Research Article

The effect of high-intensity interval swimming training on ULK and TSC1/2 proteins of hippocampus tissue in elderly rats

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Abstract

Understanding the cellular and molecular mechanisms that regulate aging reduces the possibility of dependence on age-related diseases. Exercise treatment strategies reduce the burden of aging-related illness, disability, and premature death in the elderly. The aim of this study was to evaluate the effect of high-intensity interval swimming training on ULK and TSC1/2 proteins of hippocampus tissue in elderly rats. In this experimental study, 16 elderly male Sprague Dawley rats (20 months old and mean weight 300-450 g) were divided into two groups of control and high-intensity interval swimming training. High-intensity interval swimming training consisted of 14 bouts 20-second swimming sessions with 10 seconds of rest between each session for six weeks (three days a week). The content of ULK and TSC1/2 proteins in hippocampal tissue was measured by Western blotting. Data were analyzed using independent t-test at the $P<0.05$. The results showed that high-intensity interval swimming training caused significant increase in content of ULK protein of hippocampus tissue in elderly rats ($P=0.010$). Also, high-intensity interval swimming training caused significant decrease in content of TSC1/2 protein of hippocampus tissue in elderly rats ($P=0.010$). According to the results, it seems that high-intensity interval swimming training can help improve the homeostasis regulatory pathways in hippocampal neurons in the elderly animal model.

Key Words: **Aging, Interval training, ULK, TSC1/2, Rats**

Introduction

Aging is an essential biological process that is associated with an overall decline in tissue function. In fact, as life expectancy increases, age-related disorders, such as cognitive impairment or dementia, will become a growing public health issue. Aging is also a major risk factor for age-related diseases for many people. Today, people want not only to live longer but also to be healthier. Hence, there is a fundamental need to understand the cellular and molecular mechanisms that regulate aging that allow us to reverse the aging process for healthy aging as well as reduce disease dependence on age (Fedarko, 2011). It has been shown that TSC1 / 2 complex can help increase life expectancy and improve health in animal specimens (Zhang et al., 2017). TSC1 / 2 as a molecular keyboard for multiple signals to activate or inhibit mTORC1 activity including growth hormone (insulin pathway IGF-Akt), stress (ERK / RSK), energy (AMPK pathway), hypoxia (Redd1) and cytokine (IKKβ / NFκB) (Kapahi et al., 2004; Zhang et al., 2017). Unregulated mTOR signaling is involved in many diseases, including cancer, metabolic disorders, neurological diseases, and inflammation (Huang & Manning, 2009). A previous study showed that high expression of TSC1 or TSC2 increases the lifespan of Dorsafila (Kapahi et al., 2004). In a mouse model with high expression of TSC1 or TSC2 specific for skeletal muscle, depending on the amount of gene expression, reported muscle atrophy (Wan et al., 2006). The TSC1 / 2 complex specifically inhibits mTORC1 by stimulating Rheb and converting it from the active form of Rheb-GTP to the inactive form of Rheb-GDP23 (Inoki et al., 2003). Another alternative to direct inhibition of mTOR is to target mTORC1 via the Unc-51 autophagy-activating kinase (ULK1), which inhibits mTORC1 by phosphorylation of its Raptor subunit (Dunlop et al., 2011). And regulated axon growth (Wang et al., 2018). Ulk1 acts as a regulatory protein that participates in phylogenetically protected pathways including neurite formation, synaptic transmission, and synaptic and endocytic transmission during central nervous system development (Mochizuki et al., 2011). Large families of motor proteins and transport components are structurally known as U-

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-lk1 binding partners, and they are jointly involved in the receptor internalization of the nutrient receptor, signal transduction, and intracellular vesicle renewal (DiNardo & O'Farrell, 1987). Thus, an evolutionary role for Ulk1 may indicate an association between endocytosis and neuronal survival, and Ulk1 dysfunction may be involved in the pathogenesis of various neurological disorders (Joo et al., 2016). Neural evidence in human and animal models suggests that physical activity and aerobic exercise may have positive effects on mobility and structural and functional components of the CNS. In this regard, exercise interventions are recommended as an essential activity to protect healthy elderly people from severe functional and neurological decline (Stillman et al., 2016). Exercise treatment strategies reduce the burden of aging-related illness, disability, and premature death from disease in the elderly (Merchant et al., 2021). However, the mechanism by which exercise has beneficial effects on healthy aging is still unclear. The role of TSC1 / 2 and ULK1 in health and aging is very important. Complex analysis of neuronal function of TSC1 / 2 and ULK1 genes in vitro and in animal models has changed our understanding of disease mechanisms and potential treatment options. Due to the important role of TSC1 / 2 and ULK1 in mTOR signaling, evaluating the effect of environmental stimuli on them in animal models can be very useful to better understand the role of signaling these pathways in various diseases and aging. Both proteins play important roles in several processes that are critical to the normal growth of the brain. In addition, these proteins are widely expressed throughout the adult brain, possibly having important hemostatic regulatory functions in neurons throughout adult life (Rosset et al., 2017). Exercise has a beneficial effect on the health of the elderly and is actually considered as a non-pharmacological treatment for various metabolic and neurological diseases. High-intensity intermittent exercise (HIIT) is a type of aerobic exercise that is becoming increasingly common and includes high-intensity exercise that is performed with rest periods (Gibala et al., 2014). The health benefits, risks, and optimal design of HIIT are still unclear. In addition, most research on HIIT has been performed in young and middle-aged adults and, therefore, its tolerance and effects are less well known in the elderly. Changes in TSC1 / 2 and ULK1 in brain tissue of elderly samples in response to exercise have not been well studied. Therefore, the present study intends to investigate the effect of high-intensity intermittent swimming training on the content of ULK and TSC1 / 2 proteins in hippocampal tissue in elderly rats.

Materials and Methods

Animals

The present study is an experimental study in which it was possible to control the factors affecting the research results. The present study was approved by the Ethics Committee of the institute of Physical Education and Sports Sciences with the code IR.SSRC.REC.1400.098. In this study, 16 20-month-old male Sprague Dawley rats weighing 450-300 g were purchased from the Laboratory Animal Breeding Center of Shiraz University of Medical Sciences and kept in the Sports Physiology Laboratory for one week for adaptation. The elderly rats were then randomly divided into control groups (8 rat) and intermittent high-intensity swimming exercises (8 rat). Rats in the pet house of Shiraz University of Medical Sciences in a polycarbonate cage (3 rats per cage) in controlled environmental conditions with an average temperature of 23 ± 1 ° C, humidity 55-50% and a light / dark cycle of 12.12 hours Were maintained. Free and standard rats for laboratory animals used laboratory-specific animal feed prepared from Shiraz University of Medical Sciences. In addition, the water needed by the animals was provided to them freely in a 500 ml bottle for laboratory animals. In this study, ethical principles on how to work with laboratory animals, including the availability of water and food and proper storage conditions were considered.

All experiments were performed according to the policies of the Helsinki Convention. In the present study, all rats underwent the animal pool familiarization phase (160 cm in diameter and 80 cm in height) for one week before starting the main exercise. On the first day of the rats' acquaintance with the pool, the rats were placed in an animal pool with extreme depth and calmness with a water depth of 50 cm and an average temperature of 30 ± 0.5 °C and swam at the desired speed for five minutes. In subsequent sessions, when the rats became well acquainted with the animal pool, to get acquainted with the type of periodic exercise, they were taken out of the water by the rest plate several times after a minute of swimming and put back in the water.

High intensity interval training

48 hours after the last dating session, the rats were randomly divided into two groups: control and exercise. The rats in the exercise group performed the HIIT swimming exercise, which consisted of 14-20 second swimming sessions with a 10-second rest between each exercise. The exercise program was performed for six weeks (three days per week).

In the exercise group the increasing load was: at first week , the weight was 9% of the body weight of each rat and 1% was added to it every week. So that in the last (sixth) week, the rats with a weight of 14% of their body weight exercised (Amirazodi et al., 2020) (Table 1).

Hippocampal histology and measurement of research variables

48 hours after the last training session, the rats were

Table 1. High intensity interval training protocol.

Weeks	Number (Turn)	Period (Seconds)	Rest period (Seconds)	Load amount % of body) (weight
1	14	20	10	9
2	14	20	10	10
3	14	20	10	11
4	14	20	10	12
5	14	20	10	13
6	14	20	10	14

anesthetized by observing ethical principles and intraperitoneal injection of a combination of ketamine (30-50 kg / mg) and xylazine (3-5 kg / mg). The skull was immediately dissected using a razor blade and the brain was carefully removed. The healthy brain was cut in half exactly by the surgical blade and separated from the limbic system by the clear atlas of the hippocampus according to the coordinates of the hippocampus. And was fixed inside the liquid nitrogen tank for 15-30 seconds. The hippocampi were kept in a freezer at -80 until the start of the Western blot test. First, the net weight of the tissue was calculated using a digital scale (AG245) and 3 to 5 times the weight was added to the repa buffer. After homogenizing the tissues, microtubes containing the homogenized tissue were centrifuged at 14000 rpm at 4 ° C for 20 minutes. Bradford solution, which is used to measure protein, was used to measure the protein concentration of the samples. The standard was BSA, which was used at concentrations of 0.4, 4 and 40 mg / ml. 40 µg of each sample was used for electrophoresis of proteins on SDS-PAGE gel. Electrophoresis was performed in a running buffer with a constant voltage of 100. Protein markers were used to determine the location of proteins based on molecular weight on SDS-PAGE and PVDF paper. TBS-T-Tween-20 solution was used to wash the excess material of the blocking solution on paper. After washing, PVDF paper was immersed in a solution containing the desired primary antibody. After this step, a suitable secondary IgG-HRP antibody was used. Then, using the ECL kit, the required amount of peroxidase substrate was added on PVDF paper until it was all impregnated with the substrate. To ensure the same concentration of loaded samples, the amount of β-actin protein was evaluated. Finally, digital images of the films were obtained using a scanner. The band images recorded on the film were examined for density using Imag J software (Braidy et al., 2015).

Statistical analysis

Shapiro-Wilk test was used to ensure the normal distribution of variables. After the normality of data distribution was determined, independent t-test was used to examine the differences between the groups. Significance level was considered $p \leq 0.05$ in all cases. All statistical operations were performed with SPSS software version 23.

Results

The weights of rats in the pre-test and post-test groups are presented in Table 2. The results of independent t-test showed that the mean ULK protein content in the HIIT training group was 1.47 and the mean ULK protein in the control group was 1.00. Based on the obtained t value (2.48), high-intensity intermittent swimming exercise significantly increased the ULK protein content of hippocampal tissue in elderly rats (P = 0.010) (Figure 1).

Also, the results of independent t-test showed that the mean TSC1 / 2 protein content in the HIIT training group was 0.66 and the mean of TSC1 / 2 protein in the control group was 1.00. Based on the obtained t value (2.48), high-intensity interval swimming exercise significantly reduced the TSC1 / 2 protein content of hippocampal tissue in elderly rats (P = 0.005) (Figure 2).

Discussion

The findings of this study show that six weeks of high-intensity intermittent swimming training resulted in a significant increase in ULK protein content and a significant decrease in TSC1 / 2 protein content in hippocampal tissue in elderly rats. The TSC1 / 2 complex plays an important role in the evolution of signaling pathways that regulate cell growth. This pathway enhances anabolic processes and inhibits catabolic processes in response to extracellular and intracellular factors. In the CNS, the TSC1 / 2 complex not only regulates cell growth and proliferation, but also regulates a complex and precise system that, under different conditions, Controls the stage of subcellular development and localization depending on the cell type. In general, TSC1 / 2 signaling in the CNS, through its multifaceted roles, contributes to proper neural connection (Han & Sahin, 2011). The findings of our study are consistent with the results of some previous research (Jacobs et al., 2013). In this regard, Jacobs et al. Report that mTOR and TSC2 are linked in the anterior skeletal muscle

Table 2. Rat levels of rats in pre-test and post-test groups.

Weight		Variablegroup
Post-test	pre-exam	
419.7 ± 3.53	410.2 ± 3.56	**Control**
401.0 ± 2.82	412.0 ± 3.36	**Practice**

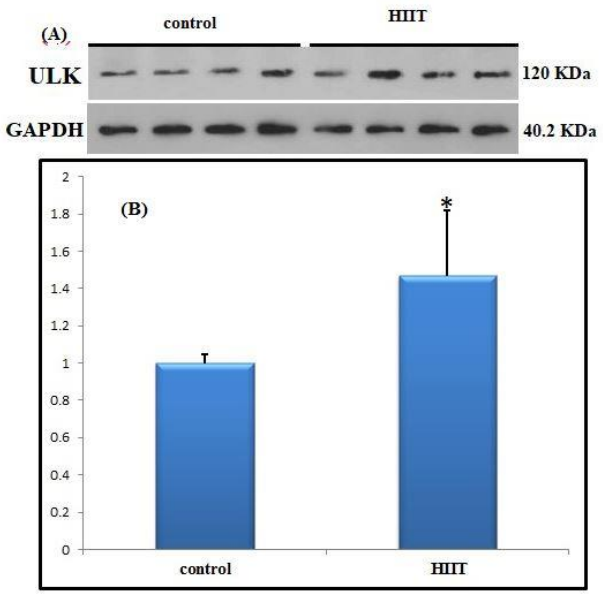

Figure 1. Comparison of ULK protein content in the studied groups; (A). Western blot images containing ULK protein and beta-actin protein as internal control (loading control) in brain hippocampal tissue; (B). Bar chart (mean and standard deviation) showing reduced values of ULK protein bands versus beta-actin (internal control).

Significant difference compared to the control group (p<0.05)

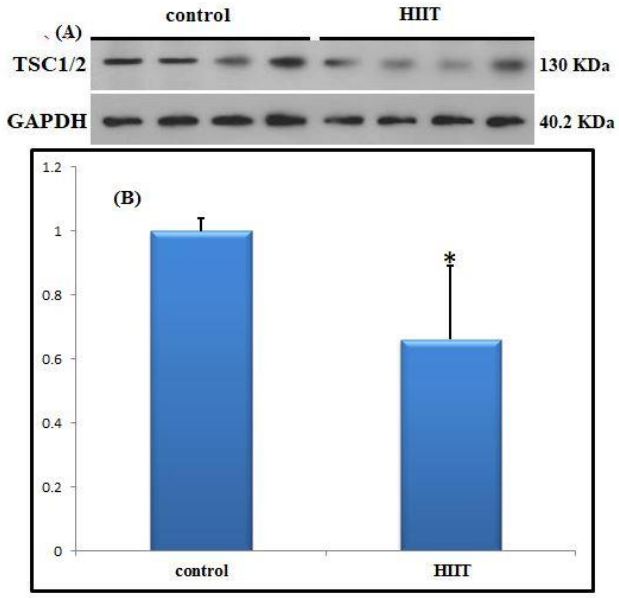

Figure 2. Comparison of TSC1 / 2 protein content in the studied groups; (A). Western blobs containing TSC1 / 2 protein and beta-actin protein as internal control (loading control) in hippocampal tissue (B). Column diagram (mean and standard deviation) showing the reduced values of TSC1 / 2 protein bands versus beta-actin (internal control).

lysosomes of tibialis-mice. In addition, extroverted contractions increase localization of mTOR-lysosomal and subsequent degradation of TSC2 in the lysosome (Jacobs et al., 2013). Studies show that HIIT may help improve brain structure and function in the elderly through more efficient neural activation and faster stimulus processing or higher levels of brain oxygenation (Erickson et al., 2014). Our findings confirm the results of previous studies that HIIT is an optimal strategy to increase brain tissue health in older rats and can help regulate the amount of proteins involved in nerve cell homeostasis in the brain. However, the results of some studies are contrary to our results (Delshad et al., 2021), Delshad et al. (2021) showed Medium intensity continuous training had no significant effect on ULK1 protein levels in skeletal muscle of elderly male rats (Delshad et al., 2021). The ULK1 response may be tissue-specific and justify the discrepancy with the above finding. There is growing evidence for a positive association between physical activity and brain function throughout life (Kao et al., 2020). Although the mechanism of change of TSC1 / 2 and ULK1 changes in brain tissue in response to exercise is unclear, however, it has been shown that the TSC1 / 2 complex can be adjusted after translation by several major signaling pathways in cells: PI3K-Akt, ERK and AMPK. The best feature of the complex TSC1 / 2 performance as a downstream target Phosphatidylinositol 3-kinase (PI3K) is activated by the binding of growth factors (eg IGF or BDNF). Activated PI3K leads to the induction of PDK1 and serine / threonine protein kinase Akt and subsequent phosphorylation / activation of Akt by PDK1. Akt activates TSC negatively by direct phosphorylation of TSC2 at five sites on human TSC (Inoki et al., 2002). The second kinase that can phosphorylate and inhibit TSC2 is extracellular signaling kinase (ERK) (Ma et al., 2007). ERK TSC2 phosphorylation seems to be very important for the regulation of ESCA-mediated TSC2. Both Akt and ERK levels are active in cortical glands associated with TSCs and SEGAs, and inhibition of TSC2 by these kinases has been suggested as a post-translational mechanism that may further enhance the loss of the first TSC gene allele (Han & Sahin, 2011). In addition, AMP-activated protein kinase (AMPK) can phosphorylate TSC2 on a set of different residues of Akt and ERK and potentially increase the ability of TSC1 / 2 to inhibit mTORC1 activity, resulting from cell Protects against excessive energy consumption in low energy conditions (Hahn-Windgassen et al., 2005). TSC1 also regulated by IKK-beta, which physically reacts with TSC1 in its Ser487 and Ser511 residues and phosphorylates in response to activation of the inflammatory pathway. Because TSC1: TSC2 acts as a dimer, the regulation of each protein is likely to affect its overall activity level (Lee et al., 2007). Changes in PI3K-Akt, ERK and AMPK in elderly specimens following exercise have been reported in previous studies (Li et al., 2019), so in our study it is likely that intermittent high-intensity swimming training has led to further effects by changing the above factors. However, further

further studies are needed to examine these indicators simultaneously in elderly samples. Inhibition of rapamycin-induced mTOR also increases ULK1 kinase activity, while mTOR activation suppresses ULK1 through high Rheb expression (Kanfer et al., 2017). In addition, it has been suggested that mTOR indirectly inhibits autophagy through autophagic phosphorylation / regulator Beclin-1 (AMBRA1), which prevents ULK1 ubiquitination and binds to ULK1, stabilizing and enhancing activity. Its kinase is in response to stimuli (Nazio et al., 2013). Periodic training of high-intensity swimming was one of the strengths of the present study; this is because this type of exercise can have different answers and adaptations than other exercise programs. There were some limitations in the present study, such as the lack of measurement of mTOR in hippocampal tissue in old age in response to exercise. Measurement of the IGF-Akt insulin pathway and the AMPK pathway can also help to understand the mechanism of changes in neuronal markers at brain tissue in response to high-intensity interval exercise. Therefore, it is suggested that a similar study examine the changes in these factors in hippocampal tissue in old age.

Conclusion

In summary, the results of our study show that six weeks of high-intensity intermittent swimming training resulted in a significant increase in ULK protein content and a significant decrease in hippocampal tissue TSC1 / 2 protein content in elderly rats. These results suggest that intermittent high-intensity intermittent training may help improve homeostasis regulatory pathways in hippocampal neurons in the elderly animal model.

What is already known on this subject?

Most research on HIIT has been performed in young and middle-aged adults and, therefore, its tolerance and effects are less well known in the elderly.

What this study adds?

Six weeks of high-intensity intermittent swimming training resulted in a significant increase in ULK protein content and a significant decrease in hippocampal tissue TSC1/2 protein content in elderly rats.

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Compliance with ethical standards

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

Ethical approval The present study was approved by the Ethics Committee of the institute of Physical Education and Sports Sciences with the code IR.SSRC.REC.1400.098.

Informed consent Animal study.

Author contributions

Conceptualization: S.Sh., M.S.Q.; Methodology: S.Sh., M.S.Q.; Software: S.Sh., M.S.Q.; Validation: S.Sh., M.S.Q.; Formal analysis: S.Sh.; Investigation: S.Sh., M.S.Q.; Resources: M.S.Q.; Data curation: S.Sh.; Writing - original draft: S.Sh., M.S.Q.; Writing - review & editing: M.S.Q.; Visualization: S.Sh.; Supervision: S.Sh.; Project administration: M.S.Q.; Funding acquisition: S.Sh.

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