Effects of Aerobic Training Combined with Green Tea Extract on Leukocyte Telomere Length, Quality of Life and Body Composition in Elderly Women

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Abstract

Background: Exercise and consumption of green tea affect the aging process. However, the effect of exercise training combined with green tea extract on leukocyte telomere length, quality of life (QoL) and body composition in aging has so far been unclear and inconsistent.

Objective: The aim of the present study was to determine the effectiveness of aerobic training intervention and green tea extracts consumption either alone or in combination on leukocyte telomere length, QoL and body composition among elderly women.

Methods: Thirty six elderly women, 60–65 years, were randomly divided into two groups, the exercise alone (placebo group) and exercise with green tea consumption (green tea group). All participants in both groups were engaged to aerobic protocol exercise three times in week for five months. The participants in green tea group received green tea extract capsule 500 mg and placebo group received toasted powder capsule 500 mg three times a day for a period of five months. At baseline and end of the study the leukocyte telomere length, QoL and body composition were measured.

Results: There were significant increase on leukocyte telomere length in green tea group compared with the baseline (P=0.004) and also placebo group (P=0.041) at the end of the study. Waist–hip ratio was also significantly decreased in green tea group compared with placebo group (P=0.016).

Conclusion: Our findings shows that combination of aerobic training with consumption of green tea has synergic effect on waist–hip ratio and leukocyte telomere length associated with aging among elderly women.

Keywords: Aerobic training, Aging, Green tea, Leukocyte Telomere length, Quality of life

Introduction

Aging is defined as the accumulation of harmful changes occurring in cells with advancing age that results in a loss of cellular and organismal fitness. Aging is marked by a substantial decrease in the regenerative potential of several cell types, including immune cells and skeletal muscle cells [1, 2]. Both genetic and environmental factors dictate the rate of tissue regeneration and the balance between accumulation and removal of cellular damage. Accumulation of unrepaired cellular damage and a lack of tissue regeneration via cell replication result not only in aging-related phenotypes (grey hair, wrinkled skin, etc.), but also in several age-related diseases such as Alzheimer's disease, cardiovascular disease, type II diabetes, and sarcopenia [3, 4]. Aging influence on body composition [5] and quality of life (QoL) (6) in elderly people. Some studies have shown a high correlation between aging, body composition and QoL [7]. It is believed that telomere length could be a biomarker of biological cellular age and thus predicts morbidity and mortality [8, 9] and it's related to body composition (10) and QoL [11, 12].

Telomeres are ribonucleoprotein complex structures localized at the end of linear eukaryotic chromosomes [13]. Telomere shortening is a natural process that occurs with each cell division. When telomeres reach a minimum critical size, they induce cell senescence or apoptosis [14]. Therefore, telomere length is a well-known indicator of mitotic replicative history and biological age [15-18]. It seem that exercise and nutrition are two important factors influence on telomere shortening [19-21].

It is well documented that exercise or physical activity increases oxidative stress. However, its continuous practice can improve the anti-oxidant activity and benefit the REDOX balance [22, 23] via influence specific signaling molecules, including PGC1α and sirt1 and sirt6 [24, 25], and improve inflammatory balance [26]. All these factors, have been shown to influence the telomere shortening [14].

Few studies have investigated the association between exercise training and leukocyte telomere length (LTL) with inconsistent results: positive, none and inverted U-shaped associations have been described [17, 27, 28]. Interestingly, a U-shaped relationship has been observed in both sedentary and extremely active individuals [4]. Most commonly moderate levels of physical activity have been associated with longer LTL [29, 30].

In the other side green tea as a favorite beverage in human nutrition has attracted considerable attention for its antioxidative, anti-inflammatory, antitumorigenic and antisenescent properties [31]. Epigallocatechin gallate (EGCG), the most abundant catechin in green tea, protects cells against ischemia/reperfusion-induced apoptotic cell death both in vivo and in vitro [32]. EGCG suppresses oxidative stress-induced cells apoptosis through inhibiting telomere dependent apoptotic pathway [33, 34].

Some studies reported controversy effects of green tea on telomere length in different cells. Li et.al. 2005 indicate epigallocatechin gallate induces telomere fragmentation in HeLa and 293 but not in MRC-5 cells that might be relevant to the apoptosis-inducing

effect of EGCG on cancerous cells but not on normal cells [35].

In addition some studies pointed out that, exercise and green tea can influence body composition and health-related QoL via influence specific signaling molecules leading to gene expression of fat metabolism enzymes [24, 25] but another study reported no effect of exercise or green tea on body composition and QoL [36].

Therefore, due to the conflicting and confusing results and lack of information on the synergic effect of both exercise and green tea consumption on aging factors, current study was conducted to evaluate the effects of aerobic training with or without green tea extracts consumption on leukocyte telomere length, QoL and body composition in elderly women.

Materials and Methods

Interventions and measures

Materials: Green tea extract was purchased from Soha Jissa Plantation, Industries and Herbal Medicine Processing Co. The green tea extract possessed total flavonoids 703.12 ± 20.21 mg/g dried extract expressed as gallic acid equivalent reported by company. Toasted powder purchased from local market. The 500 mg capsule of green tea extract and toasted powder as placebo prepared by institute of medicinal plant Karaj Iran.

Study Design: In this study, 36 healthy elderly women (determined by the health questionnaire and the following tests) housewife, who lived at Hesarak town, Karaj city Alborz, Iran, aged 60 to 65 years, were selected. After initial tests such as body mass index (BMI), waist to hip ratio (WHR), subcutaneous fat with skin fold way, blood pressure, resting heart rate, complete health questionnaire, dietary history questionnaire and physical activity level questionnaire total 36 women were participated in the study. In order to check the power of exercise training they participate in two initial session of exercise.

The inclusion criteria were Iranian healthy elderly women aged 60 to 65 years with normal blood pressure and able to complete the two initial session of exercise training of the study. The exclusion criteria were women with diabetes, psychological disorder, liver, kidney, cardiovascular and infectious lung disease, tendency to change the exercise or food regimen and cigarette smoking women.

The participant are randomly divided into herbal drug, placebo groups encoded with alphabet A or B. Block randomization with a computer generated random numbers table and sequentially numbered containers each representing a block consisting of three patients are used for the treatment assignments. Three different persons generate the random allocation sequence, enroll the participants and assign them to interventions. Care providers, participants and the person evaluating the response to treatments are blind to the interventions.

Both groups undergo to a five months period of aerobic exercise training (three sessions per week). In addition green tea group received one capsule of 500 mg green tea and placebo group one capsule of 500 mg toasted powder three times a day during the study.

Fasting blood samples were taken before and after the study for determination of leukocyte telomere length. The body composition and quality of life were also evaluated at baseline and end of the study.

This clinical trial has been approved in Medical Ethics Committee of Shahid Beheshti University of Tehran with 95/1006 code.

Exercise Protocol: The aerobic exercise protocol for both groups was based on the recommendations of the American college of sports medicine and the American heart association of physical activity and public health in older adults [37]. A session of exercise protocol was as follows: 15-minutes warm-up, stretching and movement coordination and balance training, followed by 15 minutes, running around the halls with intensity between 50 to 70 percent of maximum heart rate (from 50% started and to 70% in the middle of the period continued with the same intensity), and then 5 minutes, cooling by walking and muscles and joints stretching.

Body composition measurement: All measurements were conducted in gym in Razi town from 7:00 a.m. to 10:00 a.m.; Gym temperature was in range 20–26°C before and after five months during the study. Body height was measured in the standing position without shoes by a measuring tape against a wall to an accuracy of 0.1 cm. Body weight was measured using medical scale (Radwag WPT 100/200) with accuracy of 0.01 kg. Body mass index was calculated as body weight divided by height in meters squared. WHR was measured by measuring waist and hip circumference with tape meters with little clothing and then contacted the ratio between them. The exact age of each participant was calculated from birth and observation dates.

Fat mass, Fat free mass and % Body fat were measured by skinfold way. Skinfold thickness measurements were performed by lifting a fold of skin and subcutaneous fat away Jackson and Pollock (38) with 3-site skinfold equation:

(For Female: %BF=495/(1.089733-(0.0009245*s)+(0.0000025*s*s)-(0.0000979*a))-450

Variables: s = sum of 3 skinfold mm, a = age).

Each skinfold thickness was measured in duplicate with Harpenden skinfold caliper (Holtain Ltd, Bryberian, Crymmych, Pembrokeshire). When a difference between the first and the second measurement exceeded 6 mm, a third measurement was taken. Three site, were including Triceps, Suprailiac and Quadriceps.

Quality of life (QoL) measurement: The SF36 (Short Form with 36 questions), scoring system with 36 items, is a self-administered questionnaire. It consists of 36 questions, 35 of which are compressed into eight multi-item scales: (1) physical functioning, (2) physical capabilities, (3) bodily pain, (4) general health, (5) vitality, (6) social functioning, (7) emotional factors and (8) mental health. Hence, in the SF36 scoring system, the scales are assessed quantitatively, each on the basis of answers to two to ten multiple choice questions, and a score between 0 and 100 is then calculated on the basis of well-defined

guidelines, with a higher score indicating a better state of health [39, 40].

Leukocyte telomere length measurement: Blood samples were drawn after an overnight (12 h) fasting in the glass tube containing EDTA and centrifuged for 10 minutes at a speed of 3000 rpm for isolation of peripheral blood mononuclear cells (PBMC). After separation of plasma the residue mixed with phosphate-buffered saline (PBS) with ratio of 3 to 1. Then in a new tube the residue mixed with Ficoll solutions and centrifuged for 20 minutes at 2300 rpm and PBMC isolated by pipette as layer of white clouds. PBMC were washed with PBS two times, then moved in to the micro tubes and froze in -80ºC. DNA samples were extracted from PBMC by salt saturation - proteinase K standard methods [36]. Concentration and quality of the DNA extracted examine by the nanodrop (NanoDrop-2000) device at wavelengths of 260 and 280 nm. Telomere length was measured by quantitative polymerase chain reaction and SYBR® Green PCR master mix kits manufactured by Applied Biosystems, US. Two PCR reactions were performed for each sample: 1. Telomeric DNA fragment replication and 2. Increasing ribosomal acid phosphoprotein (control). Telomere length was calculated based on the ratio of telomeric DNA per control DNA. All the procedure for Leukocyte telomere length measurement was performed in laboratory of Razi vaccine & serum research institute of Iran and the laboratory of research institute for endocrine science of Shahid Beheshti University of medical sciences of Iran.

Statistical analyses

Statistical analyses were carried out using IBM SPSS version 21.0 (IBM Ltd, Armonk, New York, USA). Data has been reported as means ± standard deviations (SD). Variance differences were tested using Kolmogorov Simonov test. Means differences between groups in each factor measured by T independent analysis test. Means differences between baseline and endpoint of study for any factor in each groups, were compared by T paired analysis test. Longitudinal changes between groups in each factor were tested by using T independent analysis test between means different of baseline and endpoint data. $P<0.05$ were considered statistically significant.

Results

Baseline characteristics: Thirty six elderly women, 60–65 years, were participate in this study, green tea group (n=18) with mean ages=61.67±0.6 and placebo group (n=18) with mean ages=63.89±0.9. Base line characteristic include weight, height, BMI and % Body fat (BF) are shown in the Table 1. There were no significant differences in terms of any factor between groups. In this article intended $P<0.05$ and statistical power=0.80.

Leukocyte telomere length: There were significant increase on leukocyte telomere length in green tea group (P=0.004, Effect size= 0.664) compared with baseline and also compared with placebo group (P=0.041, Effect size= 0.552) at the endpoint Figure 1.

Quality of life (QoL**):** No significant interaction effect between aerobic training with (p=0.893) or without (P=0.562) green tea extracts on QoL were observed, with no different between groups (P=0.574). However Figure 2 showed insignificant increase on QoL in green tea group compared with placebo group.

Body composition: Body composition was not significantly changed in both groups, except WHR which was significantly decrease in two groups compared with baseline (P=0.003 (Effect size= 0.853) for green tea and P=0.028 (Effect size= 0.540) for placebo group). Also WHR was significantly decrease in green tea group compared with placebo group (P=016, Effect size= 0.591). Figure 3 at the endpoint.

Table 1- Base line characteristic of elderly women in the exercise alone (placebo) and exercise with green tea consumption (green tea) groups

Factor	Green tea	Placebo	P-value	T
Weight (Kg)	69.56±3.95	74.11±3.9	0.42	0.82
BMI	29.14±1.67	30.56±1.24	0.505	0.682
%BF	30.33±2.01	30.33±1.46	1	0.09
Height (m)	155.11±1.43	155.89±1.82	0.74	0.34
Age (year)	61.67±0.6	63.89±0.9	0.06	2.05
N	18	18		

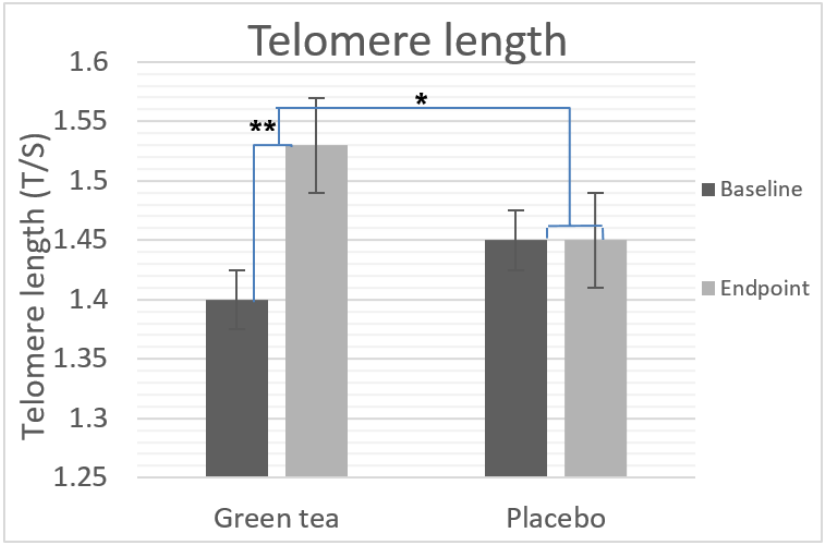


Figure 1- Effect of aerobic exercise (placebo group) alone or with consumption green tea extract (green tea group) on leukocyte telomere length in elderly women. Note. There was significant increase on leukocyte telomere length in green tea group and compared with placebo group. *= Significant with P <0.05 and **= Significant with P <0.05

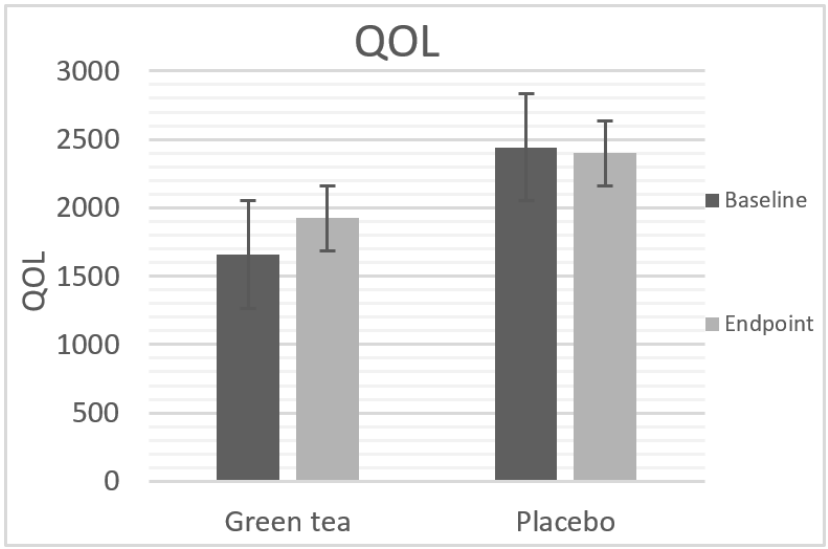
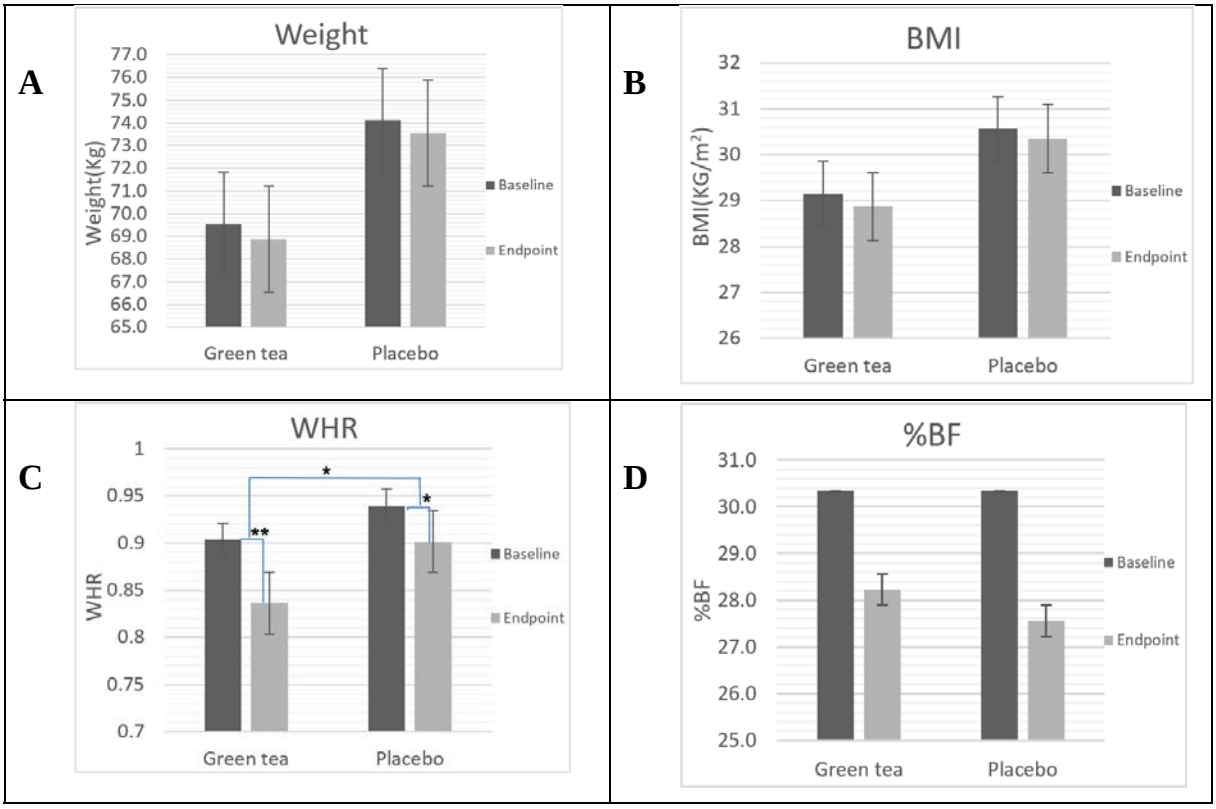


Figure 2- Effect of aerobic exercise (placebo group) alone or with consumption green tea extract (green tea group) on Quality of life (QoL) in elderly women. Note. No significant interaction effect showed between groups and between baseline and endpoint data in each groups. P-values <0.05 were considered statistically significant.



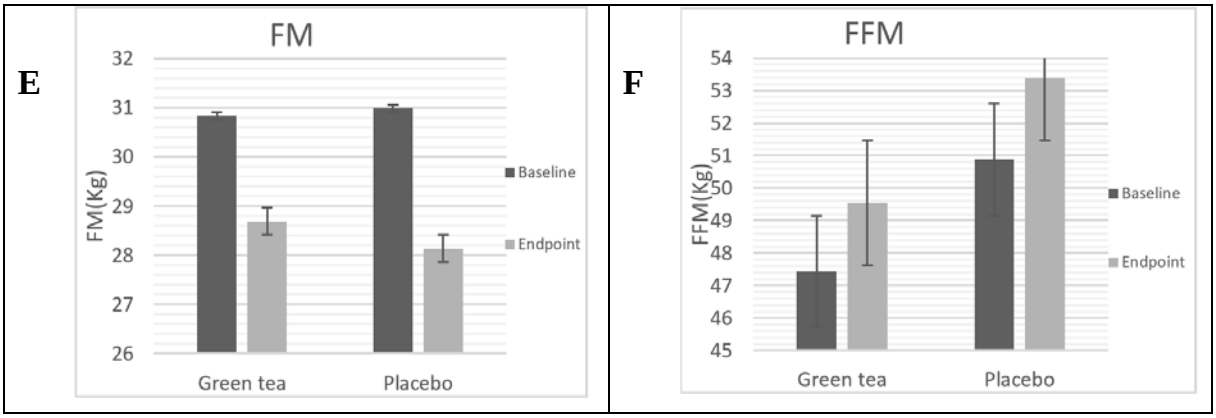


Figure 3- Effect of aerobic exercise (placebo group) alone or with consumption green tea extract (green tea group) on body composition in elderly women. A: Effect on weight. B: Effect on BMI (Body mass index). C: Effect on WHR (Waist–hip ratio). D: Effect on %BF (percent of body fat). E: Effect on FM (Fat mass). F: Effect on FFM (Fat free mass). Note. P <0.05 were considered statistically significant (*), except in WHR of green tea group that considered P <0.01(**).

Discussion

The present study suggest that aerobic exercise training combined with consumption of green tea extract significantly increase leukocyte telomere length in elderly women in a period of five months. But aerobic exercise training alone did not significantly changed leukocyte telomere length. In relation to our study Mundstock et al. (2015) [14] in a systematic review have investigated the relationship between physical activity (PA) and telomere length. In that article over half of the studies had founded no relationship between PA and telomere length, and almost a third had a positive association. However, correlations were mostly weak or moderate, with only two studies showing a strong association. In the first study conducted in post-menopause women, 73% of changes in telomere length attributed to PA [29]. In the second study the 11% increase in telomeres length were observed in the exercise group [18]. Only in two studies a "U" inverted association between PA and telomere length were reported [4, 17]. However these controversy results may be due to exercise duration, exercise intensity and cell type of the studies [17, 41].

With regard to positive effects of green tea consumption combined with aerobic exercise training on telomere length observed in our study Sheng et al. in 2010 [33] and 2013 [34] reported green tea extract or it's active ingredient (EGCG) had positive effects on telomere length or it's related signaling pathway.

The present study also suggests that combination aerobic training with green tea extract consumption had no effects on QoL. Our finding is in the favor of some studies [42], and inconsistent with other studies [43].

In the present study the combination aerobic training with green tea extract consumption has positive effect on quality of life, but was not in the range of the significant. This insignificant may be due to low number of participant.

Body composition was not changed in both groups in present study, but WHR was significantly decreases in two groups and surprisingly it was significantly decreases in green tea group compared with placebo. It means combination had synergistic effects on WHR. WHR is the most common proxy measures of visceral adipose tissue (VAT), as they demonstrate a strong correlation with increased risk of numerous health outcomes as well as mortality in the majority of populations especially elderly [44]. However exercise training and green tea catechins may influence specific signaling molecules, including PGC1α, leading to gene expression of fat metabolism enzymes [25, 45] and this mechanisms are strategies to enhance fat oxidation and body composition to the general population [25].

Of note the main limitation of the study was low number of participant, lack of another group taking only green tea extract and selection of one gender in the study. Overall the results of the present study showed that consumption green tea extract with aerobic training has significant positive effects on health outcomes particularly in elderly.

Conclusion

This study showed that exercise training with consumption of green tea extract has positive and synergetic effect on health and related aging factors such as telomere length and WHR elderly women. Further study with larger number of participant of both gender for longer time is suggested.

Conflict of Interest

The authors have declared that there is no conflict of interest.

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