

The Effect of High-Intensity Interval Training on telomere length, telomerase, and telomere related factors of Leukocytes in Sedentary Young Women

Zahra Mosallanezhad¹, Mohammad Hemmatinafar^{2*}, Abdolreza Sotoodeh³, Saeideh Erfanian⁴

Received 01 Dec 2018, Accepted for publication 26 Feb 2019

Abstract

Background & Aims: The purpose of this study was to examine the effect of eight-week high-intensity interval training (HIIT) on telomere length and telomere factors of leukocytes in sedentary young women.

Materials & Methods: A total of 21 students voluntarily participated in this study. The participants were randomly divided into two groups: the experimental group (n=11, age=23.25±2.01 years, height=163.32±5.44 cm, and weight=62.2±7.56 kg) and the control group (n=10, age=24.42±1.32 years, height=165.00±4.88 cm, and weight=66.6±6.30 kg). The experimental group performed three sessions of HIIT a week for eight weeks. Every session included three-six runs with maximum speed in a 20-metre area with 30 seconds of rest between each run. 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 analysed using independent and paired *t*-tests.

Results: The results showed that leukocyte telomere length (T/S ratio) ($p=.04$) and telomerase activity ($p=.04$) in the experimental group increased significantly. Moreover, the body fat percentage, BMI, and weight of the experimental group decreased significantly.

Conclusion: The results of this study showed that HIIT increases the leukocyte telomere length in sedentary young women, making it an efficient and appropriate method of exercise.

Keywords: Telomere, Telomerase, Telomere related factors, High intensity interval training

Address: Department of Sport Science, Shiraz University, Shiraz

Tel: +989177873803

Email: m.hemmatinafar@shirazu.ac.ir

Introduction

As vital biomarkers, telomeres are essential parts of human life. Located at the ends of chromosomes, these structures are made of a simple repeating sequence (TTAGGGn) and play an important role in the protection and functioning of genomes. In humans, the length of telomeres is increased by telomerase. This enzyme makes a template out of the existing RNA in its

structure and extends 3' terminus in the DNA (1). Telomerase activity is regulated by telomere-related factors, which include TRF1, TRF2 and POT1 in humans (2). TRF2 is physically and functionally associated with a number of repairing agents in DNA and plays an essential role in protecting telomeres from shortening (3, 4). Recent studies suggest a close relationship between short telomere length in peripheral

¹ Assistant Professor, Zand Institute of Higher Education, Shiraz, Iran

² Assistant Professor, Department of Sport Science, Shiraz University, Shiraz, Iran (Corresponding Author)

³ Assistant Professor, Jahrom University of Medical Sciences, Jahrom, Iran

⁴ Assistant Professor, Jahrom University of Medical Sciences, Jahrom, Iran

blood mononuclear cells (PBMcs), hypertension, and other cardiovascular risk factors (1,5). Hence, it is highly important to identify the factors that can protect telomere through changes in one's lifestyle. Physical activity and exercise training have not only been associated with the prevention and improvement of disease symptoms. but also with telomere length. Traditionally, it has been shown that doing the right types aerobic exercise reduces the risk of cardiovascular and metabolic diseases. However, this can be time consuming (6). Furthermore, the nature of some exercises, including endurance exercises, which require continuous exertion, pose some limitations. In this respect, it seems necessary to explore an alternative exercise programme with similar metabolic adaptations without the issue of time commitment. One of the exercising protocols that researchers of exercise physiology have recently researched is high-intensity interval training (HIIT). HIIT involves intervals of maximum-intensity exercise and resting intervals of low-intensity exercise. In HIIT, the intervals of low-intensity exercise between repeated exercise intervals mean that more exercises are performed with higher rates of effectiveness (7, 8). Therefore, with regard to diversity, very low time consumption, metabolic effects similar to endurance activities and the efficacy of these exercises, this study was conducted to examine the effect of HIIT on telomere length and telomere factors in sedentary young women.

Materials and Methods

This study quasi-experimental study was conducted with inactive normal young women studying at Shiraz University, aged 20-26 years. A total of 21 students voluntarily participated in this study and were randomly divided into two groups: the exercise group (n=11) and the control group (n=10). Subject characteristics are shown in Table 1. A questionnaire was utilized to collect

the related information about the level of physical activity and health of the participants. The participants had no cardiovascular diseases, diabetes, hereditary blood disorders, and respiratory problems and they did not use any medications. Furthermore, they had not conducted regular exercises for at least 6 months before the study. Anthropometric measures, including height, weight, body fat, and body mass index (BMI) were collected using standard procedures. VO₂max was measured before and after eight weeks of exercise using a treadmill. The participants in the experimental group executed the exercise protocol over a 20-metre distance which was marked with three cones. They did these three times a week for eight weeks, as described in Fig. 1. Upon starting the exercise protocol, the participants began running at maximum speed from the starting point (Cone 1) towards Cone 2 (Pathway A). Then, they returned and ran 20 metres at maximum speed in the opposite direction towards Cone 3 (Pathway B). They eventually returned and ran at maximum speed towards the starting point (Cone 1) (Pathway C) in order to complete the distance of 40 metres (6). The participants continued the above process at maximum speed until the 30-second period of the exercise protocol ended. Then, they then repeated the process after 30 seconds of recovery. The three repetitions of these 30-second exercises 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 up for five minutes (stretching and flexibility exercises along with slow running) before beginning the exercise protocol. They also rested for five minutes at the end of the session. The exercise protocol consisted of a 40-metre round at maximum speed which is considered as a valid test for anaerobic performance (9).

Table 1. Characteristics of the 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)	24.22±4.36	24.65±4.14	23.32±4.38	22.6±4.83*
Body fat (%)	20.52±0.95	20.42±0.78	19.96±2.21	17.87±1.13*

* Significant changes

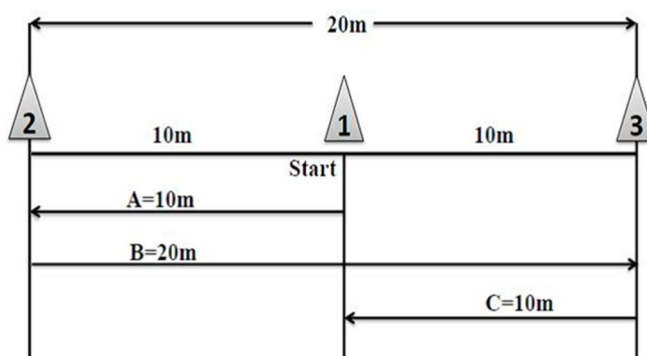


Figure 1. The exercise protocol of HIIT.

During the eight weeks of the exercise protocol, the participants in the control group did not have any regular exercising programme. The fasting blood samples of 10 cc were drawn from the brachial vein (antecubital vein) 24 hours before the first exercise session and 24 hours after the last session (8:30 am) in the laboratory. The blood samples were immediately poured into tubes containing anticoagulant (EDTA). The tubes were centrifuged with 3000 rpm for 10 minutes at 4°C.

DNA was extracted from the peripheral blood samples using a kit (Cinnagen Co., Iran) to measure telomere length. It was then dissolved in 50µl of water and kept frozen at -70°C. Optical Density (OD) was used to assess the 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). This used tel-specific and B436 (acidic ribosomal protein-coder) primers as Single Copy Gene (SCG) with the sequence that is presented in table 2.

Table 2. Primers of telomer gene and 36 B4 gen

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

A green fluorescent protein (GFP) is present in real time PCR reaction and produces a fluorescent light when the product is proliferated. Thus, the intensity of the light is directly related to the amount of product obtained. T/S (relative telomere length) is the ratio of the Cycle Threshold (CT) of the telomere gene over the B436 gene.

To measure telomerase activity, the blood mononuclear cells were removed using the concentration gradient method (through ficoll). Then, the telomeric repeat amplification protocol (TRAP) method which is based on the two techniques of polymerase chain reaction (PCR) and ELISA (kit of German Roche Company) was used with Holt's method (10). The relative activity of the participants' telomerase was calculated by putting the obtained values for absorption in the standard curve.

TRF2 Gene Expression:

To measure the expression of TRF2, the total RNA was first extracted using Thyrozol solution in accordance with the existing protocols. The extracted RNA was dissolved in 50 μ l of DEPC water and kept at -70 °C. Optical density (OD) was used to assess the quality and concentration of the extracted RNA. In a

reverse transcription reaction, 1 mg of RNA isolated from each sample was converted into cDNA using random primers and buffers in a cDNA synthesizing kit (TAKAR, Japan). A real time PCR reaction was performed using SYBR® Green PCR Master Mix kit (Applied Biosystems Company, USA). The TRF2 gene and GAPDH (as housekeeping gene) were proliferated using SYBR® Green PCR Master Mix kit (ABI, USA) and ABI step one plus system.

The collected statistical data were analysed using SPSS18 software. In this respect, the dependent *t*-test and independent *t*-test were used to examine the intragroup and intergroup changes, respectively. All of the statistical tests were performed at a significance level of $\alpha=0.05$.

Results

The effect of HIIT on telomere length:

The findings of the present study showed that there is a significant difference between the pre-test and post-test results of the training group's telomeres ($P = 0.04$). However, the difference between the pre-test and post-test values of the control group was not significant ($P=0.8$) (Table 3).

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
	exercise	1.657±0.312	1.854±0.148	0.04*

* Significant changes

HIIT effect on telomerase activity:

After analyzing the data of the training group, it was found that there was a significant difference between telomerase activity in the pre-test and posttest stage (P

= 0.04), but this difference between the pre-test and posttest levels of the control group was not significant ($P = 0.18$) (Table 4).

Table 4. Mean telomerase activity in the two blood samplings in both groups

Variables	Groups	Pretest	Posttest	Significance level
Telomerase activity(optical absorption rate)	Control	0.079±0.020	0.072±0.011	0.18
	exercise	0.082±0.042	0.098±0.035	0.04*

* Significant changes

The effect of HIIT on TRF2 mRNA expression:

Table 5 shows that in the training group, there was a significant difference between the pre-test and post-test

with regard to the mean TRF2 mRNA expression ($P = 0.03$). This difference was not significant in the control group ($P = 0.29$)(Table 5).

Table 5. Mean TRF2 mRNA expression in both groups

Variables	Groups	Pretest	Posttest	Significance level
TRF2 mRNA expression	Control	1.594±0.504	1.601±0.438	0.29
	exercise	1.531±0.438	1.883±0.548	0.03*

* Significant changes

Discussion

According to the present study, there was a significant difference in telomere length (T/S ratio) between the two groups following eight weeks of HIIT intervention. Moreover, a significant reduction was found in the body fat percentage, weight, and BMI of the trial group. Studies that concur with the present study on the positive effect of HIIT and its relationship with telomere length include Silva et al. (2016), Tucker et al. (2017), and Rehlkop et al (2016)(11,12,13). The results obtained indicate a positive relationship between telomere length and participating in intense exercise. Intense exercise prevents the erosion of telomere length caused by stress. This is due to the increased telomerase activity in the expressed leukocytes (14, 15). The present study results show the increased activity of this enzyme following eight weeks of training. This enzyme increases telomere length by adding new TTAGGG sequences. In contrast, some researchers believe in the U-turn hypothesis in relation to intense exercise and

changes in telomere. The relationship between shortened telomere and pro-inflammatory cellular environments supported the use of moderate exercises (16). Hemati et al. (2012) and Buchan et al. (2011) found improvements in fibrinogen and observed no changes in serum CRP after six weeks of HIIT in sedentary young people (6,17)., so it seems that regular HIIT is associated with slight changes in inflammatory proteins. In the present study, another possible factor affecting telomere length was the expression of TRF2 mRNA. The results of the present study showed a significant difference between the two groups in the expression of TRF2 mRNA following eight weeks of HIIT. This protein directly connects to the telomere sequence, regulates telomerase activity and strongly protects telomere length against erosion. It is possible that an increase in Shelterin proteins could occur through intermediaries IGF1, TERT and eNOS (18, 19). TRF2 increases other telomeres, since it controls mediatory signals that are associated with DNA damage. Andrew et al. (2012)

studied the response of Shelterin proteins and telomerase in various tissues, such as skeletal muscle, heart, and liver in response to 44 weeks of voluntary jogging exercise. Their results showed no reduction in TRF2 expression in heart muscle due to exercise and aging, and no significant change was observed in the liver tissue or skeletal muscle (20). These data show that, although exercise is beneficial to various tissues, the response of proteins regulating telomere length varies. In some tissues, telomerase activity increases (21) and, in others, expressions of TRF1 and TRF2 proteins and gene repair occur (22). This shows that, through telomere protection, exercise leads to specific tissue adaptation. The results of the present study disagree with those obtained by Denham et al. (2016), Laine et al. (2015) and Von et al. (2017) (23,24,25). The difference may partly be due to the difference in the measurement of intensity and duration of physical activity, and partly because of different populations and sample sizes.

The results of the present study also showed a positive relationship between telomere length and maximum oxygen intake (1,16,4) which supports the relationship between intense aerobic exercise and telomere length. This also shows the importance of aerobic preparedness for the protection of telomere length against life-long erosion. (26, 27, 28)

Exercise affects telomere dynamics and higher physical activity is associated with increased telomere length. These results show the importance of regular exercise alongside aging and subsequent reduced risk of the disease. Based on the findings, it appears that eight-week HIIT is an efficient and appropriate method of exercising in increased telomere length in sedentary young women in a normal weight range.

References

1. Arsenis NC, You T, Ogawa EF, Tinsley GM, Zuo L. Physical activity and telomere length: Impact of aging

and potential mechanisms of action. *Oncotarget* 2017;8(27):45008–19.

2. Laye MJ, Solomon TPJ, Karstoft K, Pedersen KK, Nielsen SD, Pedersen BK. Increased shelterin mRNA expression in peripheral blood mononuclear cells and skeletal muscle following an ultra-long-distance running event. *J Appl Physiol*. 2012; 112(5):773-81.
3. Ludlow AT, Roth SM. Physical activity and telomere biology: exploring the link with aging-related disease prevention. *J Aging Res* 2011;790378.
4. Werner C, Furster T, Widmann T, Poss J, Roggia C, Hanhoun M, et al. Physical exercise prevents cellular senescence in circulating leukocytes and in the vessel wall. *Circulation* 2009; 120: 2438-47.
5. Choi ES, Chang YK, Lee DH, Ko J-H, Lim I, Bang H, et al. Gender-specific associations between quality of life and leukocyte telomere length. *Maturitas* 2018;107:68–70.
6. Hemmatinfar M, Kordi M, Choopani S, Choobineh S, Gharari Arefi R. The Effect of High Intensity Interval Training (HIIT) on Plasma Adiponectin Levels, Insulin Sensitivity and Resistance in Sedentary Young Men. *ZUMS* 2013; 21 (84):1-12.
7. Smith M.J. *Sprint Interval Training - "It's a HIIT!"*. 1st Ed. 2008.
8. Noori Mofrad SR, Ebrahim Kh. The effect of high intensity interval training on telomere length and telomerase activity in non-athlete young men. *J Bas Res Med Sci* 2018; 5(2):1-7.
9. Glaister M, Hauck H, Abraham CS, Merry KL, Beaver D, Woods B. Familiarization, reliability, and comparability of a 40-m maximal shuttle run test. *J Sports Sci* 2009; 8: 77-82.
10. Holt SE, Norton JC, Wright WE, Shay JW. Comparison of the telomeric repeat amplification protocol (TRAP) to the new TRAP-eze telomerase detection kit. *Methods Cell Sci* 1996; 18:237-48.
11. Silva LCR, de Araújo AL, Fernandes JR, Matias M de ST, Silva PR, Duarte AJS, et al. Moderate and intense exercise lifestyles attenuate the effects of aging on

- telomere length and the survival and composition of T cell subpopulations. *Age (Dordr)* 2016;38(1):24.
12. Tucker L.A. Physical activity and telomere length in U.S. Men and women: An NHANES investigation. *Prev Med* 2017, 100, 145–51.
 13. Rehkopf DH, Needham BL, Lin J, Blackburn EH, Zota AR, Wojcicki JM, et al. Leukocyte Telomere Length in Relation to 17 Biomarkers of Cardiovascular Disease Risk: A Cross-Sectional Study of US Adults. *PLoS Med* 2016; 13: e1002188.
 14. Denham J, Nelson CP, O'Brien BJ, Nankervis SA, Denniff M, Harvey JT, et al. Longer leukocyte telomeres are associated with ultra-endurance exercise independent of cardiovascular risk factors. *PLoS One* 2013; 8: e69377.
 15. Zietzer A, Buschmann E.E, Janke D, Li L, Brix M, Meyborg H, et al. Acute physical exercise and long-term individual shear rate therapy increase telomerase activity in human peripheral blood mononuclear cells. *Acta Phys.* 2016, 220, 251–62.
 16. Ludlow AT, Zimmerman JB, Witkowski S, Hearn JW, Hatfield BD, Roth SM. Relationship between physical activity level, telomere length, and telomerase activity. *Med Sci Sports Exerc* 2008; 40: 1764-71.
 17. Buchan DS, Ollis S, Young JD, Thomas NE, Cooper S-M, Tong TK, et al. The effects of time and intensity of exercise on novel and established markers of CVD in adolescent youth. *Am J Hum Biol* 2011;23(4):517–26.
 18. Ludlow AT, Roth SM. Physical activity and telomere biology: exploring the link with aging-related disease prevention. *J Aging Res* 2011;2011:790378.
 19. Laye MJ, Solomon TPJ, Karstoft K, Pedersen KK, Nielsen SD, Pedersen BK. Increased shelterin mRNA expression in peripheral blood mononuclear cells and skeletal muscle following an ultra-long-distance running event. *J Appl Physiol* 2012; 112(5):773-81.
 20. Andrew T, Witkowski S, Mallory R, Jenny Wang M, Laila CJ, Lisa M. et al. Chronic Exercise Modifies Age-Related Telomere Dynamics in a Tissue-Specific Fashion. *J Gerontol A Biol Sci Med Sci* 2012;67(9):911–26.
 21. Werner C, Hauser M, Meyer T, Boehm M, Laufs U. High-intensity interval training reduces P53 expression and increases telomerase activity in circulating mononuclear cells of untrained subjects. *Circulation* 2012;126:A18220.
 22. Shadyab AH, LaMonte MJ, Kooperberg C, Reiner AP, Carty CL, Manini TM, et al. Leisure-time physical activity and leukocyte telomere length among older women. *Exp Gerontol* 2017;95:141–7.
 23. Denham J. Lack of association between pbmc telomere length and endurance exercise. *J Appl Biomed* 2016;15: 9-13.
 24. Laine MK, Eriksson JG, Kujala UM, Raj R, Kaprio J, Bäckmand HM, et al. Effect of intensive exercise in early adult life on telomere length in later life in men. *J Sports Sci Med* 2015;14(2):239–45.
 25. Von Känel R, Bruwer EJ, Hamer M, de Ridder JH, Malan L. Association between objectively measured physical activity, chronic stress and leukocyte telomere length. *J Sports Med Phys Fitness* 2017;57(10):1349–58.
 26. Puterman E, Weiss J, Lin J, Schilf S, Slusher AL, Johansen KL, et al. Aerobic exercise lengthens telomeres and reduces stress in family caregivers: A randomized controlled trial - Curt Richter Award Paper 2018. *Psychoneuroendocrinology* 2018;98:245–52.
 27. Friedenreich CM, Wang Q, Ting NS, Brenner DR, Conroy SM, McIntyre JB, et al. Effect of a 12-month exercise intervention on leukocyte telomere length: Results from the ALPHA Trial. *Cancer Epidemiol* 2018;56:67–74.
 28. Werner CM, Hecksteden A, Morsch A, Zundler J, Wegmann M, Kratzsch J, et al. Differential effects of endurance, interval, and resistance training on telomerase activity and telomere length in a randomized, controlled study. *Eur Heart J* 2019;40(1):34–46.