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Original Article

The effect of high-intensity interval training with caloric restriction on gene expression of apoptosis indices HSP70 and HSP60 in the myocardium of male rats

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Abstract

Background and aims: The purpose of this study was to investigate the effect of high-intensity interval training combined with calorie restriction on the expression of HSP70 and HSP60 in the myocardium of male rats.

Methods: A total of 27 male rats were randomly divided into three groups (9 rats in each group): control, caloric restriction, and caloric restriction with high-intensity interval training. During the study, the control group had free access to water and food, but the amount of food in the food restriction group was limited to 50% and the calorie restriction-exercise group was limited to 25% of the control group. The restriction-exercise group was subjected to intense interval training for eight weeks. The gene expression of apoptotic indicators HSP70 and HSP60 was assessed using the real-time PCR method. The data were analyzed using Shapiro-Wilk test and ANOVA test.

Results: Body weight in both calorie restriction groups significantly decreased ($P=0.001$). The results of the post hoc test showed a significant increase in the expression of HSP70 and HSP60 genes in the myocardium of male rats in the calorie-restricted-exercise group compared to the calorie-restricted and control groups ($P=0.001$). Additionally, no significant difference was observed between the two restriction and control groups in terms of HSP70 and HSP60 ($P=0.102$).

Conclusion: The combination of high-intensity interval training and mild food restriction significantly increased HSP60 and HSP70 expression.

Keywords: High-intensity, Interval training, Caloric restriction, Apoptosis, HSP70, HSP60

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Introduction

Oxidative stress caused by the production of free radicals is one of the main factors in myocardial ischemic damage, which is very important in the development of diseases such as heart attack (1,2). In an ischemic heart, the delivery of oxygen to the mitochondria of the heart muscle is not enough. This leads to an increase in the leakage of electrons from the electron transport chain, which in turn reacts with the residual oxygen and produces reactive oxygen species (3,4). On the other hand, maintaining a suitable weight is an important determining factor for survival and continuation of life. In the past decade, appetite, food intake, balance, and energy homeostasis have always been among the main and favorite topics of researchers in the field of various sciences (5). Improper diet and reduced physical activity increase reactive oxygen species, reduce antioxidant activity, and induce apoptotic mechanisms, which can lead to cardiovascular diseases (6). Apoptosis or programmed cell death is a genetic process that is an integral part of the growth and development of a living organism. It is used to eliminate unnecessary cells in a targeted way and plays an important role in normal tissue homeostasis and the development of diseases (7). One of the most specific cellular responses to stress is the production of heat shock proteins (HSPs) (8,9). The increase and accumulation of HSP70 protein levels are induced by several stimuli in natural conditions, including overheating, ischemia, hypoxia, energy depletion, acidosis, and the formation of reactive oxygen species (10). HSP60 in mitochondria helps to facilitate protein folding and is considered one of the most important proteins for cell survival due to its essential role in supporting mitochondrial function (11). HSP60 mRNA levels have been reported to be doubled in subjects with ischemic cardiomyopathy compared to normal controls (12). In one study, after 20 minutes of ischemia followed by 30 minutes of repercussion, HSP60 mRNA levels remained unchanged in the rat heart (12). The reduction of HSP60 expression in cardiomyocytes leads to the breakdown of interchaperone complexes (Bak and Bax), induction of apoptosis, release of cytochrome C, activation of caspase 3, and induction of DNA fragmentation (13). Since HSP60

can affect cardiac apoptosis, this chaperone is thought to be involved in the pathogenesis of heart failure. This condition is basically a chronic inflammation and injury to the heart muscle, which leads to cell death and thus a decrease in the pumping function of the heart (14).

On the other hand, physical activities, especially high-intensity interval training, have significant effects on weight loss, reducing calorie intake, improving cellular redox, and increasing mitochondrial biogenesis (15-17). Pouzesh Jadidi et al showed that eight weeks of interval exercise program significantly decreased the expression of the caspase 3 gene in the heart muscle of rats in the heart attack model in the exercise group (14). McMillan et al reported that 6 weeks of endurance training reduced DNA fragmentation and release of cytochrome c and Bax protein (16). Moreover, using more than 25% calorie restriction, especially intermittent calorie restriction, is one of the other methods of weight loss, which is very popular among both weightlifting athletes and ordinary people (18). However, this method has disadvantages such as reduction of muscle mass, mental discomfort caused by hunger, and lack of improvement in physical functions (19). For this purpose, some researchers have suggested that adding exercise training to the calorie restriction program can increase the benefits of each of these two methods and achieve similar benefits with the use of a lower amount of calorie restriction (20-22). Therefore, the aim of this research was to investigate the combined effect of high-intensity interval training and caloric restriction on HSP70 and HSP60 gene expression in the myocardium of male rats.

Materials and Methods

The present research was an experimental study. The statistical population consisted of 8-month-old rats obtained from the Pasteur Institute of Iran. A total of 27 rats were selected with a mean weight of 318.22 ± 18.2 g. In order to avoid stress and change in the physiological conditions, the rats were subjected to new conditions for two weeks in the central animal house of the laboratory of Islamic Azad University Central Tehran Branch. During this period, all rats had free access to standard animal food. Their diet consisted of 50% carbohydrates, 25% protein, 5% fat, 7% fiber, 13% moisture and ash (11). In the present study, all rules and regulations of handling animals (familiarization, training, anesthetizing, and killing of animals) were observed according to the Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC). Nine mice were included in the control group and the rest were included in the experimental groups. The familiarization phase lasted for 1-2 weeks, which included 5 sessions of running on the treadmill for 10 to 15 minutes at a speed of 8-10 m/min. At the end of this period, after weight matching, the rats were randomly divided into two groups: food restriction and food restriction + interval training (23).

In the food restriction protocol of the research, the mice in the control group had free access to standard food and water. During the period, in order to determine the amount of food consumed by the food restriction and food restriction-exercise groups, the exact amount of food consumed by the control group was measured and recorded daily, and the mean food consumption of these subjects was 24 ± 2 g/d. In this way, the food restriction group received 50% and the interval exercise restriction group received 25% of the food consumed by the control group, which was approximately 12 ± 1 and 18 ± 1 g/d,These restrictions were applied in two 12-hour periods (24) (Figure 1).

The high intensity interval training (HIIT) protocol used in this research was performed on a treadmill for 8 weeks and 5 sessions per week, in such a way that in each session there were ten 4-minute bouts of activity with an intensity equal to 85%-90% of VO2max and active rest periods of 2 minutes with an intensity of 40%-45% VO2 max. The training intensity increased progressively until the eighth week (by changing the treadmill speed by 0.02 m/s), but the incline of the treadmill remained unchanged (zero) throughout the training period. In each session, before starting the main training phase, the warm-up was done for 10 minutes at a speed of 5 m/s (25) (Table 1).

Aerobic capacity was estimated using a treadmill based

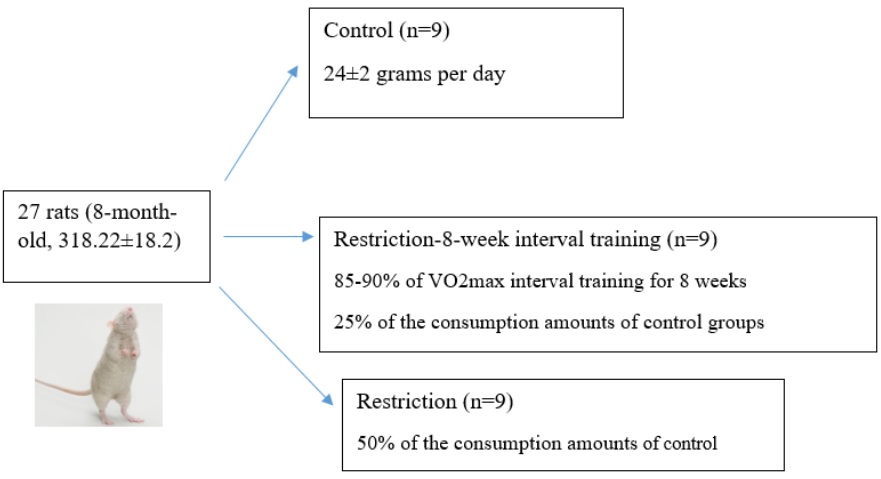

Figure 1. Study Scheme

on an indirect protocol. Accordingly, after 10 minutes of warm-up, the running test started and the speed of the treadmill increased by 0.03 m/s (1 to 2.8 m/min) every 2 minutes until the rats were not able to run. (The criteria for failure was three falls from the treadmill). The speed of reaching the resting state was recorded as vVo2 max (24).

Laboratory Animal Surgery and Sample Extraction

All rats were killed 24 hours after the last training session, after being anesthetized with Avertin (0.2 mL/kg of body weight). The heart was removed from the body after cold saline injection. Then, it was extracted and weighed. Tissue samples were extracted by homogenization in lysis buffer at a concentration of 100 mg of tissue per mL of the buffer. The homogenous samples were centrifuged on ice for 40 minutes at 12 000 rpm. Then, the supernatant was collected after centrifugation and kept at -80 degrees until analysis. After being washed in physiological serum, the sampled cardiac muscle tissue was immersed in 1.8 microtubes containing 20% RNAlater™ solution and transferred to a genetic testing laboratory. Gene expression measurement of cardiac muscle tissue was measured by real-time PCR technique and after quantification of gene expression values, it was analyzed using ctΔΔ formula (Table 2). The PCR reaction was performed using PCR master mix (Applied Biosystems) and SYBR Green in the machine (Applied Biosystems, Sequence ABI Step One Detection Systems. Foster City, CA) according to the manufacturer's protocol. To quantitatively measure the expression of the desired genes, a kit (SYBR-green Real-Time RT-PCR, TAKARA, Japan) was used and 1.20, 1.10, and 1.50 µL concentrations of cDNA were prepared. The concentration of 1.20 µL was used as the template for real-time PCR. cDNA was amplified with specific primers for HSP60 and HSP70 genes. The Ct values obtained for each sample were normalized using the internal control U6 snRNA (cat #4373381, Ambion). Specifically, the relative mRNA expression was obtained by subtracting the ct of the U6 snRNA from the Ct value of the target mRNA, which was again subtracted from the value obtained in the reference (control) sample. Fold change was calculated using the equation 2ΔΔCt. All measurements were repeated three times.

Data were collected using SPSS software version 22.0. The normality of the data distribution was checked by the Shapiro-Wilk test. It was found that the data had a normal distribution. ANOVA test was used to compare differences between groups.

Results

The distribution of data in all variables was normal according to the results of the Shapiro-Wilk test. There was no difference between the rats at the beginning of the research and some of their characteristics are presented in Table 3. The mean weight of rats in both calorie restriction groups decreased continuously during the research stages and a significant difference was observed between the control group and the two restriction groups regarding the final weight and heart muscle weight ($P=0.001$). One-way analysis of variance for the comparison of HSP70 and HSP60 gene expression level in the heart muscle of male rats in three groups of caloric restriction exercise restriction and control showed a significant difference between the means of the three groups ($P=0.001$). The results of the follow-up test showed a significant increase in the expression level of HSP70 and HSP60 genes in the

Table 1. High-intensity interval training protocol

Week	Warm-up time (minutes)	Number of repetitions	Work-rest ratio	Intensity/ exercise (vo2max)	Treadmill speed (m/min)	Intensity/rest (vo2max)	Treadmill speed (m/min)	Cooling time	Total duration
1	10	10	4:2	85-90	17	50-60	8	5	60
2	10	10	4:2	85-90	18	50-60	9	5	60
3	10	10	4:2	85-90	19	50-60	9	5	60
4	10	10	4:2	85-90	20	50-60	10	5	60
5	10	10	4:2	85-90	21	50-60	10	5	60
6	10	10	4:2	85-90	22	50-60	11	5	60
7	10	10	4:2	85-90	23	50-60	11	5	60
8	10	10	4:2	85-90	24	50-60	12	5	60

Table 2. Sequence of primers for HSP60 and HSP70 gene expression

	Sequence (5'->3')	Template strand	Length	Start	Stop	Tm	GC%	Self-complementarity	Self-3' complementarity
HSP60									
Forward primer	ATCCAAGACCAGGGTGGCTG	Plus	20	577	596	61.86	60.00	4.00	1.00
Reverse primer	CACAGTCCAAGGCAGTGGGA	Minus	20	726	707	62.06	60.00	4.00	2.00
HSP70									
Forward primer	GGAGCTTGGAACGGTACGCT	Plus	20	194	213	62.50	60.00	4.00	2.00
Reverse primer	AGTCCACTGACTTGCTCCCA	Minus	20	311	292	60.76	55.00	5.00	0.00

cardiac muscle of male rats in the restriction-exercise group compared to the calorie restriction and control groups ($P=0.001$). Besides, no significant difference was observed between the calorie restriction and control groups in terms of HSP70, HSP60, weight, and cardiac muscle weight ($P=0.102$) (Table 3 and Figure 2).

Discussion

The present study showed that two months of food restriction with and without exercise caused a significant decrease in the body weight of rats. In this regard, the body weight of the rats in the restriction and restriction-exercise groups was 35% and 33% lower compared to the control group, respectively, which was predictable due to the restriction of food intake in both groups, as observed in all previous studies. Carbone et al have reported that along with calorie restriction and negative energy balance, whole body proteolysis and oxidation of amino acids increase, which can reduce the synthesis of muscle fibers (26). In this regard, Weinheimer et al reported that the combination of exercise training and caloric restriction reduces skeletal muscle atrophy (27). However, without examining the body composition and changes in fat and lean tissue, it is difficult to discuss about the reduction of muscle mass in rats, which is one of the limitations of the present research.

Based on the findings of the present study, two months of caloric restriction and intermittent vigorous exercise caused a significant increase in the mean gene expression level of HSP60 and HSP70, which was not observed in the 50% calorie restriction group. In confirmation of the results of the present study, Delfan et al showed that 5 weeks of interval training with a relative intensity of 85%–90% of VO2max significantly increased the expression of HSP60 and decreased DNA fragmentation in the skeletal muscle of aged rats (28). These researchers stated that the significant increase in HSP60 expression level following interval exercise was associated with a decrease in the expression level of pro-apoptotic factors such as Bax protein and the ratio of Bax to Bcl-2 protein, as well as a significant increase in anti-apoptotic Bcl-2 protein. This decrease in the potential of mitochondrial apoptosis following intermittent exercise in aged rats was probably associated with a decrease in the release of apoptotic factors such as protein c and Apaf-1, which has caused a significant decrease in the expression of caspase-3 (28). Ho et al also indicated that the expression of HSP70 in the excessive endurance exercise training group was significantly lower compared to the control group (29). In the cardiac ischemia reperfusion injury that occurs in the post-stroke condition, the excessive production of free radicals, the increase of circulating HSP60, the increase of intracellular calcium, the leakage of H into the mitochondrial surfaces, and inflammation lead to the opening of the mitochondrial permeability transition pores. This can lead to the reduction of ATP, irreversible oxidation of protein, fat, and DNA in cardiomyocytes, and initiation of apoptosis (30). In this way, most researchers have considered the increase of HSP60 as a risk factor and aggravating damage, but in a new study, therapeutic benefits of protein-protein interactions between various types of HSPs, including HSP60, in the treatment of heart attack and damage caused by ischemia-reinjection have been supported. However, given that there is no certainty in this field and that the function and ultrastructure of the heart have not been investigated in the present study, it is not possible to clearly interpret the result of the increased expression of the HSP60 and HSP70 genes in the hearts of rats following intermittent training and

Table 3. Characteristics of male rats in research groups

Variables	Control	Food restriction	Food restriction-exercise	*P* value
The initial weight	313.25±20.3	317.78±22.3	321.65±23.6	$P_1=0.99$ $P_2=0.99$ $P_3=0.99$
The final weight	378.13±23.5	241.25±18.7	237.68±17.5	$P_1=0.001$ $P_2=0.001$ $P_3=0.99$
Cardiac muscle weight (g)	0.99±0.06	0.71±0.05	0.75±0.08	$P_1=0.001$ $P_2=0.001$ $P_3=0.99$

P1 = comparison between food restriction and control groups, P2 = comparison between food restriction-exercise and control groups, P3 = comparison between food restriction and food restriction-exercise groups.

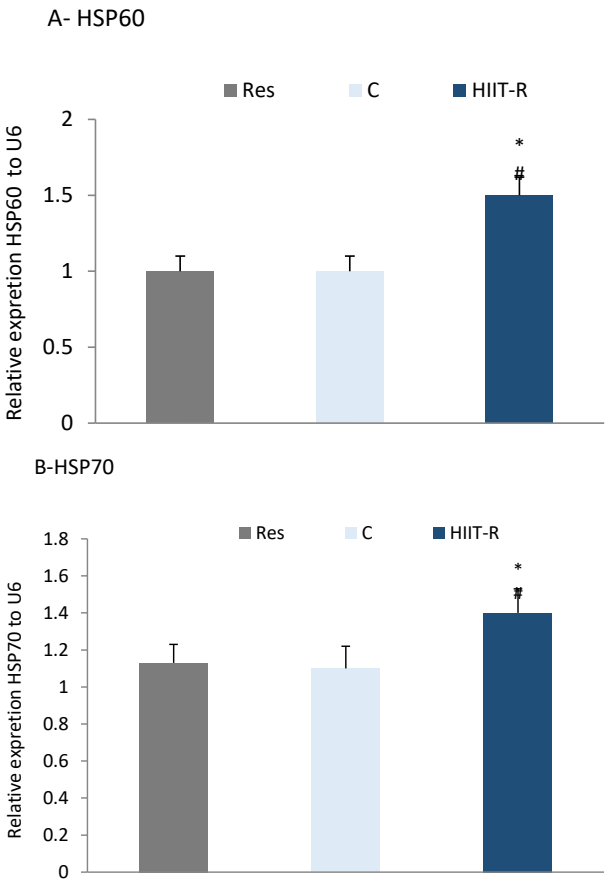

Figure 2. (A) Mean expression level of HSP60 in research groups. (B) Mean expression level of HSP70 in research groups. Res, restriction group; HIIT-R, restriction-high-intensity interval training group; C, control group. * Significant compared to Res, # significant compared to C

calorie restriction of 25%. It seems that the main reason for the contradiction between the present study and some other studies is the age of the rats, and in this regard, oxidative stress, inflammatory cytokines, and disruption of cell stress protection are possible mechanisms that contribute to the increase in apoptosis of old tissues (8). In other words, aging is associated with a significant increase in apoptosis, in which case the possibility of the effect of exercise training on apoptotic indicators becomes more and more obvious (18). Moreover, the difference in the type of tissue studied and the time of tissue harvesting can also affect the expression and occurrence of the involved variables. For example, in the present study, heart tissue was extracted 24 hours after the last training session, but in the study conducted by Ho et al, heart tissue was extracted immediately after the last training session (29). In addition, in the present study, it was not possible to evaluate inflammatory indicators such as tumor necrosis factor-alpha (TNF-α) and interleukin-6 (IL-6) due to some limitations. However, it is also possible that the increase of plasma TNF-α and IL-6 by directly activating caspase-3 is mediated through the external route (19). Because the increase in circulating TNF-α levels leads to an increase in the binding of the ligand to the TNF-α receptor in the sarcolemma and causes an increase in apoptosis through the external pathway (20). In addition, in stress conditions, factors such as glucocorticoids and cytokines cause changes in the permeability of the mitochondria by causing stress, and cytochrome c, which is located in the inner membrane of the mitochondria, is released into the cytosol and activates apoptotic protease factor 1 (Apaf-1). Therefore, this combination causes apoptosis through the activation of procaspase 9, caspase 9, and caspase 3 (18).

Some studies also indicate that food restriction and exercise can stimulate and increase the expression of proteins involved in mitochondrial biogenesis. In this regard, Zhao et al observed an increase in SOD2 and SIRT3 by applying a 30% calorie restriction (30). Although the exact mechanisms of apoptosis induced by exercise activity and caloric restriction are not precisely known, there are many possible hypotheses that require further investigation. One of the important hypotheses in this field is that exercise increases muscle metabolism and leads to ROS production by reducing fat mass in response to the limited food intake. A large amount of ROS can produce oxidative damage and thus lead to apoptosis through the intrinsic pathway (21).

According to the results of the above-mentioned study, it seems that a calorie restriction of 50% for eight weeks, despite the positive effects on the reduction of the fat mass and weight of rats, has not been able to have the same effect as a calorie restriction of 25%. However, interval training is effective on HSP values and probably causes muscle atrophy through the reduction of beta-catenin and the increase of glycogen synthase kinase 3 beta, which ultimately leads to the cessation of protein synthesis and muscle hypertrophy. Therefore, the combination of interval training and milder food restriction is likely to be effective in slowing down the process of skeletal muscle protein synthesis. Therefore, it seems that, regardless of the type and form of training, two months of interval training and caloric restriction of 25% have a good effect on apoptotic mechanisms in rat myocardium. However, it is difficult to express a definitive opinion about the effect of exercise training and caloric restriction on the indices related to apoptosis without examining the functional indices of the heart and inflammatory factors, evaluating the actual occurrence of cell death, and measuring it. Apoptotic proteins are subject to further studies and such recommendations are based on direct confirmation of results in human samples.

Conclusion

This study showed that the combination of high-intensity interval training and mild food restriction significantly increased HSP60 and HSP70 expression.

Acknowledgments

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Authors' Contribution

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Investigation: Mohammad Mazhari, Hasan Matinhomaee, Hoseyn Fatolahi.
Methodology: Mohammad Mazhari.
Project administration: Mohammad Mazhari, Hasan Matinhomaee.
Supervision: Hoseyn Fatolahi.
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Writing–review & editing: Hasan Matinhomaee, Hoseyn Fatolahi.

Competing Interests

The authors have no conflict of interests to declare.

Ethical Approval

In order to follow the principles of research ethics, the protocol of this study has been approved by the Medical Committee of Azad University, Tehran Branch (IR.IAU.CTB.REC.1401.050).

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References

1. Alansare A, Alford K, Lee S, Church T, Jung HC. The effects of high-intensity interval training vs. moderate-intensity continuous training on heart rate variability in physically inactive adults. Int J Environ Res Public Health. 2018;15(7):1508. doi: 10.3390/ijerph15071508.
2. Baghaiee B, Bayatmakoo R, Karimi P, Shannon Pescatello L. Moderate aerobic training inhibits middle-aged induced cardiac calcineurin-NFAT signaling by improving TGF-ß, NPR-A, SERCA2, and TRPC6 in Wistar rats. Cell J. 2021;23(7):756-62. doi: 10.22074/cellj.2021.7531.
3. Baghaiee B, Karimi P, Ebrahimi K, Dabagh Nikoo Kheslat S, Sadeghi Zali MH, Daneshian Moghaddam AM, et al. Effects of a 12-week aerobic exercise on markers of hypertension in men. J Cardiovasc Thorac Res. 2018;10(3):162-8. doi: 10.15171/jcvtr.2018.26.
4. Hannan AL, Hing W, Simas V, Climstein M, Coombes JS, Jayasinghe R, et al. High-intensity interval training

versus moderate-intensity continuous training within cardiac rehabilitation: a systematic review and meta-analysis. Open Access J Sports Med. 2018;9:1-17. doi: 10.2147/oajsm.s150596.
5. Jafari A, Pourrazi H, Nikookheslat S, Baradaran B. Effect of exercise training on Bcl-2 and Bax gene expression in the rat heart. Gene Cell Tissue. 2015;2(4):e32833. doi: 10.17795/gct-32833.
6. Huang CY, Lin YY, Hsu CC, Cheng SM, Shyu WC, Ting H, et al. Antiapoptotic effect of exercise training on ovariectomized rat hearts. J Appl Physiol. 2016;121(2):457-65. doi: 10.1152/japplphysiol.01042.2015.
7. Santana ET, Serra AJ, Silva Junior JA, Bocalini DS, Barauna VG, Krieger JE, et al. Aerobic exercise training induces an anti-apoptotic milieu in myocardial tissue. Motriz Rev Educ Fis. 2014;20(2):233-8. doi: 10.1590/s1980-65742014000200015.
8. Lee Y, Min K, Talbert EE, Kavazis AN, Smuder AJ, Willis WT, et al. Exercise protects cardiac mitochondria against ischemia-reperfusion injury. Med Sci Sports Exerc. 2012;44(3):397-405. doi: 10.1249/MSS.0b013e318231c037.
9. Mooren FC, Krüger K. Exercise, autophagy, and apoptosis. Prog Mol Biol Transl Sci. 2015;135:407-22. doi: 10.1016/bs.pmbts.2015.07.023.
10. Peterson JM, Bryner RW, Sindler A, Frisbee JC, Alway SE. Mitochondrial apoptotic signaling is elevated in cardiac but not skeletal muscle in the obese Zucker rat and is reduced with aerobic exercise. J Appl Physiol (1985). 2008;105(6):1934-43. doi: 10.1152/japplphysiol.00037.2008.
11. Zhong N, Chen H, Zhao Q, Wang H, Yu X, Eaves AM, et al. Effects of griseofulvin on apoptosis through caspase-3- and caspase-9-dependent pathways in K562 leukemia cells: an in vitro study. Curr Ther Res Clin Exp. 2010;71(6):384-97. doi: 10.1016/s0011-393x(10)80004-9.
12. Rodríguez-Berriguete G, Galvis L, Fraile B, de Bethencourt FR, Martínez-Onsurbe P, Olmedilla G, et al. Immunoreactivity to caspase-3, caspase-7, caspase-8, and caspase-9 forms is frequently lost in human prostate tumors. Hum Pathol. 2012;43(2):229-37. doi: 10.1016/j.humpath.2011.04.024.
13. Harada H, Hiraoka M, Kizaka-Kondoh S. Antitumor effect of TAT-oxygen-dependent degradation-caspase-3 fusion protein specifically stabilized and activated in hypoxic tumor cells. Cancer Res. 2002;62(7):2013-8.
14. Pouzesh Jadidi G, Seifi-Skishahr F, Bolboli L, Pourrahim Ghoroughchi A. Effect of high intensity interval training on cardiomyocytes HSP70 and caspase-3 gene expression levels in myocardial infarction male rat model. J Appl Health Stud Sport Physiol. 2022;9(2):1-11. doi: 10.22049/jahssp.2021.27521.1407. [Persian].
15. Siahkohian M, Asgharpour-Arshad M, Bolboli L, Jafari A, Sheikhzadeh Hesari F. Effect of 12- weeks aerobic training on some indices of skeletal muscle apoptosis in male rats. Med J Tabriz Univ Med Sci Health Serv. 2018;39(6):35-43. [Persian].
16. McMillan EM, Graham DA, Rush JW, Quadrilatero J. Decreased DNA fragmentation and apoptotic signaling in soleus muscle of hypertensive rats following 6 weeks of treadmill training. J Appl Physiol (1985). 2012;113(7):1048-57. doi: 10.1152/japplphysiol.00290.2012.
17. Zarali M, Etemad Z, Azizbeigi K, Karimi P. Effect of 8 weeks of high intensity interval training (HIIT) with and without calorie restriction on gene expression of caspase-3 and caspase-9 proteins in male rats. J Arak Univ Med Sci. 2020;23(3):300-13. doi: 10.32598/jams.23.3.5960.1. [Persian].
18. Koçtürk S, Kayatekin BM, Resmi H, Açikgöz O, Kaynak C, Ozer E. The apoptotic response to strenuous exercise of the gastrocnemius and soleus muscle fibers in rats. Eur J Appl Physiol. 2008;102(5):515-24. doi: 10.1007/s00421-007-0612-7.
19. Youle RJ, Strasser A. The BCL-2 protein family: opposing activities that mediate cell death. Nat Rev Mol Cell Biol. 2008;9(1):47-59. doi: 10.1038/nrm2308.
20. Galluzzi L, Vitale I, Aaronson SA, Abrams JM, Adam D, Agostinis P, et al. Molecular mechanisms of cell death: recommendations of the Nomenclature Committee on Cell Death 2018. Cell Death Differ. 2018;25(3):486-541. doi: 10.1038/s41418-017-0012-4.
21. Lee SD, Shyu WC, Cheng IS, Kuo CH, Chan YS, Lin YM, et al. Effects of exercise training on cardiac apoptosis in obese rats. Nutr Metab Cardiovasc Dis. 2013;23(6):566-73. doi: 10.1016/j.numecd.2011.11.002.
22. Jin H, Xie W, He M, Li H, Xiao W, Li Y. Pyroptosis and sarcopenia: frontier perspective of disease mechanism. Cells. 2022;11(7):1078. doi: 10.3390/cells11071078.
23. Long YF, Chow SK, Cui C, Wong RMY, Qin L, Law SW, et al. Regulation of mitochondrial dynamic equilibrium by physical exercise in sarcopenia: a systematic review. J Orthop Translat. 2022;35:37-52. doi: 10.1016/j.jot.2022.06.003.
24. Songstad NT, Kaspersen KH, Hafstad AD, Basnet P, Ytrehus K, Acharya G. Effects of high intensity interval training on pregnant rats, and the placenta, heart and liver of their fetuses. PLoS One. 2015;10(11):e0143095. doi: 10.1371/journal.pone.0143095.
25. Hector AJ, McGlory C, Damas F, Mazara N, Baker SK, Phillips SM. Pronounced energy restriction with elevated protein intake results in no change in proteolysis and reductions in skeletal muscle protein synthesis that are mitigated by resistance exercise. FASEB J. 2018;32(1):265-75. doi: 10.1096/fj.201700158RR.
26. Carbone JW, McClung JP, Pasiakos SM. Skeletal muscle responses to negative energy balance: effects of dietary protein. Adv Nutr. 2012;3(2):119-26. doi: 10.3945/an.111.001792.
27. Weinheimer EM, Sands LP, Campbell WW. A systematic review of the separate and combined effects of energy restriction and exercise on fat-free mass in middle-aged and older adults: implications for sarcopenic obesity. Nutr Rev. 2010;68(7):375-88. doi: 10.1111/j.1753-4887.2010.00298.x.
28. Delfan M, Amadeh Juybari R, Gorgani-Firuzjaee S, Høiriis Nielsen J, Delfan N, Laher I, et al. High-intensity interval training improves cardiac function by miR-206 dependent HSP60 induction in diabetic rats. Front Cardiovasc Med. 2022;9:927956. doi: 10.3389/fcvm.2022.927956.
29. Ho TJ, Huang CC, Huang CY, Lin WT. Fasudil, a Rho-kinase inhibitor, protects against excessive endurance exercise training-induced cardiac hypertrophy, apoptosis and fibrosis in rats. Eur J Appl Physiol. 2012;112(8):2943-55. doi: 10.1007/s00421-011-2270-z.
30. Zhao C, Liang W, Yang Z, Chen Z, Du Z, Gong S. SIRT3-mediated deacetylation protects inner hair cell synapses in a H2O2-induced oxidative stress model in vitro. Exp Cell Res. 2022;418(2):113280. doi: 10.1016/j.yexcr.2022.113280.