Effect of Endurance Training and Hydroalcoholic Extract of Black Tea on Bone Mass in Male Rats

Abstract

Background & Aim: Tea consumption and exercise loading may have favorable effects on the risk of osteoporosis. However, the effects of tea plus exercise training on bone mineral density are not clear. The present study aimed to clarify the effects of a combined treatment of tea extract plus treadmill exercise on bone mineral density in middle-aged rats.

Material and Methods: Forty 14-week-old male Wistar rats were divided into four groups randomly, including control, sham, exercise training, and tea plus exercise training. Rats in the exercise groups participated in a running exercise program (1 h/day, 5 times/week for 8 weeks). In addition, mg/kg of tea extract was intraperitoneally injected to animals, and body composition and bone mineral density of the whole body were assessed by dual-energy X-ray absorptiometry at baseline following the intervention. Data analysis was performed in SPSS using ANOVA and post hoc Tukey test.

Results: Running on treadmill significantly increased femur bone mineral density in the samples of the exercise groups, compared to the control group (P=0.04). On the other hand, tea plus exercise increased bone mineral density in the whole body (P=0.01) and femur (P=0.03) of middle-aged rats.

Conclusion: According to the results of the study, tea enhanced the bone response to mechanical loading and a synergistic effect was observed in the samples.

Keywords: Mechanical Load, Bone, Rat, Tea

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Introduction

Increased life expectancy and aging of the world’s population have made osteoporosis a serious public health concern, affecting millions of people, especially the elderly and postmenopausal women. Osteoporosis is a chronic metabolic bone disorder characterized by reduced bone mineral density (BMD) and destruction of bone microarchitecture, which can directly increase the risk of fracture and skeletal fragility. According to the reports, one in three women and one in five men might experience bone fractures during their lifetime. Fragile fractures seriously affect people’s daily activities and quality of life and are closely associated with increased mortality in patients with osteoporosis. In general, osteoporosis may involve multiple endocrine, nutritional, and genetic factors. There are many medications available to treat osteoporosis; nevertheless, they exert negative impacts on humans’ health. Given the aging of the world’s population, it is extremely crucial to find preventive solutions for osteoporosis instead of treatment in order to improve people’s health and reduce medical expenses. Interestingly, studies have shown that dietary approaches such as the consumption of tea and its chemical components can act as an effective preventative measure in maintaining bone homeostasis.
The second most consumed beverage in the world, tea is prepared from the green leaves of Camellia sinensis. Iranian black tea has antioxidant and anticancer properties owing to its components such as vitamin C, beta-carotene, tannins, epigallocatechin, and epigallocatechin gallate (EGCG) and phenolic compounds (e.g., flavonols, flavonoids and theaflavin). Furthermore, black tea contains important alkaloids such as caffeine and theine, which have sedative and nerve-strengthening properties. Even though 80% of the world consume black tea, most studies have been conducted on green tea. Since tea ingredients are different in various regions depending on the season, weather, age, and the production process, it seems vital to determine the effects of black tea on health. Animal and human lab experiments have strongly supported the protective effects of tea on bone health and its possible role in the decrease of osteoporosis\(^5,\ 6\). It is well established that excessive osteoclast activity and inappropriate osteoblastic activity can lead to osteoporosis\(^3\). Moreover, evidence shows that green and black tea extracts can suppress osteoclastic activity in vitro and reduce menopausal osteoporosis in rats\(^7\). Furthermore, the chemical components of tea (e.g., polyphenols) can exert valuable effects on bone health through improving the differentiation of osteoblasts or inhibition of differentiation and activity of osteoblasts\(^8\).

Similarly, exercise has been reported to have a positive effect on bone morphology and the mechanical load caused by physical activity helps maintain and improve bone mass and strength\(^9\). Additionally, studies have suggested that osteoblasts and osteoclasts sense and respond to mechanical stimuli, and mechanical stimulation increases the osteoblastic secretion of alkaline phosphatase, nitric oxide, and prostaglandin E2, thereby regulating the activation of runt-related transcription factor 2\(^10,\ 11\). Furthermore, mechanical stress has been reported to inhibit osteoclast differentiation and bone resorption activity\(^12\). Even though several studies have emphasized the stimulating effect of exercise on bone tissue\(^10-\ 12\), it is less suggested as an alternative to medical treatment. Moreover, quantitative studies have evaluated the combined effects of physical activity and nutrition on the prevention and treatment of osteoporosis. With this background in mind, the present study aimed to evaluate the effect of a course of treatment with tea extract along with exercise (i.e., running on a treadmill) on bone mineral density in male rats.

**Method**

In total, 40 14-week-old male Wistar rats with a mean weight of 277.1±0.2 gr at baseline were purchased from Pasteur Institute and were kept in standard conditions (temperature of 21±2 degrees and 12-12 light cycle) for two weeks to adapt to the situation. The rats were randomly divided into four groups of control, sham, aerobic exercise, and tea plus aerobic exercise. Notably, all animals had free access to a standard commercial diet and water. In addition, the animals were allowed to roam freely in the cage. It is worth mentioning that the researchers in the present study adhered to ethics in animal experimentation, and the research was confirmed by the research council of the University of Arak with the code of 97/951136.

**Exercise:** rats allocated to the groups of exercise and tea plus exercise performed aerobic activity on a treadmill (five channels and model 11, Danesh Salar Iranian Co., Iran) with a 5% incline five days a week for eight weeks. The animals were familiarized with the protocol of running on a treadmill over a one-week course, during which the speed increased from 10 to 20 m per minute gradually. In addition, the duration of each session increased from 10 to 60 minutes. For the remaining days, rats ran on a treadmill for 60 minutes at a speed rate of 22-24 m/min in each session\(^13\).
Tea extract consumption: first, 600 gr of tea was purchased from tea gardens in Fuman and ground using an electrical mill. In the next stage, the tea was mixed with 70 liters of 70% ethanol, and the solution was passed through a filter paper after 48 hours, followed preparing an injectable tea extract through the rotary device. At this stage, the tea extract was mixed with 50 mg of physiological serum and 0.3 ml of normal saline. Ultimately, 0.3 ml of the solution was injected into rats intraperitoneally\(^{(14)}\).

Evaluation of dual-energy X-ray absorption: bone mineral density of the lumbar vertebrae (L4-L5), femur, and whole body were measured in g/cm\(^2\) by the dual-energy X-ray absorptiometry (Discovery, Hologic, Bedford, USA) (DEXA). All photographs and analyzes were performed by one person, and the densitometer was calibrated weekly through appropriate phantoms. Fat and fat-free mass was evaluated by whole-body images using DEXA device and special software for small animals. All of these parameters were measured before and 48 hours after the exercise\(^{(15)}\).

Data Analysis
The results were presented as mean and standard deviation. Data analysis was performed in SPSS version 22 using the Shapiro-Wilk test (to ensure the normal distribution of the data), Levene’s test (to evaluate the equality of variances), one-way ANOVA, Tukey post hoc test, and Bonferroni test (to compare the groups). It is notable that the \(P \leq 0.05\) was considered statistically significant.

Results
Table 1 presents the average statistical indicators and standard deviation of the body composition of rats studied in grams. After eight weeks of intervention, there was no significant difference between the groups in terms of weight (\(P=0.17\)), fat mass (\(P=0.21\)), fat-free mass (\(P=0.34\)), and fat percentage (\(P=0.19\)) (Table 1). Evaluation of the whole-body bone mineral density revealed a significant difference between the groups (\(F=3.31, P=0.02\)). In this regard, the Tukey’s test results demonstrated a significant difference between the control (\(P=0.03\)) and sham (\(P=0.01\)) groups with the tea plus exercise group (Table 2). Furthermore, evaluation of femur bone mineral density in various groups demonstrated a significant difference among them (\(F=4.48, P=0.01\)). According to the results, there was a significant difference between the control (\(P=0.04\)) and sham (\(P=0.03\)) groups with the exercise group and between the control (\(P=0.02\)) and sham (\(P=0.01\)) groups with the tea plus exercise group. On the other hand, no significant difference was observed between the exercise and the tea plus exercise groups in this respect (\(P=0.34\)) (Table 2). In addition, a comparison of the bone mineral density of the lumbar vertebrae showed an insignificant difference among different research groups (\(F=1.12, P=0.41\)).

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<th>Table 1. Body composition in the research groups</th>
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<th>Table 2. Bone mineral density (g/cm(^2)) in the research groups</th>
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Exercise training and bone mass

*Shows a significant difference (P<0.05) between the control and sham groups. The amounts are presented as mean±standard deviation. Intergroup comparison by one-way ANOVA and Tukey’s test.

Discussion

A limited number of animal studies have evaluated the effect of exercise on bone mineral density. For the first, the present study showed that adding tea extract consumption to exercise increased the positive effects of each method. Osteoblasts and osteoclasts have been confirmed to sense and respond to mechanical stimuli (11). Our findings and the results of previous studies match the frost theory about mechanical loads and adaptive bone responses. According to Frost, mechanical loads can appear as tension and force on the bone, leading to bone formation and construction. Mechanical forces and traction control the synthesis and regeneration of bones and therefore affect bone mass (16). Studies show that proper mechanical stimulation increases the proliferation and differentiation of osteoblasts and prevents the differentiation and bone re-absorption activity by osteoclasts (10). According to the previous studies, exercise can increase bone calcium uptake and depletion and significantly improve estradiol and testosterone levels in the body (17). Nonetheless, a limited number of animal studies have evaluated the effect of exercise on bone status (10).

One of the most important findings in the current research was the positive effect of endurance training on bone mineral density in rats. As observed, femur bone mineral density increased in the animals of the exercise group, compared to the control and sham groups. In line with our findings, Boudenot et al. (2016) reported that 10 weeks of endurance training (one hour per day, five days a week) increased whole-body and femur bone mineral density in eight-week-old rats (18). However, Chen et al. (2004) proposed that only four weeks of high-intensity interval training (HIIT) (25 m/min, one hour a day, five days a week) was sufficient for increasing bone mineral density in rats (19). Therefore, it seems that intensity could be a key factor for bone response to exercise. In fact, human and animal studies have suggested a linear correlation between the speed of running and the increase of bone mineral density (11, 17, 19). Consistent with some human (20) and animal (21) studies, changes in bone mineral density and adaptation in the lumbar vertebrae occur later than in other bones in the current research. As such, a long-term exercise program has been suggested in order to increase bone mineral density in the lumbar vertebrae (in 12-week-old rats) (11).

Therefore, the increase in lumbar bone mineral density has been confirmed in such studies that included subjects with a relatively low level of bone mineral density (e.g., the elderly or rats with removed ovaries) (10, 11). On the other hand, in most studies, including the present study, which reported no change in bone mineral density in the lumbar vertebrae after exercise, DEXA imaging of the lumbar vertebrae of 2-4 (in humans) and 4-5 (in rats) was carried out. Given the biomechanical load distribution difference between vertebrae (22), bone mineral density varies between lumbar vertebrae. For example, in lumbar vertebrae 1, the bone mineral density is lower and the response to exercise is faster, and higher lumbar vertebrae adapt to exercise later than other parts (11, 22). As such, the lack of change in lumbar bone mineral density in the present study may be related to these factors, which require further research and considering these issues.

On the other hand, tea is one of the most popular drinks in the world and contains antioxidants that have protective effects on human health (2). Tea has been reported to have a protective effect on cardiovascular disease, Parkinson’s disease, and various types of cancer (23). In a review study encompassing two cohort studies, 11 case-control studies, and four cross-sectional studies that assessed
107819 individuals, drinking tea was reported to significantly reduce the risk of osteoporosis \cite{24}. In case-control research, Huang et al. indicated a significant relationship between drinking tea and hip and femur fractures in middle-aged and old men \cite{25}. However, some studies have suggested that tea polyphenols are not associated with an overall improvement in BMD in overweight/obese postmenopausal women \cite{26}. Moreover, several animal studies have shown that tea polyphenols are associated with increased femur bone mineral density and lumbar vertebrae in female rats \cite{23, 27}. Nonetheless, some studies have demonstrated a lack of effect of tea on animals’ bone mineral density. For instance, Minematsu et al. found a reverse correlation between tea consumption and bone mineral density \cite{28}. This lack of consistency between the results might be due to different ages (effectiveness is higher in higher ages) and gender (increase is higher in females) of samples. On the other hand, the amount of tea consumed is also important because low or high tea intake is associated with reduced or no effect on bone mineral density, while moderate consumption of tea is likely to improve bone mineral density. Furthermore, the type of tea also seems to be important in affecting bone mineral density, as increased bone mineral density is more common after consuming green and black tea. EGCG is the most abundant tea polyphenol and the main tea component. The effects of epigallocatechin have been extensively investigated, and evidence suggests that its beneficial effects occur through increased alkaline phosphate activity and increased expression and protein levels of osteoblasts \cite{29}. In addition, tea polyphenols significantly enhance osteoblastic survival and reduce osteoblastic apoptosis, thereby increasing cell proliferation and differentiation \cite{30}. Moreover, the EGCG in tea contributes to ossification through the decrease of metalloproteinase-2/9 \cite{29}. Tea is also an antioxidant that suppresses the production of pre-inflammatory cytokines, which results in increased bone mineral density in rats with weak bones \cite{31}. In fact, these results support the positive effect of tea on bone mineral density improvement consistent with our findings.

**Conclusion**

According to the results of the present study, this type of exercise prevents the deteriorating effects of aging on bone mineral density, and adding tea to this exercise program was likely to double the positive effects.

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**Authors’ Contribution**

All authors met the writing standards based on the recommendations of the International Committee of Medical Journal Publishers.

**Conflicts of Interest**

None declared.

**References**