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Eight weeks of high-intensity interval training and aerobic continues training increase serum telomerase, sirt6, and irisin level in healthy elderly men

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Abstract

Background: Telomerase activity plays a key role in preserving telomere length which is important in cellular aging. There is evidence showing the link between sirt6, irisin, and telomerase activity.

Aim: The aim of this study was to compare the effect of 8 weeks of high-intensity interval training (HIIT) and aerobic continuous training (ACT) on telomerase activity, sirt6, and irisin levels of healthy older adults.

Materials and Methods: Thirty healthy males (age: 60-70 years, weight: 68-72 kg) participated voluntarily to follow the 8-week program including 3 sessions per week. Rest ratio in HIIT training was 1:2 and the intensity corresponded to 90% of HRR. ACT intensity was progressive starting with 50% of HRR and finishing with 70% of HRR. Blood samples were taken in a 12-hour fasting situation, before and after the program.

Results: Results showed a significant effect of training on serum telomerase and sirt6 in training groups, not the control group. Also, in comparison to ACT and the control group, serum irisin was significantly higher in the HIIT group.

Conclusion: We concluded HIIT training is safe and efficient for older adults in terms of telomerase activity, sirt6, and irisin level and can be followed as a time-efficient training protocol.

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1. Introduction

Regular physical activity and increased physical fitness are known to reduce the risk of morbidity and mortality from different kinds of diseases [1]. Telomere length is a primary biomarker of cellular aging associated with several diseases such as insulin resistance and cardiovascular disease (CVD) [2]. Telomeres are the ending linear structure of chromosomes and act as a mitotic clock that becomes shorter with every cycle of cell division [3]. Thus, it is safe to say that telomeres are important aging biomarkers [4]. Recently, both telomere length and telomerase activity have been shown to be influenced by diverse environmental factors including exercise, oxidative stress, and psychological stress [5]. Telomerase is the key enzyme for preserving of telomere length by adding guanine-rich sequences [6].

SIRT6 was identified as a chromatin-associated protein, which influences the efficiency of DNA repair probably through its interaction with DNA polymerase beta [7]. After the discovery of sirt6, it has been reported to play a role in several important genomic contexts [8]. SIRT6 reduction in human cells causes structure abnormality of telomere which in turn leads to genomic instability, chromosomal fusions, and premature cellular senescence [9]. Regardless of telomeres, SIRT6 has an important role in regulating DNA repair [10].

Beneficial effects of regular physical activity on cellular regeneration and senescence have been reported [1, 2, 11]. For instance, long-term endurance training increases telomerase activity and decreases the attrition rate of telomeres in endurance athletes compared with inactive individuals [12]. Moreover, it has been shown that exercise training attenuates the age-related increase in sirt6 [7]. However, the diversity of exercise training has not been fully tested in this context and needs more studies.

After the discovery of irisin in 2012, this myokine was described as an important link between physical activity and better health [13]. Irisin content positively correlates with telomere size and negatively with human age [14]. Rana et al. (2014) reported that in healthy adults, telomere length can be predicted by plasma Irisin levels [15]. It is also reported that irisin treatment led to the elongation of telomeres [14].

Radak et al. (2001) reported that swimming training did not alter telomerase activity among young cancerous mice [16]. On the other side, Liang et al. (2022) showed that treadmill exercise improved the mitochondrial DNA damage and myocardial cell telomerase activity in aging model rats and reduced the aging process [17]. Recently, by testing three types of training, Werner et al. (2018) have shown that endurance training and interval training, but not resistance training, increased telomerase activity and telomere length [18]. Controversy still remains in the area of exercise training, aging, telomere length, and telomerase activity and also possible effective mechanism.

Myriads of mechanisms have been proposed regarding exercise training and human aging. In this study, we hypothesized that telomerase, and SIRT6 concentration in serum are affected by exercise training. Then, we tried to find out which exercise protocol is more beneficial between high intensity exercise training and conventional aerobic training. As previous studies have shown that both telomere length and SIRT6 are affected by Irisin [14, 15, 19], we tried to show the training-

related changes in serum Irisin of healthy elderly men.

2. Material and Methods

2.1. Subjects

Thirty male aged 60-70 were participated voluntary. They had not followed a regular training program before study being conducted. Based on the physical activity readiness questionnaire, we made sure that all the participants were healthy. All participants provided written informed consent before participation. All the processes and interventions were approved by University of Tehran, Faculty of Sport Sciences and Health Committee.

2.2. Procedures

All participants completed clinical background questionnaire and they were explained the study procedures. 48 hours after familiarization to exercise protocols and facilities, blood samples were collected in 12 hours-fasting situation. They were split into three groups randomly (aerobic continuous, high intensity interval, and control). Then, they followed 8 weeks of high intensity interval training and aerobic continuous training [20]. Also, they were asked to not participate in any other exercise during the study. 48 hours after the last session of training, blood sample collection was repeated.

2.3. High intensity interval training (HIIT)

The progressive HIIT protocol was performed for 8 weeks, 3 sessions a week. The intensity of each session was according to 90% of heart rate reserve (maximum heart rate– resting heart rate). Duration of each interval was 30 second and resting time between each effort was 1 min. Each set included three efforts which was followed by 4 min rest [21]. Table 1 shows the HIIT protocol. Training intensity was monitored by Beurer heart rate monitor with chest strap.

Table 1. HIIT training protocol

Stage of training	Repetition per set	Set per session
Familiarization	3	1
1st and 2nd week	3-4	3
3rd and 4th week	4-5	4
5th and 5th week	5-6	5
7th and 8th week	6	5

2.4. Aerobic continuous training (ACT)

ACT protocol was performed for 8 weeks, 3 sessions per week. Participants started with 50% of their reserved heart rate (HRR) for 20 min and finished with 65% of HRR for 35 min. Training intensity was monitored by Beurer heart rate monitor with chest strap. Table 2 shows the ACT protocol.

Table 2. Aerobic continues training protocol (ACT)

Stage of training	Intensity (% HRR)	Duration (min)
Familiarization	45	15
1st and 2nd week	50-55	20-25
3rd and 4th week	55-60	25-30
5th and 5th week	60-65	30-35
7th and 8th week	65-70	35-40

2.4. Enzyme linked immunosorbent assay

Blood samples taken in pre-test and post-test were centrifuged at 3000 RPM for 5 min. Then, serums were collected and stored in -70 until further analyzing. Commercial ELISA kit were used for SIRT6 (Cusabio: CSB-E17018h), irisin (Cusabio: CSB- EQ027943HU), and telomerase (Cusabio: CBS-E08021h). ELISA protocol was performed based on manufacture instruction.

2.5. Statistical analysis

All statistical analyses were performed using SPSS 26. Variables were compered among HIIT, ACT, and control group using

analyzing of covariance (ANCOVA) followed by Bonferroni as the post-hoc test. Values are also presented as mean±standard deviation. The significance level was considered at $P<0.05$.

3. Results

Demographic profile of participants is provided in Table 3. Descriptive statistics of telomerase, irisin, and SIRT6 level are presented in Table 4.

Table 3. Demographic profile of participants based on mean ± standard deviation

Group	Age(year)	Height (cm)	Pre-test weight (kg)	Post-test weight (kg)
ACT	65.5±3.5	171±5.4	70.85±7	69±1
HIIT	65.9±3.2	173±4.7	70±6.23	68.5±3
Control	65.2±3.4	170±7.2	72±7	72±2.7

Table 4. Descriptive statistics of study variables (mean ± SD)

Group	HIIT	ACT	Control
Telomerase-pre (ng/dl)	0.17±0.02	0.16±0.01	0.20±0.01
Telomerase-post (ng/dl)	0.29±0.03*	0.29±0.03*	0.18±0.01
Percent of variation (%)	70.5 %	81.25%	- 0.1 %
Irisin-pre (ng/dl)	57.2±6	48.03±5	54.7±7
Irisin-post (ng/dl)	62.35±5*	53.57±6*	48.5±7*
Percent of variation (%)	9 %	11.53 %	- 11.33 %
SIRT6-pre (ng/dl)	3.06±0.4	3.97±0.4	3.91±0.2
SIRT6-post (ng/dl)	4.6±0.6*	4.8±0.4*	2.93±0.4*
Percent of variation (%)	50.32 %	20.90 %	- 25.06 %

3.1. Telomerase level

In this study, we aimed to investigate the effect of 8 weeks of HIIT and ACT on telomerase, irisin, and SIRT6 level in serum of older adults. ANCOVA test showed a significant effect of training on serum telomerase ($F(2,26)=30.612$, $P=0.001$). Although exercise training increased level of telomerase in serum, pairwise comparisons did not demonstrate a significant difference between HIIT and ACT ($P=1.000$). Post-hoc tests showed that there were significant differences between HIIT and control group ($P=0.001$) and ACT and control group ($P=0.001$).

3.2. SIRT6

A significant difference was observed in serum SIRT6 following ANCOVA ($F(2,26)=32.673$, $P=0.001$) in which paired comparison showed a non-significant difference in HIIT group compared to ACT ($P=1.000$) and significant difference between HIIT vs control ($P=0.001$) and ACT vs control ($P=0.000$).

3.3. Irisin level

A significant difference was observed in serum Irisin following ANCOVA ($F(2,26)=102.245$, $P=0.001$) in which paired comparison showed a significant difference in HIIT group compared to ACT ($P=0.001$) and control ($P=0.001$).

4. Discussion

In this present study, we have reported beneficial effect of 8 weeks of exercise training on serum irisin, SIRT6, and telomerase level among healthy elderly men. We tested two different types of exercise training, HIIT and ACT. However, the difference between them was significant in irisin.

Telomeres are the ending parts of chromosomes that affect aging, and they are considered as an important indicator of

biological aging [22]. Regular training/ sedentary behavior are important elements of human life style that have undeniable effect on overall health. Previous studies have shown that exercise might have considerable effects on suppressing telomere attrition [23, 24]. Although, we were not able to measure telomere length (TL), we measured telomerase which restores short bit of DNA known as telomere [25]. Similar to Werner et al. study (2017) [18], we showed that training increased serum telomerase in in healthy elderly men, however, there was no significant difference regarding to types of exercises (HIIT vs ACT). The main difference between our study and Werner's is the study duration. The intervention in the present study was 8 weeks, while participant in Warner's study had been training for 26 weeks (6 months). Furthermore, Ludlow et al. (2008) reported no significant relationship between physical activity and telomerase activity. However, at the meantime preserving of telomere length was reported regarding to regular physical activity [2].

We also measured serum irisin in the present study in which HIIT was significantly more beneficial than ACT. Rana et al. (2014) have suggested plasma irisin level as a predictor of telomere length in healthy adults [15]. Many studies have suggested irisin as an important myokine secreted during exercise [26, 27, 28]. Lack of balance in energy metabolism is considered as a contributing factor that accelerates aging process [15]. Irisin is thought to change white adipose tissue to brown adipose tissue [13]. It has been reported to increase mitochondrial density that makes irisin as a therapeutic approach against metabolic disease and age-related decline in biological functions [26].

These results determine that irisin is a hormone with anti-ageing properties, although the precise mechanism by which irisin promotes telomere lengthening is unknown. The authors suggest that this effect can be mediated by irisin activating the p38MAPK pathway, which regulates the expression of telomerase reverse transcriptase. However, the ability of irisin to enhance telomerase expression and its precise mechanism warrants further investigation [29].

Regardless of the types of training, we observed a significant increase in serum SIRT6 after 8 weeks of training. It has been reported that SIRT6 is necessary for optimal replication of telomeres. Lack of SIRT6 in human cells causes abnormal telomeres structure [9, 19]. Exercise training is a factor that greatly affects metabolism. Generally, it is believed that aerobic exercise postpones emerging of aging phenotypes by turning NADH to NAD through mitochondria. It has also been suggested that training may tackle age-related malfunctions by stimulating NAD synthesis. SIRT6 deficiency causes the most deleterious consequences among all the sirtuin knockouts [30]. Although relationship between exercise training and SIRT6 among older adults has not been widely studied, there is evidence that exercise training can improve regulation of SIRT6 [7, 31, 32]. Also, the current evidence on the relationship between exercise and telomere length reports that regular exercise improves antioxidant activity and helps redox balance. In addition, exercise has been reported to improve inflammatory balance through a reduction in C-reactive protein, interleukin−6, and TNFα levels [33].

Available evidences show that exercise may lead to attenuate telomere attrition and

aging through regulating of metabolism, increasing antioxidant capacity, decreasing inflammatory status, and maintaining good body composition [34, 35]. Not measuring anti-inflammatory and antioxidant factors is one of the limitations of the current research.

5. Conclusion

In summary, this study shows that 8 weeks of HIIT and ACT mediate positive effects on regulators of cellular senescence. The telomerase activity and SIRT6 level are increased by both high intensity interval training and continues training suggesting general role of exercise training on regulating cellular aging key factors. In terms of irisin, HIIT is more effective than ACT representing that HIIT has more metabolic effects in regulating cellular senescence. With regard to training guidelines for controlling and postponing of cellular aging, our results support the HIIT as a safe and time-saving type of exercise for older adults.

Conflict of interest

The authors declared no conflicts of interest.

Authors' contributions

All authors contributed to the original idea, study design.

Ethical considerations

The author has completely considered ethical issues, including informed consent, plagiarism, data fabrication, misconduct, and/or falsification, double publication and/or redundancy, submission, etc.

Data availability

The dataset generated and analyzed during the current study is available from the corresponding author on reasonable request.

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