Research Paper

Training Can Potentially Accelerate Cardiac Output and Inhibit Cardiomyocyte Autophagy in Old Male Rats

Sareh Almasi Ghale[1], Mandana Gholami[2*], Behzad Bazgir[3], Hossein Abednatanzi[2]

1. Department of Exercise Physiology, Faculty of Faculty of Literature, Humanities and Social Sciences, Science and Research Branch, Islamic Azad University, Tehran, Iran.
2. Department of Physical Education and Sport Sciences, Faculty of Literature, Humanities and Social Sciences, Science and Research Branch, Islamic Azad University, Tehran, Iran.
3. Exercise Physiology Research Center, Lifestyle Institute, Baqiyatallah University of Medical Sciences, Tehran, Iran.

Use your device to scan and read the article online

Citation Almasi Ghale S, Gholami M, Bazgir B, Abednatanzi H. Training Can Potentially Accelerate Cardiac Output and Inhibit Cardiomyocyte Autophagy in Old Male Rats. Anatomical Sciences. 2023; 20(1):15-22.

Article info:
Received: 23 Dec 2021
Accepted: 30 Apr 2022
Available Online: 01 Jan 2023

Keywords:
High-intensity interval training (HIIT), Moderate-intensity continuous training (MICT), Autophagy, *FOXO3-3α*, *PGC-1α*, Aged heart

ABSTRACT

Introduction: Aging affects cardiac function and heart output. Regular exercise can improve heart function in old age. This experimental study evaluated the effects of high-intensity interval training (HIIT) and moderate-intensity continuous training (MICT) on cardiomyocyte autophagy and cardiac function.

Methods: In this study, 24 older male Wistar rats (>20 months) were divided into three groups of Control, HIIT, and MICT. The animals in the control group received no training, while the HIIT and MICT groups performed high and moderate training intensities at different intervals. Aerobic power and training capacity (VO_2max) were also assessed before the training. After eight weeks of training (5 days/week), two-dimensional echocardiography was used for the sonographic assessment of the heart, and the tissue samples of the left ventricle were dissected for assessing gene expression (*PGC-1α* and *FOXO-3α*). Data were analyzed using SPSS software, version 19 and presented using Mean±SD. P<0.05 was considered as statistically significant.

Results: The expression of the *PGC-1α* gene significantly increased, while the *FOXO-3α* gene expression significantly decreased in the HIIT and MICT groups compared to the control animals (P<0.05). Left ventricle end-systolic dimension decreased (P<0.05), while the left ventricular end-diastolic diameter, ejection fraction, and fractional shortening increased (P<0.05) in the training groups compared to the control animals. These changes were also significant in the HIIT group compared to the MICT group (P<0.05)

Conclusion: HIIT can reduce the expression of autophagy genes and improve cardiac function in aged heart more than MICT.

*** Corresponding Author:**
Mandana Gholami, Associate Professor.
Address: *Department of Physical Education and Sport Sciences, Faculty of Literature, Humanities and Social Sciences, Science and Research Branch, Islamic Azad University, Tehran, Iran.*
Tel: *+98 (912) 1491868*
E-mail: *gholami_man@yahoo.com*

1. Introduction

Aging, as a dynamic physiological process, involves all body parts [1], especially the cardiovascular system [2]. The aged heart represents unique biochemical and histological features such as apoptosis and myocyte hypertrophy [3]. Besides, the production of reactive oxygen species (ROS) also increases with age, leading to the disturbance of enzymatic function involved in the oxidative phosphorylation chain in the mitochondria of the heart [4]. Autophagy is an essential cellular process for the elimination of damaged cellular components. Disruptions of autophagy are associated with a variety of diseases, including cancer, neurodegeneration, metabolic disorders, and aging [5]. Fluctuations in oxygen concentrations caused by cellular oxidative activities are balanced using autophagy [6]. One of the main indicators of this process is the forkhead transcription factor O subfamily member-3α (FOXO-3α) protein, which prevents pathological hypertrophy of myocardiocytes [7]. The peroxisome proliferator-activated receptor-gamma coactivator-1α (PGC-1α) regulates metabolism. Any suppression in the expression of PGC-1α leads to cardiac hypertrophy and related morbidity [8]. Echocardiography is a non-invasive and safe procedure for diagnosing cardiomyopathy and grades of cardiac diseases [9]. Ejection fraction (EF) is the percentage of blood flow from the left ventricle during a heartbeat. FS is the measurement of cardiac contractility commonly used in echocardiography. It is calculated as the percentage changes in the left ventricular internal dimension from end-diastole to end-systole. LVEDD is obtained during echocardiography and provides valuable data about the size of the left ventricle during diastole, and LVESD represents the function of the left ventricle [10]. Intermittent training reduces the ROS level by accelerating Sirt-3 in mitochondria, and moderate-intensity training increases the amount of superoxide dismutase and total antioxidant capacity in cells [11]. It is reported that the Sirt1/AMPK/PGC-1α pathway is associated directly with the aging in skeletal and cardiac muscles [12]. Since exercise is important for health improvement, a particular type of training can lead to proper cardiac function. Therefore, this study aims to assess the beneficial effects of HIIT and MICT on the expression of autophagy-associated genes (*PGC-1α* and *FOXO-3α*) and cardiac function indicators (EF, FS, LVESD, and LVEDD).

2. Materials and Methods

Laboratory animals

The present experimental research was conducted on 24 older (>20 months) male Wistar rats with an average weight of 350±50 g. The animals were prepared by the Pasteur Institute (Tehran, Iran). All research processes were conducted in compliance with the ethical issues, according to the instructions of the ethics committee for animal care and use. All rats were housed in normal physiological conditions, including environmental conditions, temperature, and humidity. In all stages of the assessment, the weight of animals was recorded for future analysis. All laboratory materials were also provided by Sigma Aldrich (St Louis, MO, USA), NexGen (USA), and SinaClon companies.

Training capacity and VO_2max assessment

The VO_2max test measures the maximum level of oxygen uptake, representing an indicator of cardiovascular health and endurance capacity. The test involves measuring the O_2 consumption of rodents running on a treadmill, typically at increasing intensities, until the maximum oxygen uptake rate is reached. The VO_2max test was performed two days before the training. After 3 minutes of warming up on the treadmill at 5 m/min, the treadmilling speed was accelerated to 4 m/min every 2 minutes. The maximum speed of treadmilling was stabilized at the specific time point when the animals were unable to run continuously for 90 seconds. VO_2max can be calculated from the recorded data by dividing the highest oxygen consumption value by the body weight of the rodent. The result is typically expressed in mLO_2/kg/min [13].

Study groups and training protocols

The animals (n=24) were categorized into three groups of 8, including control, HIIT, and MICT. Treadmilling (Model 12638, Mahour Co.) was set at 3 m/min for the HIIT and MICT groups, while the control animals had no training program. Each group performed the associated protocol five days/week for eight weeks. On the sixth day of each week, the maximum oxygen consumption (VO_2max) was measured, and the last day of the week was designed for animal rest. Both MICT and HIIT protocols were applied based on Hoydal's study [14]. The HIIT protocol was having exercise for 30 minutes, including a 5-minute warm-up with four 3-minute intervals. The MICT protocol involved warming up on a treadmill with an increase in the speed by 2 m/min ev-

ery 2 minutes until the mice were unable or unwilling to continue.

Protocol of aerobic power and exercise capacity

The exercise capacity test was performed two days before the training program and at the end of each week. After 3 minutes of warming up (5 m/min), the treadmill's speed was increased to 4 m/min every 2 minutes. The maximum speed was set until the animal had no physical activity to run at a constant speed for at least 90 seconds (treadmill tilt was 0°). Since there is a high correlation between treadmill speed and VO_2max, it is possible to obtain the level of VO_2max according to the maximum running speed. The intensities were adjusted according to the obtained speed [15].

Continuous training protocol

The protocol typically involves gradually increasing the speed and duration of the treadmill running sessions to improve the rodent's cardiovascular fitness and endurance capacity. According to the design of animal exercise protocols, a typical training protocol for laboratory animals might include short runs to 70-80% of their peak treadmill speed 2 or 3 days per week. In this study, the aerobic exercise included running on a treadmill (treadmill tilt was 0°) for eight weeks and five sessions/week with an intensity of 60-65% of the speed at peak oxygen consumption (vVO_2max). The duration of each training session was designed as 10, 15, 22, 30, 36, 42, 48, and 50 min with 5 min of warming up and cooling down (intensity of 30-40% VO_2max) and 5 min of cooling (intensity of 30-40% VO_2max) [13].

Animal sacrificing and tissue sampling

24 h after the last training session, the animals were anesthetized by intraperitoneal (IP) injection of ketamine (90 mg/kg, Sigma Aldrich, CAT NO: 1867-66-9) and xylazine (10 mg/kg, NexGen, CAT NO: NC-0107). 20 min later, the cardiac puncture was applied, and the blood samples were collected directly from the heart. Centrifugation (3000 rpm, 15 min, Cent Machine Co, Product NO: 12-CM-HQ1) was conducted, and blood serum was isolated for future laboratory assays. The cardiac tissue (left ventricle) was also dissected and stored in liquid nitrogen for gene assessments [16].

Assessment of the genes expression

Total RNA was extracted using the QIAGEN RNA purification mini kit according to the manufacturer's instructions. In this procedure, 30 mg of tissue was placed in RLT buffer; ethanol (96%) was added to the lysate and centrifuged. The supernatant was added to the RNeasy mini spin column. Then, the total RNA bounded to the column membrane, the contaminants were efficiently washed away, and high-quality RNA was eluted in RNase-free water. The quality of the extracted RNA was checked by a spectrophotometer (UV1240, Shimadzu, Kyoto, Japan) in 260/280 nm wavelength absorbance ratio. DNA was synthesized using a commercial BioFact kit (BioFact RT Series, Korea). According to the kit instructions, 1 µg of total RNA, 10 µL of mastermix, 0.5 µL of oligo-d (T) primer, and 0.5 µL of Random Hexamer primers were added. Then, the final volume with RNase-free water was increased to 20 µL. The RT reaction was conducted at 70 °C (45 min), followed by heat inactivation at 95 °C (3 min). The expression of p53, BAX, and Bcl-2 were evaluated using High ROX BioFact™ 2X Real-Time PCR Smart mix SYBR Green PCR master mix. Real-Time PCR light cycler device (StepOne™ Real-Time PCR System, U.S.) was based on the manufacturer's instructions. The PCR primers were designed by Oligo software, the sequences were blasted in the NCBI database, and the genes' sequences were listed as *PGC-1α* and *FOXO-3α*. The PCR reactions for mRNA expression consisted of 95 °C for 5 min (denaturing cycle) followed by variable amplification cycles (38-42 cycle) at 90 °C for 30 s (annealing cycle) and 72 °C for 1 min (extension cycle). All qRT-PCR reactions were duplicated, and β-actin was used as a housekeeping gene. Gene expression levels were measured using the Ct ($2^{-\Delta\Delta t}$) method (fold changes). *GAPDH* was also hired as an internal control (Table 1) [17].

Two-dimensional echocardiography

24 h after the last training, two-dimensional (2D) chest-connected echocardiography was performed (M-mode model, GE-VIVID-7, V.5, USA) equipped with the 10 MHz transducer. Following anesthesia induction using IP injection of Sodium Thiopental (30 mg/kg, CAT BO: 978), various cardiac-associated indices were measured, including LVESD, LVEDD, EF, and SF [18].

Data analysis

After extracting the statistics, the Kolmogorov–Smirnov test was first conducted to confirm the normal data distribution. One-way analysis of variance (one-way ANOVA) was used for statistical analysis, and the Tukey post hoc test was used to determine the difference between the groups. SPSS software, version 19, was

Table 1. Primers used in real-time PCR

Primer	Primer Sequences	Product Size (bp)	TM	Annealing Temperature	Product Size (bp)
GAPDH	F : 50-GGCACCACACCTTCTACAATG-30 R: 50-GGGGTGTTGAAGGTCTCAAAC-30	20 19	58.53 58.32	58	82
PGC-1α	F: 50-AGCCATATTTGCCGTCCTTCTC -30 R:50-AAGGACAAGTGGTCCGAGTA-AAG-30	23 23	60.31 64.94	60	146
FOXO-3α	F:50- AAGCAAACAGGGCCAATGTC-30 R:50- GGGAAGGACAAGATCGTCCTT-30	22 22	60.5 61.3	60	132

ANATOMICAL SCIENCES

used for data analysis, and the results were expressed as Mean±SD, and P<0.05 was considered significant.

Results

Following the application of both HIIT and MICT training, no significant (P>0.05) changes were detected in total body weight among all treatment and C groups (Table 2). A significant (P<0.05) acceleration in *PGC-1α* gene expression was detected in HIIT and MICT animals than in the control group. Also, a significant incremental change in *PGC-1α* gene expression was found in the HIIT group than in the MICT group (Figure 1A). A significant (P<0.05) decrease in *FOXO-3α* gene expression was detected in the HIIT group than in the control and MICT groups. No significant difference (P>0.05) in *FOXO-3α* gene expression was found between the MICT and control groups (Figure 1B). The LVEDD index increased significantly in the HIIT and MICT groups compared to the control animals (P<0.05). Also, this index showed a significant decrease (P<0.05) in the HIIT group compared to the MICT group (Figure 1C). The LVESD index decreased significantly (P<0.05) in the MICT and HIIT groups compared to the control animals. No significant difference (P>0.05) was found between the HIIT and MICT groups regarding LVESD (Figure 1D). The EF index increased significantly (P<0.05) in the MICT and HIIT groups compared to the control animals. There was also a significant (P<0.05) higher rate of EF index in the HIIT group compared to the MICT group (Figure 1E). The FS index increased significantly (P<0.05) in the MICT and HIIT groups compared to the control animals. The FS index also significantly increased (P<0.05) in the HIIT group compared to the MICT group (Figure 1F).

Discussion

Aging is a physiological process causing a decrease in cardiac output. Numerous studies have shown that exercise can delay aging or suppress its negative effects by multiple biological mechanisms. Since different exercise

Table 2. The values of weight and echocardiographic indicators in different study groups

Variables	Mean±SD			
	Category	Control (n=8)	HIIT (n=8)	MICT (n=8)
Weight (g)	Before experiment	350±30	349±24	361±33
	After experiment	358±35	370±31	374±39
Echocardiographic indices	LVEDD	7.2±0.8	13.4±0.3*	12.9±0.5*
	LVESD	5.4±0.6	2.1±0.2*	3.4±0.3*
	EF	75.4±8.4	92.3±4.9*	87.1±8.2*
	FS	55.2±5.1	76.2±4.9*	78.3±9.1*#

ANATOMICAL SCIENCES

Abbreviations: HIIT: High-intensity interval training; MICT: Moderate-intensity continuous training; LVEDD: Left ventricular end-diastolic diameter; LVESD: Left ventricular end-systolic diameter; EF: Ejection fraction; FS: Fractional shortening.

*, #Significant differences compared to the control and HIIT groups.

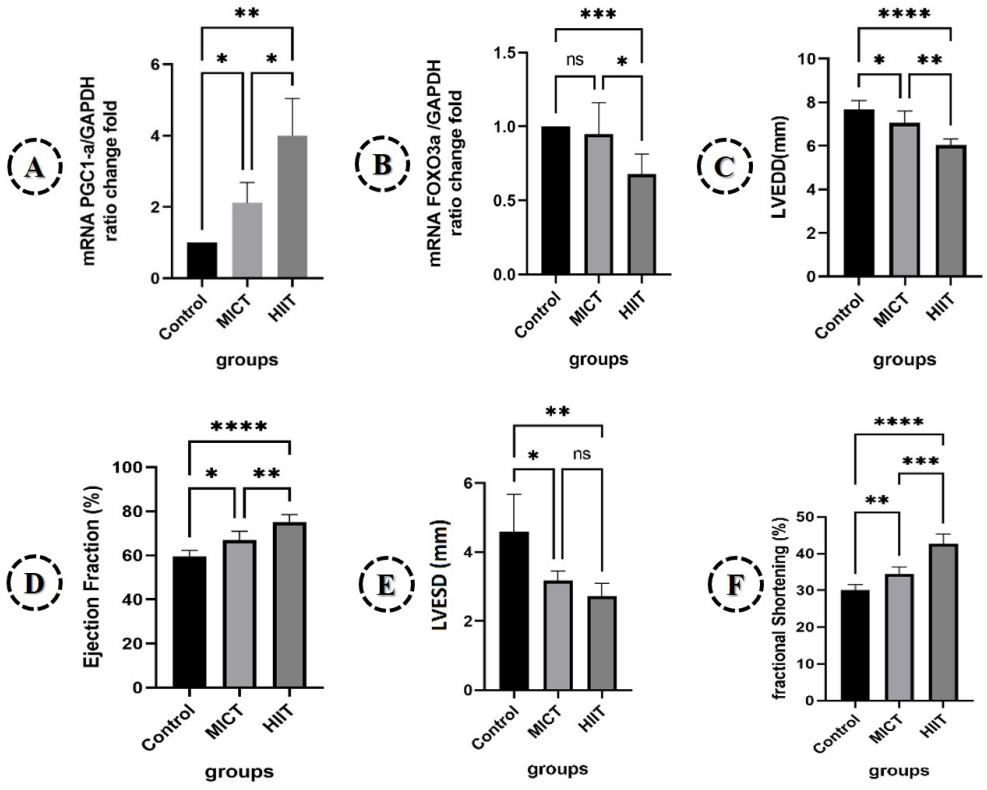

Figure 1. The findings for different study groups

A) *PGC-1α* gene expression with *GAPDH* as internal control, B) *FOXO-3α* gene expression with *GAPDH* as internal control, C) LVEDD, D) LVESD, E) EF, F) FS

Abbreviations: HIIT: High-intensity interval training; MICT: Moderate-intensity continuous training; LVEDD: Left ventricular end-diastolic diameter; LVESD: Left ventricular end-systolic diameter; EF: Ejection fraction; FS: Fractional shortening.

*P<0.05, **P<0.01, ***P<0.001, ****P<0.0001.

protocols are defined and used in the science of exercise physiology, the purpose of this study was to investigate the therapeutic effect of HIIT and MICT on cardiac function. The findings of the present study showed that HIIT and MICT can potentially increase the *PGC-1α* gene expression. The assessment of *FOXO-3α* gene expression showed that only HIIT had a considerable decremental trend compared to the C group; besides, the MICT had no significant changes compared to the C group. Following the echocardiography assessment, the results showed a significant increase in LVEDD in both HIIT and MICT training groups compared to the control animals. Concerning LVESD, the results showed a significant decrease in LVESD in both training groups compared to the control animals. Regarding EF and SF, the results showed that following training, a significant increase in EF can occur in both HIIT and MICT training groups compared to the C group. Apoptosis, a programmed cell death process, has long been considered irreversible, with caspase activation leading to cell demise. Stem cell studies have shown that enforced expression of specific genes can influence the transcription of genes relevant to different cell types. GABAergic neurons are susceptible to BAX-dependent apoptosis, with changes in microglia morphology and cytokine gene expression following exposure to certain stimuli. Studies on stroke recovery have highlighted the importance of gene expression changes post-injury, indicating critical periods for motor training and rehabilitation [19]. Additionally, research on the molecular basis of herpes simplex virus latency has shown alterations in gene expression following the reversal of silencing mechanisms, with implications for apoptosis regulation. Furthermore, investigations into pancreatic β-cell death in diabetes have revealed the role of pro- and anti-apoptotic gene expression in disease progression [20]. Genome editing technologies, such as CRISPR, have been utilized to study changes in gene expression after cancer drug therapy, offering insights into targeted therapy approaches. Overall, the regulation of apoptotic gene expression following training in animal experiments is a complex process influenced by various factors, including cell type, stimuli, and disease

conditions. Further research in this field is essential for understanding the mechanisms underlying apoptosis and developing targeted therapeutic interventions. The research findings regarding the gene expression of *FOXO-3α* showed that the implementation of HIIT and MICT induced a significant alteration in the expression of the *FOXO-3α* gene. A decrease in the expression of this gene following HIIT is consistent with the results of the study by Kavazis et al. [21] and inconsistent with the findings of Holloway et al. [22]. Also, a decreasing trend was observed in *FOXO-3α* gene expression following MICT which was inconsistent with the results of Louis et al. [23] and consistent with the findings of Slopack et al. [24]. Contrary to the findings of the present study, Tanya Holloway et al. [22] investigated the effect of CT and HIIT training on atrophy-associated factors in rats. They observed that four weeks of CT and HIIT training caused no detectable change in atrophy-related proteins. Among these, the *FOXO-3α*, muscle atrophy F-box (MAFbx), and muscle RING finger protein-1 (MuRF1) did not increase after applying HIIT and MICT [22]. In the study of Kavazis et al. [21]. the effect of 10 sessions of endurance training on doxorubicin side effects and *FOXO-3α* gene expression was investigated. They found that this type of training can potentially increase mitochondrial biogenesis through *PGC-lα* gene expression in aged rats. Also, they found that high expression levels of *PGC-1α* induce *FOXO-3α* suppression and MuRFI inhibition. Besides, the endurance exercise could not change the expression of the *MAFbx* gene or reduce the side effects of doxorubicin on cardiac muscles [21]. Slopack et al. investigated the effects of training for 7, 10, and 14 days on the *FOXO-3α* gene expression and protein levels in male and female mice [24]. They showed that *FOXO-3α* was increased considerably. They also reported that the protein levels remained unchanged following seven days and decreased after 10 and 14 days of training. This finding represented that preservation of high levels of *FOXO-3α* gene expression requires persistent training sessions [24]. In the present study, the expression level of the *FOXO-3α* gene also decreased following persistent training, which is consistent with Slopack et al.'s study. In another study, eight weeks of treadmilling could potentially increase mitochondrial adaptations, including high levels of *PGC-1α* and *Tfam* and low levels of *FOXO-3α* [25]. This finding is consistent with the results of the present study. A recent study showed that HIIT improves the cardiovascular system more than other types [26]. Also, the effects of HIIT exercises on improving mitochondrial density and insulin sensitivity were noticed [26]. According to the results of various studies, the amount and intensity of training are effective factors in energy consumption and generation of cardiac adaptation. However, some studies reported the beneficial effects of moderate-intensity aerobic training on reducing insulin resistance, which was similar to the effect of HIIT [27]. The echocardiography indices following training in animal experiments have also been explored in cardiology. However, the literature review does not directly address this specific topic. Instead, studies such as Willems et al. [28] focused on catheter ablation of persistent atrial fibrillation, while Becker examined post-stroke aphasia telerehabilitation. These studies demonstrate the diverse range of research topics within the medical field. Furthermore, the importance of randomized controlled trials in assessing the effectiveness of interventions is evident in several of the provided documents. For instance, Durand conducted a multicentric cluster randomized controlled trial to assess the impact of health literacy training for general practitioners on colorectal cancer screening in underserved areas. Overall, the literature highlights the significance of training and interventions in various medical specialties and emphasizes the need for rigorous research methodologies, such as randomized controlled trials, to evaluate their effectiveness. Further research focusing specifically on echocardiography indices following training in animal experiments could provide valuable insights into this area of study. In the present study, the EF increased and the LVESD decreased in HIIT animals, indicating the improvement of the cardiac response to this type of training. In HIIT, high activity of the cardiac and respiratory systems can increase the cardiac preload, improving both diastole and systole effects [29]. However, a recent study showed that gene regulation completely depends on the training intensity, which was observed in HIIT [30]. In an animal study, swimming training for 20 weeks can accelerate the function and structure of the heart's left ventricle [9], which is consistent with our findings. According to the effects of HIIT and MICT on biomarkers of mitochondrial biogenesis and *PGC-1α*, it is suggested to investigate other factors involved in longevity, including telomerase enzyme and other age-related factors, for future investigations.

Conclusion

The results of the present study showed non-significant changes in the weight of elderly animals following training. Also, the findings represented a high expression of *PGC-1α* and a low expression of *FOXO-3α* autophagy genes after HIIT and MICT. Investigation of the echocardiographic indicators showed high levels of EF, FS, and LVEDD and a low level of LVESD following HIIT and MICT. The HIIT is more beneficial in reducing the

expression of autophagy genes and improving echocardiographic indicators than the MICT.

Ethical Considerations

Compliance with ethical guidelines

All assessments were conducted in accordance with ethical principles and under the supervision of the Ethics Committee of Islamic Azad University, Science and Research Branch (Code: IR.IAU.SRB.REC.1400.192).

Funding

This research did not receive any grant from funding agencies in the public, commercial, or non-profit sectors.

Authors' contributions

Study design: Mandana Gholami; Laboratory assays: Behzad Bazgir and Sareh Almasi; Statistical analysis: Hossein Abednatanzi.

Conflict of interest

The authors declared no conflict of interest.

References

[1] Calcinotto A, Kohli J, Zagato E, Pellegrini L, Demaria M, Alimonti A. Cellular senescence: Aging, cancer, and injury. Physiological Reviews. 2019; 99(2):1047-78. [DOI:10.1152/physrev.00020.2018] [PMID]

[2] Lippi G, Sanchis-Gomar F. An estimation of the worldwide epidemiologic burden of physical inactivity-related ischemic heart disease. Cardiovascular Drugs and Therapy. 2020; 34(1):133-7. [DOI:10.1007/s10557-019-06926-5] [PMID]

[3] Nanayakkara S, Marwick TH, Kaye DM. The ageing heart: The systemic and coronary circulation. Heart. 2018; 104(5):370-6. [DOI:10.1136/heartjnl-2017-312114] [PMID]

[4] Triposkiadis F, Xanthopoulos A, Butler J. Cardiovascular aging and heart failure: JACC review topic of the week. Journal of the American College of Cardiology. 2019; 74(6):804-13. [DOI:10.1016/j.jacc.2019.06.053] [PMID]

[5] Aman Y, Schmauck-Medina T, Hansen M, Morimoto RI, Simon AK, Bjedov I, et al. Autophagy in healthy aging and disease. Nature Aging. 2021; 1(8):634-50. [DOI:10.1038/s43587-021-00098-4] [PMID]

[6] Yun HR, Jo YH, Kim J, Shin Y, Kim SS, Choi TG. Roles of autophagy in oxidative stress. International Journal of Molecular Sciences. 2020; 21(9):3289. [DOI:10.3390/ijms21093289] [PMID]

[7] Jokar M, Sherafati Moghadam M, Daryanoosh F. The effect of an 8-week endurance training program on the content of FOXO3a and beclin-1 proteins in heart muscle of rats with type 2 diabetes. The Journal of Qazvin University of Medical Sciences. 2020; 23(6):484-93. [DOI:10.32598/JQUMS.23.6.1]

[8] Brainard RE, Facundo HT. Cardiac hypertrophy drives PGC-1α suppression associated with enhanced O-glycosylation. Biochimica et Biophysica Acta. Molecular Basis of Disease. 2021; 1867(5):166080. [DOI:10.1016/j.bbadis.2021.166080] [PMID]

[9] Oláh A, Kovács A, Lux Á, Tokodi M, Braun S, Lakatos BK, et al. Characterization of the dynamic changes in left ventricular morphology and function induced by exercise training and detraining. International Journal of Cardiology. 2019; 277:178-85. [DOI:10.1016/j.ijcard.2018.10.092] [PMID]

[10] Li C, Dai J, Wu F, Zhang H. Impacts of different anesthetic agents on left ventricular systolic function in mice assessed by echocardiography. Physiological Research. 2019; 68(3):365-74. [DOI:10.33549/physiolres.933940] [PMID]

[11] Lin CH, Lin CC, Ting WJ, Pai PY, Kuo CH, Ho TJ, et al. Resveratrol enhanced FOXO3 phosphorylation via synergetic activation of SIRT1 and PI3K/Akt signaling to improve the effects of exercise in elderly rat hearts. Age. 2014; 36(5):9705. [DOI:10.1007/s11357-014-9705-5] [PMID]

[12] Yuan Y, Shi M, Li L, Liu J, Chen B, Chen Y, et al. Mesenchymal stem cell-conditioned media ameliorate diabetic endothelial dysfunction by improving mitochondrial bioenergetics via the Sirt1/AMPK/PGC-1α pathway. Clinical Science. 2016; 130(23):2181-98. [DOI:10.1042/CS20160235] [PMID]

[13] Kraljevic J, Marinovic J, Pravdic D, Zubin P, Dujic Z, Wisloff U, et al. Aerobic interval training attenuates remodelling and mitochondrial dysfunction in the post-infarction failing rat heart. Cardiovascular Research. 2013; 99(1):55-64. [DOI:10.1093/cvr/cvt080] [PMID]

[14] Høydal MA, Wisløff U, Kemi OJ, Ellingsen O. Running speed and maximal oxygen uptake in rats and mice: Practical implications for exercise training. European Journal of Cardiovascular Prevention and Rehabilitation. 2007; 14(6):753-60. [DOI:10.1097/HJR.0b013e3281eacef1] [PMID]

[15] Haram PM, Kemi OJ, Lee SJ, Bendheim MØ, Al-Share QY, Waldum HL, et al. Aerobic interval training vs. continuous moderate exercise in the metabolic syndrome of rats artificially selected for low aerobic capacity. Cardiovascular Research. 2009 1; 81(4):723-32. [DOI:10.1093/cvr/cvn332] [PMID]

[16] Roshankhah S, Abdolmaleki A, Salahshoor MR. Anti-inflammatory, anti-apoptotic, and antioxidant actions of Middle Eastern Phoenix dactylifera extract on mercury-induced hepatotoxicity in vivo. Molecular Biology Reports. 2020; 47(8):6053-65. [DOI:10.1007/s11033-020-05680-4] [PMID]

[17] Abdolmaleki A, Jalili C, Mansouri K, Bakhtiari M. New rat to mouse xenograft transplantation of endometrium as a model of human endometriosis. Animal Models and Experimental Medicine. 2021; 4(3):268-77. [DOI:10.1002/ame2.12181] [PMID]

[18] Hagar JM, Matthews R, Kloner RA. Quantitative two-dimensional echocardiographic assessment of regional wall motion during transient ischemia and reperfusion in the rat. Journal of the American Society of Echocardiography. 1995; 8(2):162-74. [DOI:10.1016/S0894-7317(05)80405-5] [PMID]

[19] Gonzalez-Ramos A, Waloschková E, Mikroulis A, Kokaia Z, Bengzon J, Ledri M, et al. Human stem cell-derived GABAergic neurons functionally integrate into human neuronal networks. Scientific Reports. 2021; 11(1):22050. [DOI:10.1038/s41598-021-01270-x] [PMID]

[20] Petrovic I, Pejnovic N, Ljujic B, Pavlovic S, Miletic Kovacevic M, Jeftic I, et al. Overexpression of galectin 3 in pancreatic β cells amplifies β-cell apoptosis and islet inflammation in type-2 diabetes in mice. Frontiers in Endocrinology. 2020; 11:30. [DOI:10.3389/fendo.2020.00030] [PMID]

[21] Kavazis AN, Smuder AJ, Powers SK. Effects of short-term endurance exercise training on acute doxorubicin-induced FoxO transcription in cardiac and skeletal muscle. Journal of Applied Physiology. 2014; 117(3):223-30. [DOI:10.1152/japplphysiol.00210.2014] [PMID]

[22] Holloway TM, Bloemberg D, da Silva ML, Simpson JA, Quadrilatero J, Spriet LL. High intensity interval and endurance training have opposing effects on markers of heart failure and cardiac remodeling in hypertensive rats. Plos One. 2015; 10(3):e0121138. [DOI:10.1371/journal.pone.0121138] [PMID]

[23] Louis E, Raue U, Yang Y, Jemiolo B, Trappe S. Time course of proteolytic, cytokine, and myostatin gene expression after acute exercise in human skeletal muscle. Journal of Applied Physiology. 2007; 103(5):1744-51. [DOI:10.1152/japplphysiol.00679.2007] [PMID]

[24] Slopack D, Roudier E, Liu ST, Nwadozi E, Birot O, Haas TL. Forkhead BoxO transcription factors restrain exercise-induced angiogenesis.The Journal of Physiology. 2014; 592(18):4069-82. [DOI:10.1113/jphysiol.2014.275867] [PMID]

[25] Lee I, Hüttemann M, Kruger A, Bollig-Fischer A, Malek MH. (-)-Epicatechin combined with 8 weeks of treadmill exercise is associated with increased angiogenic and mitochondrial signaling in mice. Frontiers in Pharmacology. 2015; 6:43. [DOI:10.3389/fphar.2015.00043]

[26] Little JP, Gillen JB, Percival ME, Safdar A, Tarnopolsky MA, Punthakee Z, et al. Low-volume high-intensity interval training reduces hyperglycemia and increases muscle mitochondrial capacity in patients with type 2 diabetes. Journal of Applied Physiology. 2011; 111(6):1554-60. [DOI:10.1152/japplphysiol.00921.2011] [PMID]

[27] Motiani KK, Savolainen AM, Eskelinen JJ, Toivanen J, Ishizu T, Yli-Karjanmaa M, et al. Two weeks of moderate-intensity continuous training, but not high-intensity interval training, increases insulin-stimulated intestinal glucose uptake. Journal of Applied Physiology. 2017; 122(5):1188-97. [DOI:10.1152/japplphysiol.00431.2016] [PMID]

[28] Willems S, Borof K, Brandes A, Breithardt G, Camm AJ, Crijns HJGM, et al. Systematic, early rhythm control strategy for atrial fibrillation in patients with or without symptoms: the EAST-AFNET 4 trial. European Heart Journal. 2022; 43(12):1219-30. [DOI:10.1093/eurheartj/ehab593] [PMID]

[29] Haykowsky MJ, Timmons MP, Kruger C, McNeely M, Taylor DA, Clark AM. Meta-analysis of aerobic interval training on exercise capacity and systolic function in patients with heart failure and reduced ejection fractions. The American Journal of Cardiology. 2013; 111(10):1466-9. [DOI:10.1016/j.amjcard.2013.01.303] [PMID]

[30] Rosenblat MA, Granata C, Thomas SG. Effect of interval training on the factors influencing maximal oxygen consumption: A systematic review and meta-analysis. Sports Medicine. 2022; 52(6):1329-52. [DOI:10.1007/s40279-021-01624-5] [PMID]