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High-Intensity Interval Training Effects with Genistein on Serum Osteocalcin and Bone Alkaline Phosphatase in Female Elderly Rats

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ABSTRACT

Introduction: Exercise and nutrition can be two factors influencing bone metabolism in old age. The present study aimed to investigate the effects of high-intensity interval training (HIIT) with genistein (Ge) on serum levels of osteocalcin (OCN) and bone alkaline phosphatase (BALP) in female elderly rats.

Methods: In this experimental study, 40 elderly female rats with a mean age of 18-24 months and mean weight of 220.15 ± 15.28 g were divided into five groups of eight rats including 1) control (C), 2) sham (Sh), 3) HIIT, 4) HIIT+Ge, and 5) Ge. During eight weeks, groups 3 and 4 performed HIIT for three sessions per week with an intensity of 90-95% of maximum oxygen consumption (VO2max) in high-intensity intervals and 40-45% of VO2max in low-intensity intervals as well as groups 4 and 5 received 60 mg/kg/day Ge peritoneally. OCN and BALP were measured by the ELISA method.

Results: HIIT significantly increased BALP (P=0.001) and OCN (P=0.04); Ge and HIIT + Ge significantly increased BALP (P=0.001); although Ge had a more favorable effect on increasing BALP compared to HIIT (P=0.001) HIIT had a more favorable effect on increasing OCN compared to Ge (P=0.008).

Conclusion: Although HIIT simultaneously with Ge consumption can increase serum BALP levels in female elderly rats the effects of HIIT and Ge alone on BALP and OCN are different from each other.

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PTH/PTH1R in the first stage. Ge has the potential to prevent and treat osteoporosis in postmenopausal women, which has a high clinical advantage and fewer adverse effects (5). In addition to proper nutrition, exercise with the proper intensity and duration can reduce age-related bone disorders, especially in postmenopausal women. High-intensity interval training (HIIT) is defined as periods of exercise activity that is characterized by fluctuations in the intensity of exercise at a given time (6). HIIT involves the repetition of periods of very intense activity (close to the maximum or ultra-maximum) that are separated by low-intensity or moderate-intensity exercises or, in some cases, complete inactivity (complete rest) (7). In a review article, the effects of various types of exercise on bone tissue and body performances of postmenopausal women were examined, and the results showed that physical activity is an effective stimulant for the treatment and prevention of osteoporosis (8). Osteocalcin (OCN) and alkaline phosphatase (ALP) have been reported to be the most important markers of bone formation. Alkaline phosphatase is an enzyme derived from the bone (bone alkaline phosphatase or BALP) and liver that shows the activity of osteoblasts and bone formation (9). Osteopontin and OCN are organic and non-collagenous proteins in bone metabolism (10). In a study, it was found that the combined effect of mechanical stresses with erythropoietin led to an increase in proliferation, and osteogenic differentiation in BMSCs, as well as an increase in ALP and mineral deposition (11). It has been reported that ALP levels significantly increased with very intense interval training (12). In another study, exercise significantly increased OCN in young men (13) also the effects of walking on bone biochemical markers in women aged 30-70 years were studied, and the results showed that OCN was not significantly different between premenopausal and postmenopausal women (14). Another study investigated the short-term effects of exercise on young men and showed an increase in OCN (13). Considering the contradictory results of the above researches in this field and also that osteoporosis is one of the common diseases in old age as well as the lack of studies on the interactive effects of HIIT and Ge consumption, the present study aimed to investigate the effect of HIIT with Ge administration on serum levels of OCN and BALP in female elderly rats.

Materials and Methods
In this experimental study, 40 Sprague Dawley rats with a mean age of 18-24 months and weight of 220.15 ± 15.28 g were purchased and kept in an animal laboratory (with humidity of 45 to 55%, a dark-light cycle of 12-12 hours and temperature of 23 ± 2 °C). To adapt with new environment, the rats were kept for one week under standard conditions and free access to food (including crude protein 23%, crude fat 3.5%- 4.5%, crude fiber 4%- 4.5%, ash maximum 10%, calcium 0.95%- 1%, phosphorus 0.65%- 0.75%, salt 5%- 5.5%, humidity maximum 10%, lysine 1.15%, methionine 0.33%, methionine + cysteine 0.63%, threonine 0.72%, and tryptophan 0.25%) and water. Then rats randomly divided into five groups of eight rats including: 1) control (C), 2) sham (dimethyl sulfoxide (DMSO) + normal saline) (Sh), 3) HIIT, 4) HIIT + Ge, and 5) Ge. During eight weeks, groups 3 and 4 performed HIIT for three sessions per week with an intensity of 90-95% of maximum oxygen consumption (V02max) in high-intensity intervals and 40-45% of V02max in low-intensity intervals (15) as well as groups 4 and 5 received 60 mg/kg/day Ge dissolved in DMSO + normal saline peritoneally (16). Forty-eight hours after the last training session and Ge administration rats were anesthetized with ketamine (50mg/kg) and xylazine (10mg/kg). After blood sampling and transferring to the laboratory; BALP and OCN were measured by the ELISA method.

HIIT protocol
HIIT performed three sessions per week on rodent treadmill for eight weeks. The main training was as follow: based on the calculated aerobic power at the beginning of each week, the rats performed nine 2-minute high-intensity intervals with an intensity of 90-95% of V02max along with 1-minute active recovery intervals with an intensity of 50% of V02max. It should be noted that at the beginning and end of the training program, 4 minutes of warm-up and
cool down with an intensity of 45- 55% of \( V_{O_2}\text{max} \) was added to the time of the main training (15).

**Ge administration**

To prepare Ge, at first Ge (Sigma Aldrich; with economic code of CAS NO: 446-72-0 and BACH NO: 20150605) was dissolved in DMSO and then diluted using normal saline and 60 mg/kg/day was injected intraperitoneally (16).

**Aerobic power**

To evaluate the aerobic power, at first, the rats warmed up for five minutes on a treadmill at speed of six meters per minute with a slope of zero degrees, then every three minutes, the speed increased by three meters per minute until the animals reached exhaustion and could no longer continue. The criterion for reaching \( V_{O_2}\text{max} \) was the inability of the rats to continue the training protocol and three consecutive collisions (within one minute) to the end of the treadmill, so the \( V_{O_2}\text{max} \) was obtained by using the running speed (15).

**BALP and OCN measurement method**

The serum samples were collected after centrifugation and stored in the minus 80°C freezer. The experimental method was conducted according to the ELISA kits instructions of OCN and BALP (OCN and BALP; Hangzhou Eastbiopharm Co., Ltd., Hangzhou, China). In brief, the rat samples were added into a 96-well plate to reaction with the capture antibody and incubated for 60 minutes at room temperature. After washing the 96-well plate, chromogenic solutions added. Then, add the stop solution to stop the reaction. Finally, the optical density (OD) was read at 450 nm by the ELISA reader by using a FLUOstar OMEGA microplate reader and software (BMG Labtech Ltd., Aylesbury, UK). The OCN and BALP protein concentrations (U/L) were assessed compared with a standard curve.

**Statistical Analysis**

Shapiro-Wilk test was used for investigation of the normal distribution of data and one-way ANOVA with Tukey's post-hoc tests were used for the analysis of data in Graph pad PRISM 8.3.0 software (P≤0.05).

**Results**

BALP and OCN levels are presented in Figures 1 and 2, respectively. The results of one-way ANOVA test showed that there were significant differences in BALP (P=0.001) and OCN (P=0.005) levels in five groups of research. The results of Tukey's post-hoc test showed that there were no significant differences in BALP levels between C and Sh groups (P=0.41); nevertheless, BALP levels in HIIT, Ge, and HIIT+Ge groups were significantly higher than C group (P=0.001) and in Ge group were significantly higher than HIIT and HIIT+Ge groups (P=0.001) (Figure 1).

![Figure 1](image1.png)

**Figure 1.** BALP levels in five groups of research

*** P≤0.001 Significant increase compared to C group; +++ P≤0.001 Significant increase compared to HIIT and HIIT+Ge groups

(C: control; Sh: sham; Ge: genistein; HIIT: high-intensity interval training)
The results of Tukey’s post-hoc test showed that there were no significant differences in OCN levels between C and Sh groups (P=0.98); nevertheless, OCN levels in the HIIT group were significantly higher than C (P=0.04) and Ge (P=0.008) groups (Figure 2).

![Figure 2. OCN levels in five groups of research](image)

* P≤0.05 Significant increase compared to C group; ++ P≤0.01 Significant increase compared to Ge group
(C: control; Sh: sham; Ge: genistein; HIIT: high-intensity interval training)

**Discussion**

The results of the present study showed that HIIT significantly increased serum BALP and OCN levels in female elderly rats. According to existing theories in the regulation of bone cell homeostasis, one of the most important theories is mechanical pressure. In other words, researchers believe that mechanical stress following exercise can increase bone calcium/phosphate levels and lead to the formation of bone salts, and increase hydroxyapatite levels as well as by activating oxygen-dependent pathways, it reduces the activity of osteoclasts and increases the activity of osteoblasts also produces BALP and OCN. It also causes the synthesis of bone collagen; also, the exercises lead to the bone formation by increasing anti-inflammatory factors and reducing inflammatory factors (17). Besides, exercises increase the activity of osteoblasts by increasing the Wnt signal pathway and release of sclerostin; therefore exercises can increase the BALP and OCN (18). In this regard, 12 weeks of walking and stepping significantly increased OCN and type 1 collagen (CTX-1) in women with osteoporosis; also stepping training had a more favorable effect on increasing CTX-1 compared to walking training (19); as well as, higher physical activity per day was positively associated with increased OCN levels, but no association was observed between physical activity and BALP (20). Also, gene expression levels of ALP and parathyroid hormone increased significantly after 10 weeks of walking training and low intensity-running in sedentary postmenopausal women, while serum levels of ALP and calcium did not change significantly (21). It seems that the changes in these biomarkers depend on the type and intensity of exercise, the basic levels of these variables, the method of measurement, and the duration of the training. Researchers have shown that resistance training and walking did not have a significant effect on BALP levels in patients with osteoporosis, but resistance training significantly increased sclerostin, which improved bone metabolism (18); also, 10 weeks of aerobic training did not have a significant effect on BALP and OCN changes in patients with type 2 diabetes (22).

The present study showed that Ge administration significantly increased serum levels of BALP in female elderly rats but did not increase OCN levels. Studies have shown that soy and its isoflavones, such as Ge and daidzin, due to their estrogenic-like effects, can increase bone density and minerals by increasing insulin-like growth factor-1 (IGF-1) levels and its receptors. It has been reported that Ge inhibits translation of estrogen receptor alpha (ERα), topoisomerase type I and II as well as 17β-
hydroxysteroid dehydrogenases via binding to estrogen receptor beta (ERβ). Continuing this process increases the transcription of runt-related transcription factor 2 (Runx2) and increases the expression of mRNA BALP and OCN in bone tissue (23). In this regard, 40 mg soy or casein consumption for three months significantly increased IGF-1 levels, improved lipid profile, C-reactive protein (CRP), and increased Runx2 gene expression in men and women aged 27-87 years (23). Ge consumption can increase the activity of osteoblasts by the mechanism of MAPK and PI3K, as well as by increasing the levels of ER and nitric oxide synthase; it increases the activity of ALP and eventually osteoblastogenesis (24). Ge consumption enhanced RANKL/OPG pathway, ALP, and OCN in ovariectomized rats (25). In line with the results of the present study, some researchers have shown that soy and its isoflavones increase the ALP levels but have no significant effect on OCN levels and some other metabolic markers (26). In addition, 4, 8, and 12 weeks of 20 mg/day Ge consumption reduced menopausal symptoms in postmenopausal women (27).

Regarding interactive effects in the present study HIIT+Ge significantly increased serum levels of BALP in female elderly rats nevertheless did not increase OCN levels also the effects of Ge alone on increasing serum BALP levels were higher than HIIT and HIIT+Ge. Studies have shown that the consumption of minerals such as calcium, vitamin D, vitamin K, estrogens, and isoflavones as well as an exercise by improving bone metabolism (with an increase of osteoblasts) can increase bone density, mass, and strength (28). In this regard 600 mg/kg Ge consumption along with moderate-intensity endurance training increased bone strength density in obese rats (29). Evidence suggests that exercises increase the osteoblast activity, BALP, and OCN levels by an increase of calcium/phosphate, hydroxyapatite, collagen synthesis (17), and Wnt signaling pathway as well as decrease of LRP5 activity (18). Also, Ge increases bone density and minerals by estrogen-like effects, increasing IGF-1 levels, and its receptors, antioxidant effects, and increasing the transcription of the Runx2 gene (23). Therefore, it seems that exercise and Ge with different mechanisms can improve bone metabolism. Enabling to measure bone mineral mass and bone strength were the research limitations of the present study, therefore it is recommended to study the mentioned variables in future studies. It is also recommended to study the gene expression levels of estrogen receptors, RANKL/OPG, BALP, and OCN gene expression pathways in future studies.

**Conclusion**

HIIT and Ge consumption appears to improve serum bone resorption markers with different mechanisms. Although HIIT simultaneously with Ge consumption can increase serum BALP levels in female elderly rats the effects of HIIT and Ge alone on BALP and OCN are different from each other.

**References**


