

The effect of taking green tea extract for 12 weeks on telomerase enzyme content in heart tissue of old rats in response to acute exhaustive exercise

Mirza Hossein Norouzi Kamareh¹, Mohammad Reza Zolfaghari², Firouz Ghaderi Pakdel³, Javad Tolouei Azar^{4*}

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Abstract

Background & Aims: The prevalence, incidence, and complications of heart disease increase with age. Green tea is an antioxidant which can prevent cellular senescence and cardiovascular diseases. Telomerase enzyme decreases with aging. Acute exhaustive exercise results in disturbances in the oxidative system and production of active oxygen species in the heart tissue. Thus, the present study aims to investigate the effect of taking green tea extract for 12 weeks on telomerase enzyme content in heart tissue of old rats in response to acute exhaustive exercise.

Materials & Methods: In this study, 20 male rats were randomly categorized into two groups of supplement and non-supplement each consisting of 10 rats. The supplement group received green tea extract for 12 weeks. At the end of 12 weeks, each of these groups was further divided into two groups of rest and acute exhaustive exercise. The non-supplement with rest group was called control group. The non-supplement with exercise group was called exercise group. Further, the supplement group with rest was called supplement group and finally the last group was entitled supplement with exercise. After 12 weeks of taking green tea extract, one session of acute exhaustive exercise was held on the treadmill. Sandwich ELISA method was used to measure telomerase content. The data were analyzed by ANOVA and Tukey tests.

Results: The results showed that telomerase enzyme content in the supplement with exercise group was significantly more than the exercise group ($P=0.021$). Also, it was significantly more in the supplement group than the control group ($P=0.008$). The difference between the exercise and control groups ($P=0.241$) as well as the difference between the supplement with exercise and supplement groups ($P=0.112$) were not statistically significant.

Conclusion: The study showed that using green tea extract for 12 weeks can increase telomerase enzyme content in rest or after acute exhaustive exercise and accordingly prevent cellular senescence.

Keywords: Exhaustive exercise, Green tea, Telomerase content

Address: Faculty of Sport Sciences, Urmia University, Urmia, Iran

Tel: +989143410949

Email: j.toloueiazar@urmia.ac.ir

Introduction

The outbreak and effects of cardiovascular diseases increase with aging. Cardiovascular disorders are

reported four times more in individuals aged over 85 years old than those between 65-75 years old (1). In the cellular level, senescence affects the cell's ability to live

¹ PhD Student in Exercise Physiology, Faculty of Sport Sciences, Urmia University, Urmia, Iran

² Assistant Professor of Exercise Physiology, Faculty of Sport Sciences, Urmia University, Urmia, Iran

³ Associate Professor, Physiology Department, Faculty of Medicine, Urmia University of Medical Sciences, Urmia, Iran

⁴ Assistant Professor of Exercise Physiology, Faculty of Sport Sciences, Urmia University, Urmia, Iran (Corresponding Author)

through telomeres and its proteins. Short telomere length is a sign for the death resulting from cardiovascular diseases. Telomerase enzyme is a ribonucleoprotein which is located in telomeric complex center (2). The most important factor in telomere length increase is telomerase. Telomerase enzyme is a Reverse transcriptase which can take the RNA in its structure as a pattern and increase telomere length (3). The activity of telomerase enzyme in the heart tissue decreases with aging (4).

Senescence is the result of the balance between damage and amendment which leads to low performance and improper response to external stress. Free radical theory, as one of the most accepted theories among aging theories, considers free radical production as the main factor for cellular damage. Oxidative stress reflects an imbalance between the systemic manifestation of reactive oxygen species and a biological system's ability to readily detoxify the reactive intermediates or to repair the resulting damage (5). Over manifestation of reactive oxygen species can lead to cellular stress, DNA damage, Short telomere length, and finally cellular senescence (6). Doing regular exercises decreases cardiovascular diseases. It leads to the improvement of blood pressure, sensitivity to insulin, temperament, body weight, and inflammatory variables (7). Studies show the positive effects of regular aerobic exercise on increasing the antioxidant capacity and preventing apoptosis in the heart tissue (8-11). However, acute exhaustive exercise leads to disturbance in oxidation system and manifestation of reactive oxygen species in the heart tissue. The increase in free radicals resulting from exercises with high intensity (12), leads to DNA damage and the death of heart cells. Gul et al. (2006) showed that activities of glutathione peroxidase, glutathione reductase, and Superoxide dismutase enzymes decreased by acute exhaustive exercise on a treadmill in rat heart (12). Trofin et al. (2014) reported that increased serum oxidative levels

such as malondialdehyde and protein carbonyls in rats after five minutes of running on a treadmill, and decreased levels of serum superoxide dismutase (13). Olah et al. (2015) showed that an exhaustive exercise session in rats caused the fragmentation of myocardial structure, decreased ejection fraction, impaired contractility (preload recruitable stroke work) and mechanoenergetics (ventriculoarterial coupling, mechanical efficiency) of LV after exercise. Myocardial expression of major antioxidant enzymes was increased along with increased myocardial nitro oxidative stress. Bax/Bcl-2 ratio and TUNEL staining showed enhanced apoptotic signaling (14). From the results of studies, aging and acute exhaustive exercise can disrupt the oxidative system and cellular aging. Thus, it is important to identify a factor able to decrease cellular senescence despite aging and acute exhaustive exercise.

Green tea is a popular drink which has caught the attention of many people because of its antioxidant, anti-inflammation, anti-tumor and anti-sensitivity properties. Epigallocatechin gallate (EGCG) is the most catechin in the green tea. Recent studies show the protective effects of green tea and EGCG in cardiovascular diseases (15). Li et al. (2005) reported that EGCG leads to the destruction of cancer cells but has no effect on normal cells (16). Sheng et al. (2009) showed that EGCG controls apoptosis and oxidative stress in heart cells when extra pressure is imposed during hypertrophy (17). Moreover, EGCG suppresses oxidative stress of apoptosis cells by controlling telomere change dependent on apoptosis path (18). Given the positive effects of green tea in controlling oxidative stress, it seems that it can prevent cellular senescence and Short telomere length as a result of aging and acute exhaustive exercise in the heart tissue. However, as far as the researchers found, no study has focused on this issue. Thus, the present study investigates the effect of taking green tea extract for 12 weeks on telomerase enzyme

content in heart tissue of old rats in response to acute exhaustive exercise.

Materials and Methods

This study investigated the effect of taking green tea extract for 12 weeks on telomerase enzyme content in heart tissue of old rats in response to acute exhaustive exercise. Twenty 40-week-old male Wistar rats were the population of this study. The rats were first categorized into two groups of supplement and non-supplement each consisting of 10 rats. The supplement group received green tea extract for 12 weeks. At the end of the 12 weeks, each of these groups was further divided into two groups of rest and acute exhaustive exercise. After the 12 weeks, all exercise groups did one session of acute exhaustive exercise while the rest groups did no exercise. The non-supplement with rest group was called control group. The non-supplement with exercise group was called exercise group. Further, the supplement group with rest was called supplement group and finally the last group was entitled supplement with exercise. At the beginning of the last week of supplement protocol, all groups ran on the treadmill for 5 days with the speed of 15 meter/minute for 10 minutes to get familiar with the treadmill and exhaustive exercise. These 5 days were only done to make rats familiar with the treadmill and it had no significant exercise effect (12). After one session of acute exhaustive exercise, necessary samples were taken from the rats.

Exercise Protocol:

At the end of the protocol, the exercise groups did one session of acute exhaustive exercise on the treadmill. First, it was done for 5 minutes with the speed of 15 meter/minute in order to warm up; then the treadmill's speed increased gradually in a way that it reached 35 meter/minute in the 30th minute. This speed was maintained until the animals were exhausted. At the end, 5 minutes of running with 15 meter/minute was

done in order to cool down. The treadmill's slope was zero throughout the session (19).

Feeding Supplements:

Rats in the supplement and exercise with supplement groups received 12 weeks of green tea extract (5 days a week). The extract was in the form of 400 mg capsules made the American company NOW FOODS. Each capsule contained 40 percent catechin. The capsules were dissolved into 1 cc of distilled water (5).

Results

Sandwich ELISA method was used to study telomerase content in the heart tissue. Here, Rat TE (telomerase) ELISA Kit by the Elabscience Company of U.S.A. was used. The minimum detectable dose of kit is 46.88 pg/ml and its detection range is 78.13 to 5000 pg/ml. The tissue was lubricated based on the guidelines of the manufacturer. First, 300 mg of the heart tissue was taken. Then, it was placed into the microtube with 1 ml of PBS and 100 mg of glass homogenizer. Then, homogenizing was done by Mikro-dismembrator for two minutes and 3000 shake per minute. It was done in the ice for 2 minutes. The solution was centrifuged in 4 degrees centigrade's for 8 minutes at 5000×g. The solution was then assessed. Next, Sandwich ELISA was used to measure telomerase content according to the manufacturer's instructions. Telomerase enzyme content of the tissue was stated with respect to tissue's total protein. Total protein was measured by Bradford method.

Statistical Methods:

Descriptive statistics was used to describe data and draw graphs. The normal distribution of data was measured by Shapiro-Wilk test. The comparison between groups was done by ANOVA and Tukey tests. Homogeneity of variance was investigated through Leven test. The level of significance was $P \leq 0.05$. Spss 24 was used to analyze data and Excel was used to draw graphs.

Table 1. Results of Tukey test

Groups	Mean difference	Level of significance
Exercise - Supplement with exercise	401.749	P=0.021*
Exercise - control	239.840	P=0.241
Exercise - supplement	697.849	P=0.000*
Control - supplement	458.009	P=0.008*
Control - supplement with exercise	161.909	P=0.560
Supplement - supplement with exercise	296.100	P=0.112

* Level of significance $P \leq 0.05$

Telomerase enzyme content with respect to total protein in the heart tissue in different groups shows in figure 1. As this figure shows the content of telomerase enzyme with respect to total protein was more in supplement with exercise group as compared with the exercise group. This increase was statistically significant ($P=0.021$). As a result, 12 weeks of using green tea extract increases telomerase enzyme content in response to one session of acute exhaustive exercise. Also, this enzyme was significantly more in the

supplement groups as compared with the control group ($P=0.008$). Thus, 12 weeks of using green tea extract increased telomerase enzyme content in the heart tissue. The difference between exercise and control groups ($P=0.008$) and supplement with exercise group with supplement group ($P=0.008$) was not statistically significant. Thus, one session of exhaustive exercise decreased telomerase enzyme amounts but this decrease was not statistically significant.

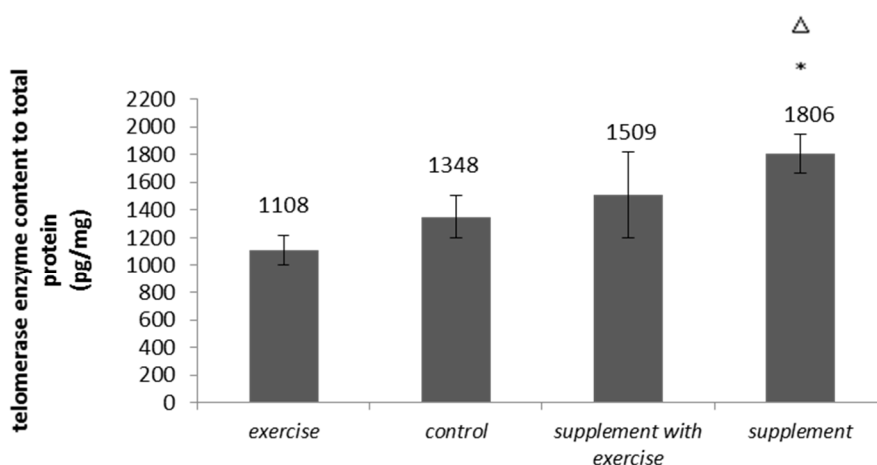


Figure 1. Telomerase enzyme content to total protein in the heart tissue of different groups

* Significant difference compared with control group $P \leq 0.05$

△ significant difference compared with exercise group $P \leq 0.05$

Discussion and Conclusion

The results of the present research showed that 12 weeks of using green tea extract increases telomerase

enzyme content in response to one session of acute exhaustive exercise. Also, 12 weeks of using green tea extract increased telomerase enzyme content in the heart tissue. One session of exhaustive exercise decreased

telomerase enzyme amounts but this decrease was not statistically significant, which is in line with previous studies (12-14). Acute exercise leads to disruption of the oxidation system and appearance of ROS in the cardiac tissue. The production of ROS can create stress in the cell, tissue, or the organ and leads to DNA damage, apoptosis, or senescence (6). Starr et al. suggested some oxidative stress genes which are related to telomere change and senescence biology. Steady oxidative stress leads to DNA damage and short telomere length (20). Olah et al. (2015) reported that exhaustive exercise in rats leads to fragmentation of myocardial structure, decreased ejection fraction, impaired contractility, and Bax/Bcl-2 ratio and TUNEL staining showed enhanced apoptotic signaling (14). Thus acute exhaustive exercise can disrupt the oxidative system, short telomere length, and decrease telomerase enzyme amounts.

Few studies have focused on the effect of green tea on telomere's biology. As far as researchers reviewed the related literature, this is the first study on the effect of green tea extract on telomerase enzyme in response to acute exercise. Sheng et al. (2013) investigated the effect of EGCG on apoptosis telomeric path in rats' heart tissues. They showed that EGCG decreased apoptosis, DNA damage, and P53 and P21 amounts (18). The results of those two studies are in line with the present research which can be due to the same samples (male rats). Telomere length decreases in both senescence process and chronic diseases. However, it is possible to maintain its length by using antioxidant supplements.

According to the results of the studies, it can be concluded that both aging and acute exhaustive exercise can disrupt the oxidative system, cellular aging, and decrease telomerase enzyme amounts. Some mechanisms can be studied in relation to the effects of antioxidant supplements on telomere length, the most important of which is telomerase enzyme and oxidative stress. When the length of telomere shortened as the

result of aging, telomerase enzyme replaces a two-phase pattern from its structure. One study showed that both telomere length and telomerase enzyme activity gradually decrease from 4 to 39 years old. Also, 65 percent of those over 40 years old who face Short telomere length, showed steady but low telomerase enzyme activity and the remaining 35 percent had no telomerase enzyme activity (6). Using antioxidant supplements can make balance in oxidant-antioxidant system and prevent the production of ROS (18).

30 percent of the weight of green teas, as a popular drink, is made up of tea polyphenols. EGCG is the most active catechin in green tea and enters blood via small intestine quickly. Polyphenols of green tea can release and reduce free radicals. They can even prevent the harmful effects of free radicals (5). Studies report the benefits of some antioxidants such as vitamin C, glutathione, and N-Acetylcysteine in preventing apoptosis (15). Rice-Evans (1998) showed that antioxidant capacity of EGCG is more than vitamin C and vitamin E (21). Given the effects of oxidative stress on telomere biology and senescence process and the antioxidant property of green tea, it seems that drinking green tea can have positive effects on telomere biology and the activity of telomerase enzyme. Thus, the increase of telomerase enzyme content due to using green tea extract, as this study shows, can be because of antioxidant properties of green tea.

This study concludes that using green tea extract for 12 weeks can increase telomerase enzyme content while resting or after acute exhaustive exercise in the heart tissues of old rats and prevent Short telomere length and cellular senescence.

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