Research Article

Investigating the effect of exercise training in different periods of growth on protein synthesis (4E-BP1) and proliferation of cardiac cells (S6K1) in male rats

Bahman Mirzaei[1], Hossein Ghavami Amin[2], Mohammad Reza Fadaei Chafy[3*]

Abstract

We investigated the effect of exercise training in different stages of growth on protein synthesis (4E-BP1) and proliferation of heart cells (S6K1) in male rats. 30 male Wistar rats were prepared in three age groups of 2 weeks, 8 weeks, and 90 weeks (10 in each group), and each age group was divided into two control and training groups (5 in each group). In the exercise training group, the animals performed the resistance and aerobic training program every day (interval). The amount of overload for the resistance-training program was determined based on the body weight of the animals. For the aerobic training group, the training intensity increased from 50% of maximum speed in the first week to 80% in the last week. The results showed that there is no significant difference between the control and training groups in each age, as well as between the training groups in the three age ($p>0.05$). In contrast, the 2-week exercise groups ($p = 0.022$) showed a significant increase and the 90 weeks control group ($p = 0.002$) showed a significant decrease in S6K1 protein in cardiac tissue compared to the 2-week control groups. In the analysis of gene expression, it was also found that the 2-week training group showed a significant increase in S6K1 gene expression compared to the 2-week control group ($p=0.018$). It seems that doing combined exercise at different ages, especially early stage of life has a greater effect on the proliferation of heart cells (with increasing S6K1). However, studies with longer training durations should also be considered.

Key Words: **Exercise training, Growth periods, Cardiac protein synthesis, Cardiac cell proliferation**

1. Professor of Exercise Physiology, University Of Guilan, Rasht, Iran. 2. PhD Student of Exercise Physiology, University Of Guilan, Rasht, Iran. 3. Assistant Professor, Department of Physical Education and Sport Science, Faculty of Humanities, Rasht Branch, Islamic Azad University, Rasht, Iran.

*Author for correspondence: mfadaei2000@yahoo.com

B M: 0000-0001-3723-7434; H GA: 0009-0004-1785-7809; MR FC: 0000-0003-0265-5329

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Introduction

Remodeling is an active tissue process that occurs at different ages in order to adapt each tissue to a specific age (Tracy et al., 2020). Tissue regeneration may occur physiological or pathophysiological according to genetic and environmental conditions (Mihl et al., 2008). In old age, many environmental risk factors can lead tissue regeneration to pathophysiology. This is despite the fact that at a younger age, conditions such as exercise training can increase tissue regeneration towards the physiological side. Cardiac tissue is among the most important tissues that are affected by regeneration at different ages (Tracy et al., 2020). Physiological or pathological reconstruction of the heart, usually at the cellular level, can affect the proliferation of myocytes.

At early stages of life, cardiac myocytes exit the cell cycle in the perinatal period. Subsequent maturation or adaptive (hypertrophic) growth in the heart results from an increase in cell size. Cardiac hypertrophy is also affected by exercise (Chen et al., 2021). One of the key features of hypertrophy is increasing the rate of protein synthesis (Sugden & Fuller, 1991). Insulin-like growth factor 1 (IGF-1) strongly stimulate cardiac protein synthesis (Fuller et al., 1992). Multiple transcription factors regulate the protein synthesis pathway in many cells. The overall rate of protein synthesis is partly related to the efficiency of translation, in other words, protein synthesis is primarily regulated at the level of translation. In eukaryotes, a key step is binding of eIF4F to mRNA molecules with a 5′-terminal 7-methylGTP cap. Another mechanism that regulates protein synthesis from the PI3K/PKB pathway is 4E-BP1 (Dennis et al., 1999). Dephosphorylation of 4E-BP1 by eIF4E prevents it from binding to the cap and inhibits protein synthesis, therefore, the phosphorylation of this transcription factor through the PI3K⟶PKB⟶mTOR pathway allows the initiation complex to be formed and translation continues, therefore protein synthesis is also expands (Pham et al., 2000). Increasing or decreasing changes of this transcription factor can affect protein production in heart. Another transcription factor that affects the proliferation of heart cells is S6K1.

S6 kinases (S6K) are proteins that are controlled by mTORC1 through the ability of rapamycin. S6Ks contain a TOS motif near their N terminus that allows them to associate with their receptor and be recruited to mTORC1 to be phosphorylated by mTOR (Fingar et al., 2002). S6Ks are usually activated by hormones and growth factors, and this activation (in many types of cells) leads to center expansion and cell proliferation (especially in heart tissue) (Ming et al., 2012). While increasing age leads to a decrease in the activity of this factor and the spread of cardiovascular aging symptoms (Wu et al., 2015). The incremental changes of these transcription factors in heart tissue at different ages can have cardiovascular protection effects. Exercise training is one of the positive agent that can affect these transcription factors at the cellular level. In this regard, Shabani et al. (2021) investigated the effect of 8 weeks of endurance training on the content of S6K1 and 4E-BP1 proteins in the left ventricle of diabetic rats and stated that the implementation of this endurance protocol in rats for 2 months resulted in a significant increase of S6K1 and 4E-BP1 proteins in the heart. These researchers show that the increase of these factors associated with protein synthesis and cardiac hypertrophy (Shabani et al., 2021). While in pathological conditions, Jain et al. (2022) showed that variants in the S6K1 gene are associated with cardiomyopathy hypertrophy, and early detection of S6K1 type carriers can help identify family members at risk of this hypertrophy (Jain et al., 2022). In the skeletal muscle tissue, researches showed various findings related to this factor. Liao et al. (2017) stated that in S6K1 (but not 4E-BP1) with the reduction of type A lamin, it leads to the expansion of muscle tissue pathology (Liao et al., 2017). On the other hand, Koopman et al. (2006) stated that performing resistance training by increasing the phosphorylation of S6K1 leads to hypertrophy of type II fibers (Koopman et al., 2006). Since different exercises at different ages have different effects on these transcription factors and limited studies have investigated these factors with combined exercise at different ages, the purpose of this study is to investigate the effect of exercise training at different growth periods on protein synthesis (4E- BP1) and cardiac cell proliferation (S6K1) in male rats.

Materials and Methods

Animals

In this study, 30 male Wistar rats were purchased from the Pasteur Institute of Iran in three age groups. Rats were placed in the animal laboratory under controlled conditions with 12 hours of light and 12 hours of darkness (starting light at 6 in the morning and turning off at 6 in the evening), temperature (3 ±22 C), and humidity (about 45%). A number of 3-5 rats were kept in plexiglass cages with mesh lids and dimensions of 25 x 27 x 43 cm. Animals had free access to standard food and water. All sta--ges of keeping and slaughtering rats were carried out according to the rules of the Animal Ethics Committee of Islamic Azad University, Rasht Branch, Rasht, Gilan Province, Iran (ethical code: IR.IAU.RASHT.REC.1399.024)

After one week of familiarization with the laboratory environment, the animals were divided into three age groups (two control and training groups for each group). Groups were consist of: 2-week control group (entered the research at the age of two weeks and did not do physical activity during the period), 2-week training group (started their physical activity at the age of two weeks), the 8-week control group (entered the research at the age of eight weeks and did not do physical activity during the period), the 8-week training group (started their physical activity at the age of eight weeks)), the 90-week control group (entered the study at the age of ninety weeks and did not do physical activity during the period) and the 90-week exercise group (started their physical activity at the age of ninety weeks) (each group includes 5 was rat).

Combined exercise training

The exercise program was performed for 6 weeks in the form of a combination of resistance training on a ladder and aerobic training on an animal treadmill (Tejhiz Gostar Iranian, 2016 model). The resistance training program included 3 training sessions per week (Saturday, Monday, Wednesday) for 6 weeks. Each session consisted of 3 sets and each set consisted of climbing a special ladder 4 times, with a height of 1 meter and 26 steps with a distance of 4 cm between the steps. 30 seconds of rest was provided for the animals between each sets. After attaching a weight to the tail, the animals were forced to climb a vertical ladder. The principle of overloading was carried out by increasing the percentage of body weight on a weekly basis in such a way that in the first week the amount of weight attached to the animal's tail was 30% and gradually from the second week 70%, the third week 100%, the fourth week 120%, the fifth week 140% and 160% of their body weight in the sixth week (Kim et al., 2015). Aerobic exercises were also performed for 3 sessions a week (Sunday, Tuesday and Thursday) and on alternate days with resistance exercises for 6 weeks. The training intensity in the first week was equal to 50% of the maximum speed. Which gradually reached 80% in the sixth week. The duration of each training session was 30 minutes.

Laboratory measurements

48 hours after the last training session, all rats were anesthetized with an intraperitoneal injection of xylazine (10 mg/kg) and ketamine (75 mg/kg). Then a blood sample was taken from the heart of the animal. After making sure that the animal was sacrificed, tissue removal was done in compliance with ethical principles. Heart tissue was removed from the mediastinum area with the highest sensitivity.

For tissue sampling, first, the heart tissue was quickly removed and washed in 9% normal saline to remove the amount of blood inside the tissue, then it was divided into two upper and lower parts to measure measurement cellular factor with different methods. The apex of the heart was preserved in 9% formalin to measure protein expression by immunohistochemistry. Also, a part of the heart tissue was transferred to a temperature of -80°C in a microtube. In the following, the gene expression values of the study variables were checked by the RTPCr method in the laboratory.

Expression of 4E-BP1 and S6K1 genes in heart tissue by RTPCr method

For molecular investigations at the level of gene expression (4E-BP1 and S6K1), RNA extraction was first performed from the tissue in all the studied groups, according to the manufacturer's protocol (Qiagen, Germany). For this purpose, the amount of 200 Landa Kiazol was added to the samples and incubated at -80 for 24 hours. The plaque in the cryotube was crushed in the semi-frozen state, and in order to lyse the samples, the amount of 100 Landa chloroform was added to them for 1 minute. The resulting solution was centrifuged at 12000 rpm for 10 minutes. The clear liquid from the top of the tube containing the RNA was gently removed and placed in a DEPC microtube. 1 mL of isopropanol was poured onto the clear RNA and mixed by hand for 1 min. The samples were centrifuged at 12,000 rpm for 10 minutes. Then the supernatant was discarded and 1 ml of 70% alcohol was added to the sediment. After vortexing, the mixture was centrifuged for 10 min at 7500 rpm. The supernatant was drained and the plaque was dried inside the microtube. The amount of 20 L of distilled water at 60 degrees was poured on the plate and placed on the plate at 60 degrees for 5 minutes. After extracting RNA with high purity and concentration from all studied samples, cDNA synthesis steps were performed according to the manufacturer's protocol (Fermentas, USA) and then the synthesized cDNA was used to perform the reverse transcription reaction. Measurement of cardiac expression levels of 4E-BP1 and S6K1 was done by real time-PCR quantitative method. Primers were designed based on the information of 4E-BP1 and S6K1 genes in the NCBI gene bank by Macrogen. Glyceraldehyde-3-phosphate dehydrogenase gene (GAPDH) was used as a control gene, and the expression level of the desired gene was calculated with the formula $2^{-\Delta\Delta CT}$ in the following way.

In this way, first, the threshold cycle of the desired gene of each sample was subtracted from the threshold cycle of the house-turning gene of the same sample

(ΔCt= Ct Target-Ct Housekeeping)

In the next step, we subtract the ΔCt of each sample from the sample that needed to be compared, and multiply the negative number obtained to the power of two and obtain the relative expression of 4E-BP1 and S6K1 genes.

($\Delta\Delta$Ct=ΔCt Target-ΔCt Reference) $E= 2^{-\Delta\Delta Ct}$

The sequences of the primers used are reported in Table 1.

Immunohistochemistry

A part of the animal's heart tissue was immediately extracted and placed in 9% formalin. After fixing the samples, the tissues molded in paraffin were cut with a microtome device with a thickness of 5 micrometers. The obtained slices were incubated in TBS1X buffer (pH: 9.2) for 20 minutes at 70 degrees for antigen retrieval. Then, Triton 0.3% was used for 30 minutes in order to permeabilize the cell membrane. After washing with PBS, 10% goat serum was added for 30 minutes to block the secondary antibody reaction in the form of additional background color. Anti-S6K1 diluted primary antibody (1:100) with PBS was added to the sample overnight and incubated at 2-8 degrees. After washing with PBS, FITC-conjugated secondary antibody was added and incubated at 37 degrees for 2 hours in the dark. Finally, in order to stain the nucleus, DAPI was added to the samples. In the last step, the samples were observed by a fluorescent microscope (CX23, Olympus, Japan) with a 400 lens to confirm the markers (Montes et al., 2015).

Statistical analysis

The normality of data distribution was checked and confirmed using the Shapiro-Wilk test. In order to determine the significance of the difference between the variables of the research groups, one-way analysis of variance and post hoc Tukey's test were used. Mean and standard deviation were used for descriptive data reporting. After collecting the required information, it was analyzed using SPSS version 26 statistical software at a significance level of at least $p \geq 0.05$.

Results

Cardiac 4E-BP1 gene expression

Changes in the expression of the heart protein synthesis factor (4E-BP1) gene are shown in Figure 1. According to the results of the one-way ANOVA test, there is a significant difference between the different research groups in the expression of the h-

Table 1. Primer sequences for 4E-BP1 and S6K1 genes

Gene	Primer sequence 5'-3'
4E-BP1	F: 5' GAAGAGTGACAGTTTGAGATG 3' R: 5' CCTGAGTGAGGAGCAGGA 3'
S6K1	F: 5' GTGTTGTGGATTGGTGGAGT 3' R: 5' TTGTTGTGTGAGGTAGGGAGG 3'
GAPDH	F: 5' AAGTTCAACGGCACAGTCAAGG 3' R: 5' CATACTCAGCACCAGCATCACC 3'

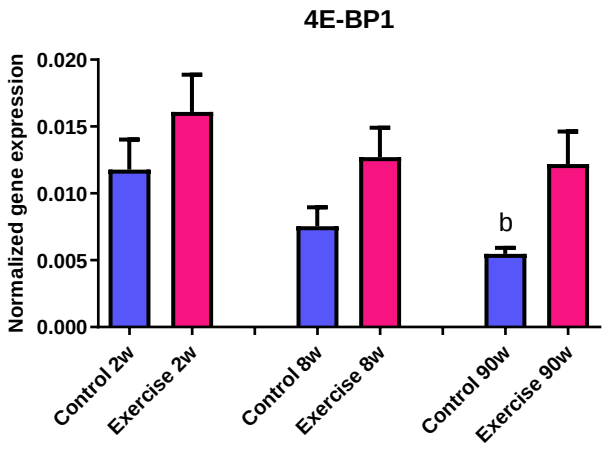

Figure 1. Changes related to the expression of the 4E-BP1 gene in the heart tissue for the research groups. Data are shown as mean ± standard deviation. Significance level: p<0.05 is considered, b: significant difference compared to the 2-week training group. , Exercise: resistance-aerobic exercise, w: week

-eart tissue protein synthesis factor (4E-BP1) (p=0.0372 and F=3.431). Nevertheless, the results of Tukey's post hoc test showed that there is no significant difference between the control and training groups in each age group, as well as between the training groups in the three categories (p>0.05). Meanwhile, only the 90-week control group showed a significant decrease in 4E-BP1 compared to the 2-week training group (p=0.032) (Figure 1).

Cardiac S6K1 protein and gene expression

Changes in protein and gene expression of cardiac cell proliferation factor (S6K1) are shown in Figure 2 (A, B, C). According to the results of the one-way ANOVA test between different research groups, there is a significant difference at protein (p<0.0001 and F=19.18) and gene (p=0.0010 and F=8.886) expression of cardiac cell proliferation factor (S6K1). The results of Tukey's post hoc test showed that the 2-week training groups (p=0.022) showed a significant increase and the 90-week control group (p=0.002) showed a significant decrease in S6K1 protein of the heart, compared to the 2-week control groups. Compared to the 2-week exercise group, the 8-week control group and the 90-week exercise control group showed a significant decrease in S6K1 protein expression in heart tissue (p<0.001). Compared to the 8-week control group, the 90-week control group showed a significant decrease in S6K1 protein expression in the heart tissue (p=0.048). Also, compared to the 8-week training group, the control and 90-week training groups showed a significant decrease in S6K1 protein expression in heart tissue (p=0.001 and p=0.028). In the examination of gene expression, the results of Tukey's post hoc test showed that the 2-week training group showed a significant increase in S6K1 gene expression compared to the 2-week control group (p=0.018).

The 8-week training group also showed a significant increase in S6K1 gene compared to the 2-week control group (p=0.013). In the study between training groups in three age groups, it was found that the 2-week training and 8-week training groups had a greater increase in S6K1 gene expression than the 90-week training group (p=0.017 and p=0.012).

Discussion

The weight of the heart in men and women increases very rapidly until the age of 20, and after this time, it grows slowly until the age of 90. Only after 90 years, there is a decrease in heart weight (Leutert, 1976). The thickness and length of heart muscle cells are similarly changing. Capillaries also multiply very quickly in the first and second decades and do not decrease in old age. Interstitial connective tissue increases only in the first decade of life. In old age, part of the collagen fibers are degraded. The fibrous framework of heart valves becomes a solid and stable organ in the first and second decade. After the 50th year, collagen and elastic fibers show signs of degeneration (Leutert, 1976). Therefore, the tissue of the heart is very changeable during age, and aging can affect the cellular and molecular structure of the heart. On the other hand, exercise is one of the most important factors affecting the proliferation and growth of cardiomyocytes, which also minimizes the destructive processes caused by age. Therefore, the aim of this study is to investigate the effect of exercise training in different periods of growth on protein synthesis (4E-BP1) and proliferation of cardiac cells (S6K1) in male rats.

Cardiac myocytes are affected by various conditions after the embryonic period and can continue their protein production. Maturational or adaptive growth (physiological hypertrophic induced by exercise or pathological hypertrophic induced by disease) in cardiac tissue affects myocytes and cell size (Sugden & Fuller, 1991). One of the key features of hypertrophy is increasing the speed of protein synthesis, which is regulated by some factors such as IGF-I of the mTOR pathway or in the pathways of transcription factors such as 4E-BP1. In the current study, among the factors involved in tissue synthesis, only 4E-BP1 was evaluated and the results showed that only the 90-weeks control group showed a significant decrease in 4E-BP1 compared to the 2-week training group. Although resistance-aerobic exercise did not have a significant effect in different age groups, but higher expression was evident in trained groups, and with increasing age, the expression values of this factor were much lower in heart tissue. As mentioned, myocytes have a much higher reproduction rate during the embryonic period, however, after birth, with aging. Since the heart has limited growth, insignificant changes in this factor seem reasonable, but since exercise training affect the heart tissue on structural and functional, significantly increased cardiac 4E-BP1 values were e-

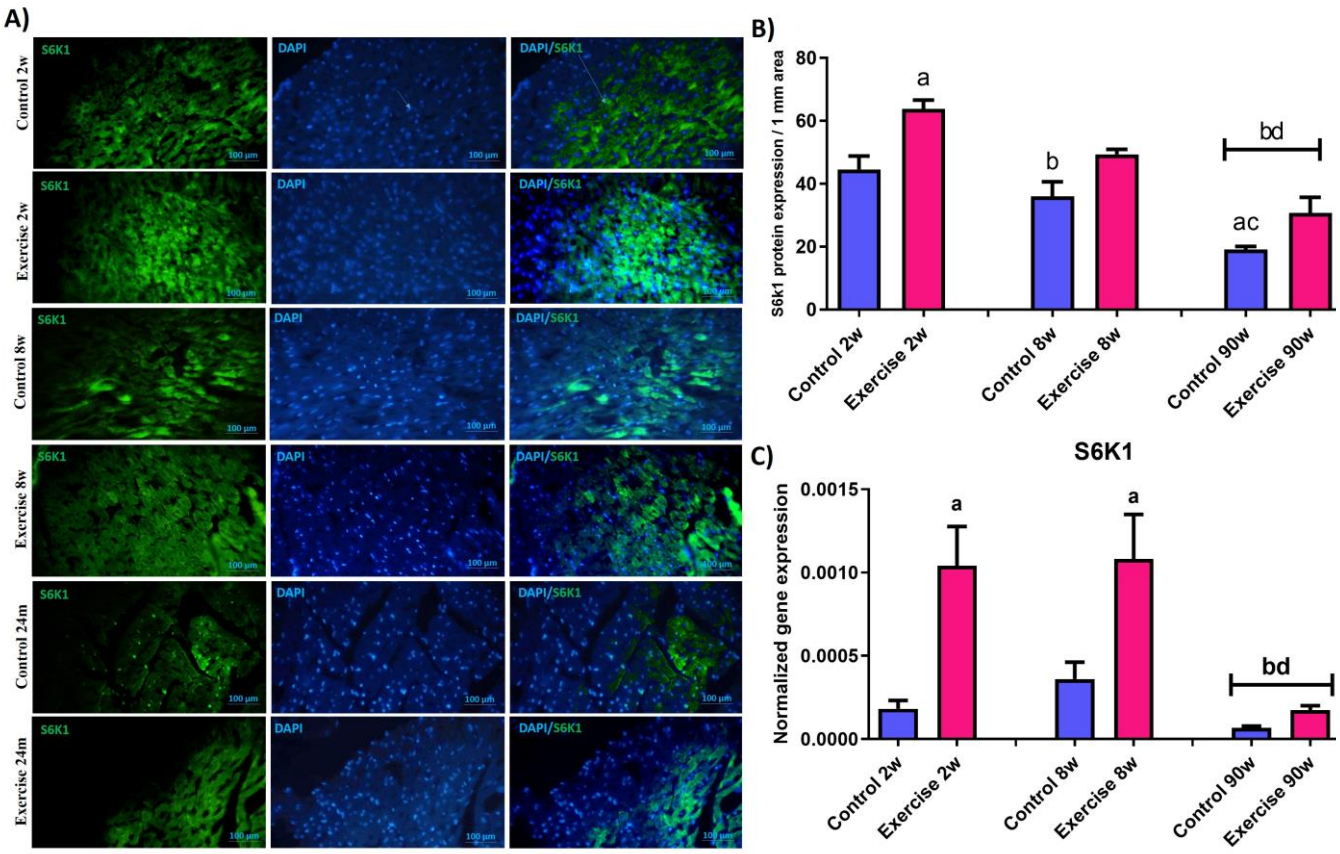

Figure 2. Changes related to protein expression (A and B) and gene (C) S6K1 heart tissue for research groups. Magnification 100 um. DAPI staining was used to analyze the results. In this staining, blue cell nuclei were observed. The number of green cells relative to the total number of blue nuclei was reported as a percentage. The results of S6K1 protein expression in the heart tissue were evaluated with green color, which was due to the reaction of the antigen with the corresponding antibody. In the tissues with more activity, including in the tissue that exercised, this amount of protein increased with exercise. The results showed that the presence of this protein in the cytoplasm of cardiomyocytes cells, and in the tissue layers of the heart muscle in the groups with more activity of the heart tissue showed an increase. In figure B, protein expression values are shown by Image J software, and the expression values of cardiac S6K1 gene are also shown in picture C. Data are shown as mean ± standard deviation. Significance level: p<0.05 is considered, a: significant difference compared to the 2-week control group, b: significant difference compared to the 2-week exercise group. c: significant difference compared to the 8-week control group, d: significant difference compared to the 8-week training group. Exercise: resistance-aerobic exercise, w: week

-xpected with exercise training. In this regard, the principle of adaptation and cardiac hypertrophy with longer duration, especially endurance training, should be mentioned. In this study, unfortunately, the values of left ventricular hypertrophy were not evaluated, but considering that long-term endurance training has a greater effect on cardiac hypertrophy (Małek et al., 2019), it does not seem reasonable to expect cardiac hypertrophy with 6 weeks of combined training.

In relation to 4E-BP1, it has been stated that the phosphorylation of this factor may not be the main mechanism for translation regulation and probably increases protein synthesis through other mechanisms (PI3K) (Pham et al., 2000). In the present study, it would be better to measure other factors involved in protein synthesis in myocytes. Another mechanism caused by exercise is the increase of free radicals (Bejma & Ramires, 2000). Therefore, in the present study, it would be better to measure other factors involved in protein synthesis in myocytes. Another mechanism caused by exercise is the increase of free radicals (Bejma & Ramires, 2000), which can be involved in cellular adaptations, but it has been shown in heart tissue that H2O2 increases the dephosphorylation of 4E-BP1. In hepatocytes exposed to hypoxia and in various cells (including cardiac myocytes) with heat shock protein, 4E-BP1 is also affected. Therefore, it seems that exercise prevents the increase and proper functioning of 4E-BP1 in cardiac myocytes by increasing these factors, including free radicals. Therefore, these factors can justify the non-significant increase of 4E-BP1 in myocytes with exercise training in the three age groups of the present study. Contrary to the results of the present study, Medeiros et al. showed that 12 weeks of swimming training caused a significant increase in cardiac muscle 4E-BP1 in diet-obese rats (Medeiros et al., 2011). Meanwhile, in the present study, the combined exercise on the treadmill was evaluated for 6 weeks in non-obese

rats in 3 age groups, so the difference in the results of the present study with Medeiros' study seems reasonable.

In the present study, S6K1 factor was used to investigate the proliferation of cardiomyocytes. To investigate the effect of growth in the comparison between the 3 control groups, it was found that only the S6K1 protein in the old control group (90 weeks) had a significant decrease compared to the 2 and 8 week control groups. In the comparison between the effect of exercise in each age group, it was found that the expression of cardiac S6K1 gene and protein was significantly increased in the 2-week exercise group compared to the 2-week control group. In the study between training groups in three age groups, it was found that the 2-week training and 8-week training groups had a greater increase in S6K1 expression and protein than the 90-weeks training group. Based on these results, it is clear that increasing age, especially old age, causes a significant decrease in S6K1. Aging is a major risk factor for cardiovascular diseases and is associated with increased oxidative stress and accumulation of senescent endothelial cells in the heart and blood vessels (Rajapakse et al., 2011). It seems that the increased changes in oxidative stress caused by aging can also be effective in reducing the proliferation factors of cardiomyocytes. S6K1 is critical for cell growth and its activation is reported during cardiac hypertrophy (Balasubramanian & Kuppuswamy, 2003). At the same time, increasing age, especially old age, due to the less mobility of the person and the weakness of the heart muscle, the tissue of the heart muscle deteriorates (Kitzman & Edwards, 1990). Therefore, the reduction of cardiomyocyte proliferation factor (S6K1) in the 90-weeks-old rats of the present study also seems reasonable.

The S6K1 factor is one of the main factors in cardiomyocyte growth signaling, and its activation has been shown to increase the biogenesis of translation components. S6K1 has been reported to increase translation of mRNAs containing unique 5-terminal oligopyrimidine sequences in the 5-untranslated region. 5-terminal oligopyrimidine mRNAs generally encode ribosomal proteins, thereby increasing the overall growth and migration capacity of cells (Volarević & Thomas, 2000). Therefore, the increase of cardiac S6K1 in the child and adolescent groups of the present study with resistance/aerobic exercise can indicate the positive effect of exercise on the strengthening and protection of cardiac tissue. Because genetic deletion of S6K1 has been shown to lead to developmental phenotypes in mice (Shima et al., 1998) and Drosophila (Montagne et al., 1999). Such findings emphasize the important role of S6K1 in determining cell growth and size. Considering the prominent role of S6K1 in cell growth, it can be predicted that this kinase also plays an important role during cardiac hypertrophy. In the present study, as previously stated, although the training modality was not effective for cardiac hypertrophy, it seems that cardiac hypertrophy was also observed if the training duration was increased. Because with the positive regulation of S6K1, various signaling pathways start cell growth. There are two isoforms of S6K1, both expressed by the same gene due to alternative start sites: a 70-kDa isoform that is predominantly cytoplasmic and an 85-kDa isoform that has an additional 23 N-terminal residues that direct it to the nucleus (Balasubramanian & Kuppuswamy, 2003). It seems that the exercise training of the present study was also able to activate other pathways of cardiomyocyte proliferation by increasing S6K1 in the cell nucleus, especially in 2-week-old rats, which needs to be investigated more closely.

Consistent with the results of the present study (groups of 2 and 8 weeks of training), Shabani et al. (2021) showed that 8 weeks of endurance training increases the content of S6K1 and 4E-BP1 proteins in the left ventricle of the heart of diabetic rats treated with streptozotocin. In the present study, although the values of the 2-week training group increased compared to the control group, no significant changes were seen in S6K1 and 4E-BP1 in other age groups. Which seems to have more control and regulatory effects on heart tissue in disease models. Liao and colleagues also showed that moderate-intensity endurance training leads to an increase in S6K, but in the high-intensity group, there was no change in S6K activation in the heart of Sprague-Dauli rats (Liao et al., 2015). According to this research, intensity and duration are important factors in cardiac protein changes that should be taken into account. Along with the young and old groups of the present study, DeSouza and colleagues investigated the effect of endurance and strength training on S6K1 protein content in the skeletal muscle of animal samples, which did not report significant changes (De Souza et al., 2013). Although cardiac tissue was investigated in the present study, it seems that the length of the training period is an important influencing factor on S6K1. Apart from the effect of the type and duration of exercise on this expressed factor, the activation of S6K1 is initiated by a PKC/c-Raf/MEK/ERK pathway, in other words, the stable activation of this kinase can also be protected by the integrins pathway (Iijima et al., 2002). Integrins, which are ubiquitously expressed as heterodimers on the cell surface, mediate outside-in and inside-out signaling. Integrins, apart from being critical for cell adhesion, regulate a wide array of signaling events including mechanosensing, transcriptional activation, cell growth, and tissue regeneration (Kuppuswamy et al., 1997). Integrins contribute, at least in part, to the sustained activation of S6K1. However, the activity of integrins was not investigated in the present study.

Conclusions

According to the results of the present study, it seems that since cardiac cells have a higher proliferative capacity at younger ages, combined exercise have better effects on the cardiac cell proliferation, especially S6K1. However, it is possible that exercise training through other routes is effective in controlling h-

-eart disease at older ages. In this field, the need for more research is suggested, especially with higher periods of exercise training.

What is already known on this subject?

At early stages of life, cardiac myocytes exit the cell cycle in the perinatal period. Subsequent maturation or adaptive (hypertrophic) growth in the heart results from an increase in cell size. Cardiac hypertrophy is also affected by exercise training.

What this study adds?

combined exercise at different ages, especially early stage of life has a greater effect on the proliferation of heart cells (S6K1).

Organ Cross-Talk Tips:

- Combined endurance-resistance exercises can have an impact on the communication between different growth periods and cardiac protein synthesis factors.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval Animals had free access to standard food and water. All stages of keeping and slaughtering rats were carried out according to the rules of the Animal Ethics Committee of Islamic Azad University, Rasht Branch (ethical code: IR.IAU.RASHT.REC.1399.024).

Informed consent Animal study.

Author contributions

Conceptualization: B.M., H.G.A.; Methodology: MR.FC.; Software: B>M.; Validation: H.G.A.; Formal analysis: MR.FC.; Investigation: .B.M.; Resources: H.G.A.; Data curation: H.G.A., B.M.; Writing - original draft: MR.FC.; Writing – review & editing: B.M.; Visualization: B.M., H.G.A.; Supervision: MR.FC.; Project administration: B.M., H.G.A.; Funding acquisition: MR.FC.

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