The effect of high intensity interval training on telomere length and telomerase activity in non-athlete young men

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Abstract

Introduction: Telomeres are DNA portions that are located on the two ends of the chromosome. Telomeres play an important role in cellular life. Exercise is one of the factors that contributes to their control. The purpose of the present study was to investigate the effect of 8 weeks of high intensity interval training (HIIT) on telomere length and telomerase activity in non-athletic young men.
Materials and methods: 30 inactive students were selected as sample and randomly divided into two groups of exercise (15 people) and control (15 people) in this semi-experimental study. The exercise group performed 8 weeks of HIIT exercise in 3 sessions per week with an intensity of 150 to 175% of their maximum power (Pmax). Control group subjects did not do regular sport activities. To measure telomere length and telomerase activity, 10 ml of blood was taken from the brachial vein of the subjects 24 hours before the first and after the last exercise session. The dependent t was used to analyze intra-group and independent t for within-group differences.
Results: The findings of this study showed that 8 weeks of HIIT training in non-athlete young men resulted in a significant increase in telomere length (P = 0.001) and telomerase activity (P = 0.001).
Conclusion: It seems that HIIT can alter telomerase activity and telomere length. Therefore, these training may have a positive effect on cell biology.

Keywords: Telomere, Telomerase, High intensity interval training, Non-athlete

Introduction

Telomeres are DNA-specific structures that are made up of the sequential repetition of DNA strands, TTAGGGn, (in humans) and are at the end of eukaryotic chromosomes. Telomeres have vital functions, including the protection and stability of the chromosome, preventing the end of the chromosomes from joining each other.
Due to the inability of DNA polymerase to completely reproduce the 3' end of DNA strand, telomere lose 30 to 150 base pair in each cell division. The telomere length is closely related to the chronological agenda and is even considered as an indicator of the biological age or cell aging. When the telomere length reaches a critical level, cell proliferation stops and cell function may be impaired and cell death may overtake. Not only telomere length, but also the disorder in its related proteins can cause chromosomal instability, cell aging or apoptosis (1).
Telomeres and their lengths are not constant, but dynamic (2). The telomerase ribonucleoproteins enzyme increases

telomere length by adding nucleotides to the end of telomeres. In most adult cells, telomerase activity is almost undetectable, but is active in embryonic stem cells, germline cells, cancer cells, and pro-cellular cells of epithelial cells and lymphocytes. In fact, the telomerase enzyme maintains or extends the telomere length and allows the cell to continue dividing (3).

Some of the effects of telomeres shortening include: weakening of vision, atherosclerosis, wound healing, heart disease, hair bleeding and wrinkling of the skin (4).

Several studies have reported a positive relationship between physical activity and telomere length in which active subjects have a longer telomere length compared to inactive people. Cherkas et al. (2008) reported a positive correlation between increased physical activity and longer telomere (5). Others confirm the findings of Charkas et al. by demonstrating that individuals with maximum amount of higher consumed oxygen have a lower telomere length than those with maximum amount of less oxygen (6).

Most people often know the benefits of endurance training by doing a series of long-term exercises. And because such activities are time-consuming, they often refuse to participate in these types of training programs because of the time limit. Therefore, part of recent studies have examined the replacement exercise program with similar metabolic adaptations without the need to spend much time.

One of the suggested solutions is the use of short-term practice protocols called high intensity interval training (HIIT). HIIT has been defined as an intense (maximum Intensity or intensity close to VO2max) short interval training (7). Several studies have shown the beneficial effects of this type of exercise on various aspects of health and well-being (8). But whether HIIT affects telomere length and telomerase activity is not well defined. Therefore, the purpose of this study was to investigate the effect of 8 weeks of HIIT on telomere length and telomerase activity in non-athletic young men.

Materials and methods

In this Quasi-experimental pre-post intervention study, a sample of 30 students from non-athlete students of the University of Kashan was selected as an available sample (Table 1). Subjects were randomly divided into experimental (n = 15) and control (n = 15) groups after selecting, and announcing their readiness and obtaining written consent from them.

Only those with the following criteria were included in the sample: Participant were expected to meet the qualities of no regular exercise in the last six months, no smoking, no illness, no prescription drugs or sports supplements. Exclusion criteria of this study were: smoking, using drugs or supplements, inability to exercise, and orthopedic problems

The experimental group performed 8-week HIIT practice protocol in three sessions per week. The exercise program is designed to first determine the maximum aerobic power of people on a powered bike. For this purpose, the subjects performed a 5-minute warm-up of 50 watts on a powered bicycle (Monark 874E, Sweden). Then, 1 watt was added to the exercise resistance every two seconds.

This increase continued until the person was exhausted.

The ride speed was considered between 65 and 75 rpm. Subjects performed 6 to 10 turns of HIIT 30 seconds at an intensity of 150 to 175 percent of their Pmax one week after determining the Pmax (maximum power).Four minutes of rest was in the form of pedaling without load between two repetitions. Subjects completed the first two weeks of each session 6 times, the next four weeks of each sessions 8 times and 10 times in the last two weeks (9). The control group did not have regular sports activities and continued their normal life during this course.

Blood sampling: Half an hour before the start of the training, the first blood samples were taken. The second ones were taken 24 h after the last training session. In every attempt for blood collection, 10 mL of blood was taken from the antecubital vein of the subjects' arm and placed into the test tube containing anticoagulant (EDTA).

Telomere length: Peripheral blood mononuclear cells (PBMC) were used to measure the telomere length. The DNA from these cells was extracted using standard salting out- proteinase K method. The concentration and quality of the extracted DNA were examined using Nano drop (NanoDrop-2000) at wavelengths of 260 and 280 nm, and the ratio of the two wavelengths were used. The researchers assessed telomere length by quantitative polymerase chain reaction (qPCR). Two PCR reactions were performed for each sample, the first reaction for telomeric DNA fragment and the second for its control gene, acid ribosomal phosphoprotein. The primers for the telomere PCR were as follows: CGGTTTGTTTGGGTTTGGGTTTGGGTTTGGGTTTGGGTT and reverse GGCTTGCCTTACCCTTACCCTTACCCTTACCCTTACCCT. The primers for the single-copy gene (acid ribosomal phosphoprotein) PCR were 36B41 CAGCAAGTGGGAAGGTGTAATCC and 36B42 CCCATTCTATCATCAACGGGTACAA. DNA telomeric length was calculated based on the ratio of telomere to control gene. Real time PCR kit was SYBR® Green PCR Master Mix manufactured by Applied Biosystems (USA). Real Time PCR using specific primers for telomere and primer for 36B4 (acidic ribosomal protein-coding) as a single-copy gene (SCG). Briefly, the primers concentration was 100 nM and DNA concentration was 20 ng of DNA in the reaction. Temperature cycle for the reaction was as follows: a 95 °C for 10 min,which followed by 40 cycles consisting of 95 °C for a minute and 60 °C for 15 s. After determining of C_T related to telomere and 36B4, for telomere length, T/S calculated.

Telomerase activity: Using a real-time polymerase chain reaction (qPCR) assay (Quantitative Telomerase Detection [QTD] kit, US Biomax), Telomerase activity was determined according to the manufacturer's instructions. The blood sample PBMC was isolated and lysed, the lysate was centrifuged for 30 min at 12,000g and 4 °C, and the supernatant kept at -80 °C for sensitive protein content determination (Bradford method). The extracted sample used as a template for the qPCR assay. The increase in fluorescence caused by the SYBR Green I dye binding to the double-stranded DNA monitored for a direct detection of the PCR product. Briefly, the qPCR master mix comprised 12.5 µL of the QTD premix containing telomere primers, 1 µL of PBMC extract, and heat-inactivated extracts (negative control) and with water for a final volume of 25 µL. Forty cycles with 20 min at 25°C, 10 min at 95 °C, 30 s at 95 °C, 30 s at 60 °C and 30 s at 72 °C, was the qPCR program. Data collections were performed at three steps: during annealing, extension, and during melt curve analysis. For estimation of telomerase activity, a positive control used to produce a standard curve, consisting of six serial dilutions. The analysis of samples consisted of extracted sample with and without heat-treated.

Statistical analysis

By using SPSS software version 18, Shapiro Wilk test was first used to check the distribution of data. Independent t-test was used to compare the values inter-groups and dependent t-test was used for intra groups. The significance level for all calculations was considered as $P < 0.05$. The present study was conducted under the control of the University's Ethics Committee.

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 of the length of the training group's telomeres (P = 0.002). However, the difference between the pre-test and post-test values of the control group was not significant (P = 0.492). Independent t-test also showed that there was a significant difference between the values of the telomere length of the training group and the control group (P = 0.01) (Table 2).

HIIT effect on telomerase activity: After analyzing the data, it was found that there was a significant difference between telomerase activity in the pre-test and post-test stage, training group (P = 0.001), But this difference between pre-test and post-test levels of the control group is not significant (P = 0.783). Independent t-test indicated that there was a significant difference between the activity of telomerase in the training group and the control group (P = 0.001) (Table 2).

Table 1. Demographic data in training and control subjects.

Variable	Control group	Training group	P value
Age (year)	20.13±0.64	19.7±1.1	0.62
Height (cm)	176.8±5.9	176.6±5.4	0.45
Weight (kg)	71.1±7.2	69.9±6.6	0.73
Body mass index(Kg/m^2)	23.1±1.6	22.4±1.4	0.56

Data are shown as means ± SD.

Table 2. Mean and standard deviation of Telomere length and Telomerase activity.

Variable	Training		Control	
	Pre-test	Post-test	Pre-test	Post-test
Telomere length (T/S)	1.43±0.20	1.60±0.13	1.47±0.2	1.45±0.15
Telomerase activity (CT)	27.39±0.83	26.04±0.70	27.25±0.83	27.34±0.92

Data are shown as means ± SD.

Discussion

The results of this study showed that 8 weeks of HIIT increased telomere length and telomerase activity. A few studies have been performed on the effect of HIIT training on telomere length. Werner et al. (2017), showed that 6-month HIIT training in individuals aged 30-60 years increased the activity of telomerase and decreased P53 and did not change the telomere length (10). Also, Mosalanejad and colleagues studied the effect of 8 weeks of HIIT on non-athletic women's telomerase activity. Their findings showed that 8 weeks of HIIT significantly increased the activity of non-athletic women's telomerase (11). On the other hand, Mathur et al. (2013), did not observe a relation between the maximum oxygen consumption, physical activity levels and telomere length, despite a large difference in physical fitness by studying a group of marathon athletes (12). In a study by Layer et al. (2012), following the 7-day ultraruning, the amount of the mRNA expression of the three proteins TRF1, TRF2, Pot1 in peripheral blood mononuclear cells (PBMCs) increased, but a change in telomere activity and telomerase activity failed (13). The age of the subjects, the amount of physical activity, telomere length measurement methods and other uncontrolled factors (such as diet and psychological stresses) can contribute in difference of findings (14). One of the main causes of telomere stress is oxidative stress. The telomere of smooth muscle cells of the vascular and endothelial cells becomes shorter when exposed to oxidative stress in the medium. Also, the activity of telomerase decreases in response to oxidative stress (15). Although the use of antioxidant

supplements has been successful in reducing oxidative stress and improving health, Adjunctive regulation of endogenous antioxidant capacity by regular exercise has led to favorable adaptations in most studies (16). Interestingly, in order to achieve the desired adaptations of the anti-oxidant defense system as a result of exercise, the stimulant should be strong enough to significantly increase the production of active oxygen species (ROS).

Hence, both short-term aerobic and anaerobic activities lead to oxidative stress (17). According to the principle of hormesis, it should be accepted that such a short-term increase in ROS in some people is an essential stimulant for the incremental regulation of anti-oxidative defense (16). However, short-term sports activities will increase the production of ROS, this increase in ROS can actually increase antioxidant defense as a result of exercise. This means that repeated exposure to an exercise stimulant increases the antioxidant defense (16) and also reduces the accumulation of oxidized biomolecules at rest and after exercise. It seems that improving the antioxidant capacity of the cells can play a role in telomere control and telomerase activity (18). Stress is accompanied by the release of glucocorticoid hormones in the adrenal gland. It has been shown that these hormones reduce the amount of antioxidant proteins (19) and, therefore, can increase the oxidative damage to DNA (20) and accelerate the erosion of telomeres (21).

In confirmation, women with a stressful life were more likely to have more oxidative stress, lower telomerase activity and shorter telomeres in peripheral blood mononuclear cells compared with those in the control group (22). Since telomere length can represent a person's biological age, stress has a harmful effect on one's health and lifetime. Physical activity with positive effects on the quality of life of individuals and reducing their stress can be considered as a defense mechanism against stress hormones and their harmful effect on telomere biology .In addition to oxidative stress-related directions, chronic inflammation associated with age can also play a role in telomere biology. It is observed that chronic inflammation also plays a role in telomere shortness (23). Exercise is associated with an increase in serum levels of IGF-1 (24). IGF-1 increases telomerase activity, delay cellular aging and cell death (16, 18).

Conclusion

Overall, it was observed that HIIT can increase telomerase activity and thus have a beneficial effect on the telomere length, 8 weeks of HIIT may have a positive effect on cell biology. Nutrition affects physiological processes, including the status of antioxidant oxidation of the body. Oxidative pressure plays an important role in telomere biology. In the present study, the nutritional status of subjects was not completely controlled. Therefore, it is suggested that the nutritional status of the subjects be controlled in order to obtain better results in subsequent studies.

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