H.N, M.M.K; Data curation: H.N, M.M.K; Writing - original draft: F.G, H.N, M.M.K; Writing - review & editing: H.N, M.M.K; Visualization: F.G, H.N, M.M.K; Supervision: F.G, H.N,; Project administration: F.G, H.N, M.M.K; Funding acquisition: : F.G, H.N, M.M.K.

References

1. Dai DF, Rabinovitch PS, Ungvari Z. Mitochondria and cardiovascular aging. Circ Res. 2012 Apr 13;110(8):1109-24. doi: 10.1161/CIRCRESAHA.111.246140. PMID: 22499901; PMCID: PMC3867977.

2. Lu L, Guo J, Hua Y, Huang K, Magaye R, Cornell J, Kelly DJ, Reid C, Liew D, Zhou Y, Chen A, Xiao W, Fu Q, Wang BH. Cardiac fibrosis in the ageing heart: Contributors and mechanisms. Clin Exp Pharmacol Physiol. 2017 Dec;44 Suppl 1:55-63. doi: 10.1111/1440-1681.12753. Epub 2017 Sep 20. PMID: 28316086.

3. Mihl C, Dassen WR, Kuipers H. Cardiac remodelling: concentric versus eccentric hypertrophy in strength and endurance athletes. Neth Heart J. 2008 Apr;16(4):129-33. doi: 10.1007/BF03086131. PMID: 18427637; PMCID: PMC2300466.

4. Bernardo BC, Weeks KL, Pretorius L, McMullen JR. Molecular distinction between physiological and pathological cardiac hypertrophy: experimental findings and therapeutic strategies. Pharmacol Ther. 2010 Oct;128(1):191-227. doi: 10.1016/j.pharmthera.2010.04.005. Epub 2010 May 12. PMID: 20438756.

5. Riyahi Malayeri, S., Nikbakht, H, Gaeini (2014). Serum Chemerin Levels and Insulin Resistance Response to High-Intensity Interval Training in Overweight Men. Bulletin of Environment, Pharmacology and Life Sciences, 3(2), pp. 385-389..

6. Horn MA, Trafford AW. Aging and the cardiac collagen matrix: Novel mediators of fibrotic remodelling. J Mol Cell Cardiol. 2016 Apr;93:175-85. doi: 10.1016/j.yjmcc.2015.11.005. Epub 2015 Nov 11. PMID: 26578393; PMCID: PMC4945757.

7. Leask A. TGFbeta, cardiac fibroblasts, and the fibrotic response. Cardiovasc Res. 2007 May 1;74(2):207-12. doi: 10.1016/j.cardiores.2006.07.012. Epub 2006 Jul 21. PMID: 16919613.

8. Parker L, Caldow MK, Watts R, Levinger P, Cameron-Smith D, Levinger I. Age and sex differences in human skeletal muscle fibrosis markers and transforming growth factor-β signaling. Eur J Appl Physiol. 2017 Jul;117(7):1463-1472. doi: 10.1007/s00421-017-3639-4. Epub 2017 May 10. PMID: 28493029.

9. Liu XH, Zhang QY, Pan LL, Liu SY, Xu P, Luo XL, Zou SL, Xin H, Qu LF, Zhu YZ. NADPH oxidase 4 contributes to connective tissue growth factor expression through Smad3-dependent signaling pathway. Free Radic Biol Med. 2016 May;94:174-84. doi: 10.1016/j.freeradbiomed.2016.02.031. Epub 2016 Mar 3. PMID: 26945889.

10. Biernacka A, Dobaczewski M, Frangogiannis NG. TGF-β signaling in fibrosis. Growth Factors. 2011 Oct;29(5):196-202. doi: 10.3109/08977194.2011.595714. Epub 2011 Jul 11. PMID: 21740331; PMCID: PMC4408550.

11. Chiao YA, Ramirez TA, Zamilpa R, Okoronkwo SM, Dai Q, Zhang J, Jin YF, Lindsey ML. Matrix metalloproteinase-9 deletion attenuates myocardial fibrosis and diastolic dysfunction in ageing mice. Cardiovasc Res. 2012 Dec 1;96(3):444-55. doi: 10.1093/cvr/cvs275. Epub 2012 Aug 22. PMID: 22918978; PMCID: PMC3500048.

12. Liu T, Chan AW, Liu YH, Taylor-Piliae RE. Effects of Tai Chi-based cardiac rehabilitation on aerobic endurance, psychosocial well-being, and cardiovascular risk reduction among patients with coronary heart disease: A systematic review and meta-analysis. Eur J Cardiovasc Nurs. 2018 Apr;17(4):368-383. doi: 10.1177/1474515117749592. Epub 2017 Dec 19. PMID: 29256626.

13. Haskell WL, Lee IM, Pate RR, Powell KE, Blair SN, Franklin BA, Macera CA, Heath GW, Thompson PD, Bauman A. Physical activity and public health: updated recommendation for adults from the American College of Sports Medicine and the American Heart Association. Med Sci Sports Exerc. 2007 Aug;39(8):1423-34. doi: 10.1249/mss.0b013e3180616b27. PMID: 17762377.

14. Guzzoni V, Marqueti RC, Durigan JLQ, Faustino de Carvalho H, Lino RLB, Mekaro MS, Costa Santos TO, Mecawi AS, Rodrigues JA, Hord JM, Lawler JM, Davel AP, Selistre-de-Araújo HS. Reduced collagen accumulation and augmented MMP-2 activity in left ventricle of old rats submitted to high-intensity resistance training. J Appl Physiol (1985). 2017 Sep 1;123(3):655-663. doi: 10.1152/japplphysiol.01090.2016. Epub 2017 Jul 6. PMID: 28684598.

15. Ahmadi-Noorbakhsh S. Sample size calculation for animal studies -with emphasis on the ethical principles of reduction of animal use. Research in Medicine 2018; 42 (3):144-153. URL: .

16. Nucci RAB, Teodoro ACS, Krause Neto W, Silva WA, de Souza RR, Anaruma CA, Gama EF. Effects of resistance training on liver structure and function of aged rats. Aging Male. 2018 Mar;21(1):60-64. doi: 10.1080/13685538.2017.1350157. Epub 2017 Jul 11. PMID: 28696823.

17. Baghaiee B, Siahkouhian M, Karimi P, Teixeira AM, Kheslat SD. Weight gain and oxidative stress in midlife lead to pathological concentric cardiac hypertrophy in sedentary rats. Journal of Clinical Research in Paramedical Sciences. 2018 Jun 30;7(1). URL: http://jcrps.com.

18. Aihara KI, Ikeda Y, Yagi S, Akaike M, Matsumoto T. Transforming growth factor-β1 as a common target molecule for development of cardiovascular diseases, renal insufficiency and metabolic syndrome. Cardiology research and practice. 2011 Oct;2011. https://doi.org/10.4061/2011/175381.

19. Akhurst RJ. Targeting TGF-β Signaling for Therapeutic Gain. Cold Spring Harb Perspect Biol. 2017 Oct 3;9(10):a022301. doi: 10.1101/cshperspect.a022301. PMID: 28246179; PMCID: PMC5630004.

20. Hinz B. The extracellular matrix and transforming growth factor-β1: Tale of a strained relationship. Matrix Biol. 2015 Sep;47:54-65. doi: 10.1016/j.matbio.2015.05.006. Epub 2015 May 8. PMID: 25960420.

21. Liu RM, Desai LP. Reciprocal regulation of TGF-β and reactive oxygen species: A perverse cycle for fibrosis. Redox Biol. 2015 Dec;6:565-577. doi: 10.1016/j.redox.2015.09.009. Epub 2015 Oct 10. PMID: 26496488; PMCID: PMC4625010.

22. Riyahi Malayeri, S., Mirakhorli, M. The Effect of 8 Weeks of Moderate Intensity Interval Training on Omentin Levels and Insulin Resistance Index in Obese Adolescent Girls. Sport Physiology & Management Investigations, 2018; 10(2):59-68. https://www.sportrc.ir/article_67070.html?lang=en .