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The Effect of High-Intensity Interval Training on telomere length of Leukocytes in sedentary Young Women

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ABSTRACT

Telomere is at the end of chromosome and is shortened through every cell division until the cell grows old. Nucleoprotein structures protect chromosome ends against damage. This purpose of study was to examine the effect of an 8-week high-intensity interval training (HIIT) on telomere length of leukocytes in sedentary young women. For this reason, 21 students voluntarily participated in this study and were randomly divided into two groups, Experimental group (n=11, age=23.25±2.01 years, height=163.32±5.44 cm, & weight=62.2±7.56 kg) and control group (n=10, age=24.42±1.32 years, height=165.00±4.88 cm, & weight=66.6±6.80 kg). The Experimental group performed the HIIT, 3 sessions a week for 8 weeks. Every session included 3-6 times of running with maximum speed in a 20-m area with 30 s rest from each other. The fasting blood samples were collected immediately before and after the exercise protocol. The telomere length was measured using Real Time PCR method. The data were analyzed using independent and paired t- tests. The results showed that leukocyte telomere length (T/S ratio) in the Experimental group increased significantly (P=0.001). Moreover, the body fat percentage, BMI, and weight in the Experimental group decreased significantly. The results of this study showed that a HIIT- as an efficient and appropriate method of exercising- increased the leukocyte telomere length in sedentary young women.

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INTRODUCTION

Aging is defined as the accumulation of cellular damages resulting in incompatibilities of the organism [11]. Aging a physiological process in which the telomere length is shortened, and the ability of cell growth and division is reduced as well [11]. Telomeres are repetitive DNA-protein complexes that protect the ends of linear chromosomes and maintain genomic stability. The predominant mechanism of increased telomere sequence length in most eukaryotes, and especially in human, is telomerase. Telomerase is a ribonucleoprotein that consists of two central components: a protein reverse transcriptase component (TERT) and an RNA template (TERC). From a functional standpoint, telomerase is thought to be preferentially recruited to short telomeres [10,15]. At each cellular division, telomeric DNA terminal regions are not fully replicated, which, if not counteracted by elongation by telomerase, can lead to telomere shortening [1]. Epidemiologic studies have shown links between shortened leukocyte telomere length (LTL) , telomerase activity and increased risk of age-related outcomes, such as cancer incidence and mortality, type 2 diabetes, and cardiovascular disease [4,1]. Many of these same diseases and risk factors have also been associated with an inactive lifestyle. Physical activity and exercise training have been associated not only with prevention and improvement of disease symptoms but also with telomere length, indicating a possible role for PA influencing telomere biology as a potential mechanism for prevention or delay of age-related disease [1,2]. Traditionally High volume of aerobic exercise reduces the risk of cardiovascular and metabolic diseases though it is time consuming [7]. Furthermore, the nature of some exercises including endurance exercises, which require continuous exercising and regular

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presence, poses some limitations. In this respect, the study of an alternative exercising program with similar metabolic adaptations and without time commitment seems necessary. Furthermore, one of the exercising protocols that have recently been attended to by researchers of exercise physiology is high-intensity interval training (HIIT). The HIIT involves intervals of maximum-intensity exercise and resting intervals of low-intensity exercise [13]. The intervals of low-intensity exercise between intervals of repeated exercise in interval training cause to perform more exercises and receive higher effectiveness. Therefore, regarding the diversity, very low time consumption, metabolic effects similar to endurance activities, and efficacy of these exercises, this study was conducted to examine the effect of HIIT on telomere length in sedentary young women.

Methods:

The study was performed as quasi-experimental and subjects were inactive normal young women studying in Shiraz University and aged 20-26 years. Of the population, 21 students voluntarily participated in this study and were randomly divided in two groups, the exercise group (n=11) and the control group (n=10). The information on the level of physical activity and health of participants was acquired using a questionnaire. Participants had no any of cardiovascular diseases, diabetes, hereditary blood disorders, and respiratory problems, nor used medications, nor had regular exercises at least 6 months before the study. Anthropometric measures, including height, weight, body fat, body mass index (BMI) were collected using standard procedures. VO2max was measured before and after eight weeks exercise by treadmill. The participants in the Experimental group executed the exercise protocol in a 20-meter distance marked with three cones three times a week for eight weeks as described below (Figure 1). Upon the start of exercise protocol, the participants began to run at maximum speed from the starting point (Cone 1) toward Cone 2 (Pathway A), then returned and ran 20 meters at maximum speed and in the opposite direction toward Cone 3 (Pathway B), and eventually returned and ran at maximum speed toward the starting point (Cone 1) (Pathway C) in order to complete the distance of 40 meters. The participants continued the above process at maximum speed until the 30-second period of the exercise protocol ended, and then, they repeated the process after 30 seconds recovery. The three repetitions of 30-second exercise in the first and second weeks increased to four repetitions in the third and fourth weeks, five repetitions in the fifth and sixth weeks, and six repetitions in the seventh and eighth weeks. In each session, the participants warmed themselves up for five minutes (stretching and flexibility exercises along with slow running) before beginning the exercise protocol and cooled themselves down for 5 minutes at the end of the session. The exercise protocol consisted of a 40-meter round at maximum speed that was a valid test of anaerobic performance. During the eight weeks of exercise protocol, the participants in the control group did not have any regular exercising program. The fasting blood samples of 10 cc were drawn from brachial vein (antecubital vein) 24 hours before the first session of exercise and 24 hours after the last session (8:30 am) in the laboratory. The blood samples were immediately poured into the tubes containing anticoagulant (EDTA). The tubes were centrifuged with 3000 rpm for 10 minutes at 4°C.

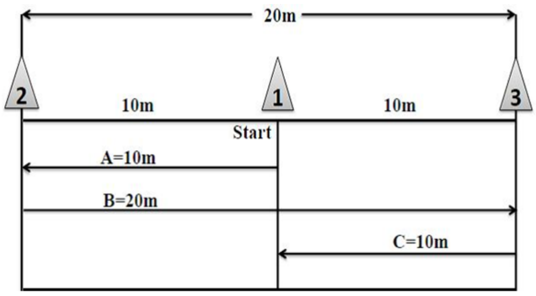

Fig. 1: The exercise protocol of HIIT.

DNA was extracted from peripheral blood samples using a kit (Cinnagen Co., Iran) to measure telomere length. It was then dissolved in 500µl of water, and kept frozen at -70°C. Optical Density (OD) was used to assess quality and concentration of DNA. Real Time PCR reaction was performed using SYBER® Green PCR Master Mix kit (Applied Bio-system Co., the U.S.A), using tel specific and B436 (acidic ribosomal protein-coder) primers as Single Copy Gene (SCG), with the sequence presented in table 1.

Table 1: primers of telomer gene and 36 B4 gen.

Telomer gene primer	forward	CGGTTTGTTTGGGTTTGGGTTTGGGTTTGGGTTTGGGTT
	reverse	GGCTTGCCTTACCCTTACCCTTACCCTTACCCTTACCCT
36B4 gene primer	forward	CAGCAAGTGGGAAGGTGTAATCC
	reverse	CCCATTCTATCATCAACGGGTACAA

Because a green fluorescent protein (GFP) is present in real time PCR reaction, and produces a fluorescent light if the product is proliferated. Thus, the intensity of the light is directly related to amount of product obtained. T/S (relative telomere length) is the ratio of Cycle Threshold (CT) of telomere gene over B436 gene. The collected statistical data were analyzed using SPSS18 software. The Kolmogorov-Smirnov test was used to determine the normal distribution of data. Considering that the result of Kolmogorov-Smirnov test showed the normal distribution of data, the parametric statistical tests were used. In this respect, the dependent *t* test and independent *t*- test were respectively used to examine the intragroup changes and intergroup changes. All the statistical tests were performed at significance level of α=0.05.

3. Results:

Subject characteristics are shown in Table 2.

Table 2: Characteristics of study participants (Mean ± standard deviation).

Variables	Control group		Case group	
	Pretest	Posttest	Pretest	Posttest
Age (year)	24.42±1.32	-	23.25±2.01	-
Height (cm)	165.22±4.88	-	163.32±5.44	-
Weight (kg)	66.67±6.30	67.10±6.94	62.20±7.56	60.41±6.80*
Body mass index (kg/m^2)	23.72±4.36	23.95±4.14	24.42±4.38	23.18±4.83*
Body fat (%)	20.52±0.95	20.42±0.78	19.96±2.21	17.87±1.13*

* Significant changes

Table 3: Mean telomere length (T/S ratio) in the two blood samplings in both groups.

Variables	Groups	Pretest	Posttest	Significance level
telomere length (T/S ratio)	Control	1.647±0.227	1.661±0.302	0. 8
	Case	1.657±0.312	1.854±0.148	0.04*

* Significant changes

Discussion:

The present study results showed a significant difference in telomere length (T/S ratio) between the two groups after 8 weeks of HIIT intervention. Body fat percentage, weight, and BMI also significantly reduced in the exercise group.

In line with the present study, Zhu et al. [16] reported that women with high-intensity training (MET<6) had longer telomere lengths than women with low- and moderate-intensity training, which may be due to estrogen effect in up regulation of telomerase [16] In the present study, increased telomerase activity due to intense exercise could explain increased telomere length. However, many studies support the inverted U theory in relation to telomere length and intensity of exercise [2]. Andrew et al. [1] showed a relationship between moderate exercise and increased telomere length, and argued that high intensity exercise was associated with shortening of telomere length due to increased oxidative damage and inflammatory factors. These results indicate a relationship between shortened telomere and pro-inflammatory cellular environments, and support moderate exercise with desirable inflammatory effects, and cite possible protective effects of physical activity (PA) in shortening telomere through lower expression of inflammatory genes. These results disagree with the findings in Ponsout et al. study on telomere muscle length in elderly men and women, which showed equal telomere length in people who trained and those who did not. These results showed that moderate intensity exercise cannot act as a proliferative stress in muscle tissue. A study on a large group of over 72 year-old Chinese elderly showed that telomere length was shorter in people with low PA compared to other group [Woo et al., 2008]. However, in the present study, high intensity training was used, which was perhaps a reason for increased telomere length.

Researchers and trainers alike seek the best exercise intensity and duration, with the least inflammatory stimulation. According to studies by Hemmatinafar et al. and Bochan et al. [3] showing reduced fibrinogen and no change in serum CRP following 6 and 7 weeks of HIIT in sedentary youths [36, 37], it seems regular HIIT is associated with small changes in inflammatory proteins, and it could probably be used as a beneficial training in sedentary people.

In a study by Mathew et al. no change was observed in telomere length in PBMC following 7 days of ultra-running, which was attributed to no increase in telomerase, and perhaps emergence of new lymphocytes or elimination of old ones. They also argued that this intensity of exercise may not sufficiently affect telomere length in people who trained. Thus, whether exercise protects or damages telomere length depends on intensity and duration of exercise, and the tissue type because athletes with "fatigued athlete myopathic syndrome"

(FAMS) had shorter telomeres in their skeletal muscles compared to other athletes, which results from their huge proliferative stress on satellite cells and increased ROS

Mason et al. [8] studied telomere length in obese postmenopausal women over 12 months of moderate to vigorous aerobic activity, and found no significant change in telomere length due to exercise, which disagrees with the present study results. This was attributed to type, intensity and duration of exercise that may have been insufficient to alter dynamic changes in telomere. Higher intensity Interval training with greater effect on dynamics of telomere in the present study may be the reason for disagreement with above study. Mason et al. cite genetic factors as a possible reason, since people with initially longer telomeres respond less to exercise.

In line with the present study, cherkas et al. [4] showed that twins with high-intensity training had longer telomeres compared to twins low intensity training. They also argued that adolescents, especially girls, should do more vigorous physical activity (VPA) for more anti-aging effects of exercises., and that telomere length is directly related to vigorous exercise (which agrees with the present study), due to reduced mental stress, oxidative stress and inflammation. It has been shown that people with extreme stress and minimal activity have low telomerase activity due to secretion of stress hormones, leading to shortened telomeres.

Study limitations:

Information relating to nutrition and mental stress, which are known to affect telomere dynamics were only obtained through a questionnaire. Study population was from an Asian country that may have different telomere dynamics from African, American or European people. Thus, the results cannot be globally extended to women, but pave the way for future studies on exercise and frequent measurement of telomere length at different occasions in greater depth.

Conclusion:

Exercise affects telomere dynamics, and higher physical activity is associated with increased telomere length. These results show the importance of regular exercise with aging and subsequent reduced risk of diseases. Although various studies have shown a relationship between exercise and telomere length, the present study is the first to investigate a particular type of HIIT in adult women.

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