Original Article

The Effects of 8-Week Resistance and Endurance Trainings on Bone Strength Compared to Irisin Injection Protocol in Mice

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Abstract

Background: Osteoporosis is a prevalent elderly complication that is characterized by decreased bone mineral density and increased fracture risk because of dysregulation in bone mineralization and resorption. Physical activity can enhance bone strength by exerting mechanical forces and myokines. Irisin is a myokine that is increased following physical exercise and can affect bones. In this study, 8 weeks of resistance and endurance exercises are applied in mice compared to irisin injection to assess the contribution of the protocols and this myokine to bone strength. **Materials and Methods:** Thirty-five male NMRI mice were separated into five groups; control, placebo, irisin injection, resistance exercise, and endurance exercise. 8-week of exercise protocols and irisin injection protocol (100 μg/kg/week) was applied. Plasma irisin concentration and bone strength were measured using enzyme-linked immunoassay and 3-point bending assay, respectively. Statistical analyses were done through one-way ANOVA and Tukey test, and $P < 0.05$ was considered the significant difference. **Results:** Serum irisin concentration and bone strength in resistance exercise and irisin-injected groups were significantly higher than control and placebo groups ($P < 0.0001$). Serum irisin concentration, but not bone strength, of the endurance exercise group was also significantly higher than control and placebo groups ($P < 0.0001$) but lower than resistance and irisin-injected groups. **Conclusion:** Resistance exercise and irisin injection, but not endurance exercise, are likely to be effective in increasing bone strength. There may be a threshold for plasma irisin level to affect bones which the applied protocols of irisin injection and resistance exercise but not endurance exercise can reach.

Keywords: *Bone mineral density, exercise tolerance, osteoporosis, resistance training*

Introduction

Osteoporosis is marked by fracture risk because of decreasing bone mass and hardness after metabolic balance disturbance and decreasing bone mineral density (BMD).[1,2] It becomes a global problem, and a dramatic increase is expected in the decades ahead, affecting approximately 319 million people by 2040.[3] Disuse and the decrease of physical activities, Vitamin D deficiency, chronic treatment by glucocorticoids, and postmenopause hormone alterations can result in severe and progressive loss of bone strength and osteoporosis.[4-7]

Different treatment strategies are recommended to confront this complication. The prevention strategies such as limiting lifestyle risk factors such as alcohol consumption, smoking, calcium and Vitamin D malnutrition, and receiving adequate supportive physical activity are the priority of public health in many countries. Physical exercise is a widely used treatment strategy not only to prevent osteoporosis but also to improve a variety of age-related diseases.[8-11]

It seems that different characteristics of an exercise such as modality, intensity, duration, number of bouts, and mechanical force intensity have various beneficial effects on the body. Many attempts have been accomplished to uncover the impact of different exercise protocols on bone parameters in animals and humans. The results show the beneficial effects of the various protocols of resistance and endurance exercises on bone density and strength. Shiguemoto *et al*. assessed the biomechanical features of bones after resistance exercise in ovariectomized rats. A significant increase in BMD was observed in exercise-treated groups compared to the control group.[12] The

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effect of continuous supervised resistance training suggests the beneficial effects of this training on fracture prevention in osteoporotic women.[13] Iwamoto *et al*. have shown the volume enhancement of the proximal and distal tibial metaphysis after a 12-week endurance exercise program.[14]

There is some evidence highlighting muscle and bone crosstalk to mediate the events by which physical exercise maintains the bone mass. It is assumed that physical exercise affects bones via mechanical force-activated signals or endocrine regulation.[15-17]

Irisin secretion after physical exercise is a candidate factor to mediate direct muscle and bone endocrine crosstalk.[18] Irisin was first evidenced to be an inducer of osteoblast proliferation, differentiation, and the formation of mineralized nodules *in vitro*.[19,20] Irisin effects on bone were analyzed by assessment of osteogenesis and expression of bone maintenance genes.[21] Similar to the effects of mechanical loading forces, irisin increases osteopontin (OPN) expression[22,23] and decreases sclerostin (SOST) expression.[24,25]

There are also direct *in vitro* and *in vivo* reports that confirm the irisin effects on bone structure.[26] Some studies have displayed an association between serum irisin level and osteoporotic fractures.[24,27] Colaianni *et al*. reported positive correlation of irisin with BMD in a cohort study in older adult patients.[28] When a low dose of recombinant irisin (r-irisin) was injected into the mouse, cortical bone mass and strength were increased.[25] Irisin administration reverses androgen deficiency-induced trabecular BMD decrease in mice.[29]

All of the *in vivo* studies around the effects of irisin on the bone, as a mediator of exercise, have compared the results to the reports from other studies, and there is no direct comparison of irisin effects with a specific exercise protocol. To assess the irisin mediation in beneficial effects of exercise on bones, we directly examined the effects of two main exercise modes (resistance exercise and endurance exercise) on femur strength compared to an irisin-injected group in mice. Moreover, a few studies have used direct examinations for strength effects of irisin or exercise on the bone. In this study, we used a direct method (bending load) to assess bone strength after exercise and irisin-injection treatments.

Materials and Methods

Animal handling

According to Bennell *et al*.'s study, which reported the beneficial effects of exercises on the bones in young mice, not in older ones, we used young 5-week-old male mice.[30] Thirty-five male NMRI mice (weight 18 ± 2 g) were purchased from Pasteur Institute of Iran and kept in standard condition (12:12 h daily cycle, 23°C ± 1°C temperature, and 50% ± 3% humidity) for 2 weeks of adaptation (when they were 5-week old) before they were joined to the study. Every seven ones were assigned randomly into one of five groups consisting of control, placebo, resistance and endurance exercises, and irisin treatment. A specialized individual took care of animals before and during the study. The study was approved by the Ethical Committee of the Isfahan University of Medical Science for animal studies.

Resistance exercise protocol

The first exercise group was adapted with a specific resistance protocol. The training protocol was based on a previously reported one that was suggested as a highly suitable model for the investigation of hypertrophy mechanisms.[31,32] Briefly, a 1 m ladder inclined at 80° was used for climbing of mice while weights were attached to their tail. The weights were gradually increased throughout the 8 weeks of training from initially 30% of their body weight to 200% in the final week [Table 1]. The exercise schedule was designed as three sets of five repetitions for every single bout which was performed 3 times a week for totally 8 weeks. 1 min rest was considered for repetition and 3 min between the sets.[33]

Endurance exercise protocol

The endurance exercise program was designed by the treadmill (Exer6M, Columbus Instruments) running, which was performed 3 times a week for 8 weeks. In the 1st and the 2nd week, the treadmill incline was set to 0°, and the mice run at the speed of 5 m/min for 10 min. In the 3rd and 4th weeks, the speed was 7 m/min, the incline was 2° incline, and the duration of exercise was 12 min, in the 5th and 6th weeks, the speed was 8 m/min, the incline was 4°, and the duration of exercise was 13 min. Finally, in the last 2 weeks, mice run at the speed of 10 m/min and 5° incline for 15 min.[14,34,35]

Irisin administration protocol

Irisin powder (Phoenix Pharmaceuticals, Canada) was dissolved in 1% dimethyl sulfoxide. Injections were performed intraperitoneal 3 times a week (using 100 μg/kg dose per week for each mouse) for 8 weeks. This dose was previously reported to have an impact on BMD.[25]

Plasma irisin concentration measurement

Blood sample collection in EDTA tubes was done by cardiac puncture, 72 h after the last bout to eliminate the acute effect of training. Samples were centrifuged at 4000 rpm for 15 min, and the supernatant was collected to evaluate irisin level.

Table 1: Weights used during resistance training

Week	1	2	3	4	5	6	7	8
Weight /kg(%)	30	50	80	100	125	151	175	200

Plasma irisin concentration was measured by mouse Irisin ELISA kit (Aviscera Biosciences, Santa Clara, CA, USA).

Bone strength measurement

3-point bending measurement was done using Electromechanical Universal Testing Machine (Walter-Bai, Löhningen, Switzerland). The main application of this machine is measuring the strength of hard body tissues such as bone and tooth and tissue engineering synthetic materials which are going to be substitute tissue. Briefly, mice were sacrificed, and the femur was removed.

The two sides of the excised bone were fixed between machine clamps and the mechanical arm exerts pressure onto the midpoint. The pressure was gradually increased to fracture of the bone. The threshold of force-induced fracture was recorded by the machine.

Statistical analysis

Statistical analysis was performed using SPSS version 20 software (Chicago, IL, USA). Variance analysis was done by one-way ANOVA method. Tukey posttest was used to find different groups.

Results

The results were statistically analyzed, and the test of homogeneity of variance shows that variances in both tests of plasma irisin concentration and bone strength have homogeneity, and there is no variance error. $P < 0.05$ was considered as the significance level.

Plasma irisin concentration

Plasma irisin concentration was measured to assess its alterations after physical training and irisin injection. There is a significant difference between mean plasma irisin concentration in physical training and irisin-injection groups compared to control or placebo groups ($P < 0.0001$). It displays an increase of approximately 45% (6.45 ± 0.78 ng/mL) in the endurance training group, 64% (7.3 ± 0.51 ng/mL) in the resistance training group, and 60% (7.16 ± 0.58 ng/mL) in the irisin injection group compared to the control group. The resistance training group induces the highest plasma irisin concentration increase [Figure 1].

Bone strength evaluation

There is a significant difference between the mean of bone strength in resistance training and irisin-injected groups compared to the control or placebo groups ($P < 0.0001$). It displays an increase of approximately 9% (0.0555 ± 0.00477) in the endurance training group, 22% (0.062 ± 0.01127) in the resistance training group, and 17% (0.059 ± 0.00579) in the irisin-injection group compared to the control group. There is no significant increase in bone strength in the endurance training group compared to the control group and the resistance training group induces the highest increase in bone strength [Figure 2].

Discussion

Physical training has received the most attention to prevent age-related complications such as osteoporosis. Physical exercise can affect bones through signals activated by mechanical forces or secreted muscle myokines. Literature reports have indicated the beneficial effects of irisin on different parts of the body, and it is considered a probable mediator of the exercise beneficial effects. Findings display some beneficial effects of this myokine on bone health.[19,20,24,25,27] In the current study, we used the direct examination of bone strength after resistance exercise and endurance exercise and irisin injection to assess the irisin effect and its contribution to exercise beneficial effects on bone strength.

According to other studies, 8-week protocols are selected for the study groups.[14,30,33,35,36] Colaianni *et al*. observed beneficial effects on mouse BMD by applying 4-week irisin administration, and Zhang *et al*. did the same by 2-week of irisin administration.[37] The injection dose of irisin is selected based on the previously reported minimum dose that induces beneficial effects on bones.[38]

The serum irisin level elevation was first reported by Boström *et al*. after 10 weeks of endurance exercise in healthy obese humans.[39] Many other studies have verified this increase in serum or muscles following physical training. For instance, Iwamoto *et al*. have shown that treadmill exercise can increase serum irisin level,[14,40]

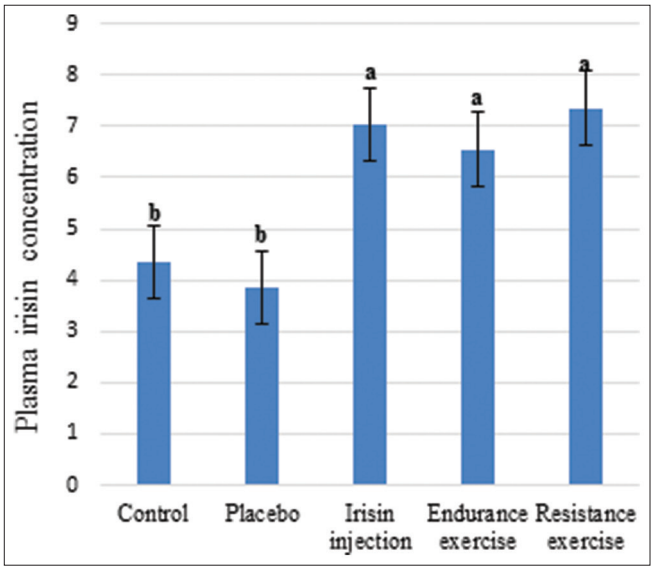

Figure 1: Serum irisin concentration (mean) in 5 groups of control, placebo, irisin-injection, endurance exercise, and resistance exercise. There is a significant difference between resistance exercise, endurance exercise, and irisin-injection groups compared to the control and placebo group ($P < 0.0001$) (Groups with label a/b have no significant difference, while groups with label b have a significant difference with the groups with label a)

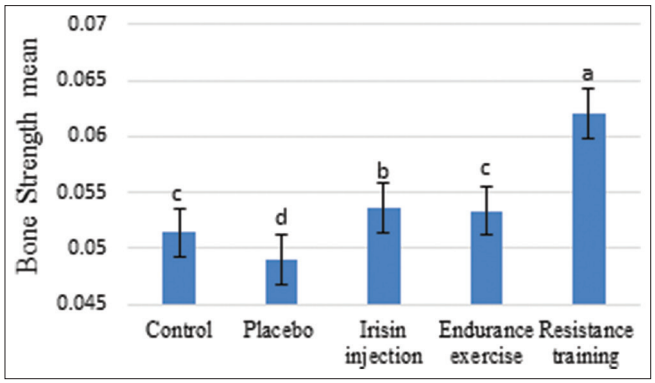

Figure 2: Bone strength mean in 5 groups of control, placebo, irisin-injection, endurance exercise, and resistance exercise. There is a significant difference between the mean of bone strength in resistance training and irisin injected groups compared to the control or placebo groups ($P < 0.0001$). There is no significant increase in bone strength in the endurance training group compared to the control group (Groups with label a/b have no significant difference, while groups with label b have a significant difference with the groups with label a)

or Pekkala *et al*. reported an increase of 1.4-fold of FNDC5 mRNA in muscles after resistance exercise.[41] Huh *et al*. reported an elevation of serum irisin in response to both endurance and resistance trainings, whereas the increase in the second type of training was more significant.[42] Similar to other studies, the results of this study have verified the serum irisin elevation after both endurance exercise and resistance exercise compared to the control group. However, inconsistent with the Huh *et al*.'s study, no significant difference between serum irisin concentrations of two exercise groups was observed. A considerable increase in serum irisin concentration was also observed in the irisin-injected group.

The effects of different exercise protocols or pharmacologic agents on bones are usually evaluated using imaging techniques, histomorphometry, and mechanical properties analysis. Many studies assess the exercise effects on the bone of mice using histomorphometry though it does not indicate the bone strength exactly. Mechanical measurement is the best evaluating method that addresses bone strength directly. It can be measured in three different ways of compression, tensile, and bending. In this study, the 3-point bending method was used to assess the effect of irisin injection and exercise protocols on the bone as closely as possible. The geometry and strength of the bone can be directly inferred from the result.

There are a few studies that used 3-point bending to evaluate the impact of exercise on the bones. Notomi *et al*. assessed the effects of 4-week and 8-week protocols of resistance training on bone using 3-point bending. BMD and bending load increased in the femur and tibia in both exercise protocols.[43] Another study assessed the impact of a combination of resistance training and hormone therapy in ovariectomized mice.[44] Ramon *et al*. also revealed that resistance training has beneficial effects on bone strength. Finally, the 3-point bending test was carried out for the direct effect of irisin on the bone in Colaianni *et al*.'s study.[25]

In the current study, the 3-point bending result of femur strength in resistance training has the highest. The impact of this protocol of exercise is in line with the results of Bennell *et al*.'s study of 10-week, and Shiguemoto *et al*.'s study of 12-week resistance training protocol, indicating beneficial effects of the protocol on bones.[12] Despite a significant increase in plasma irisin concentration following endurance exercise, bone strength had no significant difference with the control group.

As it is evident, the increase in irisin level is observed in both exercise groups and also in irisin-injected group compared to the control or placebo groups, while the bone strength increased significantly in two groups of resistance exercise and irisin-injected not in endurance exercise.

Plasma irisin concentration in the irisin-injected group was approximately 7 ng/ml. About 1.6-fold increase in serum irisin concentration was observed in parallel with a significant increase in the strength of the bone. It means that the bone-related benefits of this peptide were met approximately at this plasma irisin concentration, here in this study. It can be inferred that this increase is necessary for bone effects, though assigning the amount limit needs further studies. According to our results, resistance exercise has the most effects on bone strength that is consistent with other reports. Our results are indicating that as the plasma irisin concentration is higher in the resistance exercise group compared to the irisin-treated group, the bone strength is higher as well. Plasma irisin concentration and 3-point bending results of the resistance exercise group can be interpreted as a capability of the applied resistance exercise protocol to increase plasma irisin concentration to reach the extent that affects bones.[25] In the current study, bone metabolism stimulation by irisin and resultant effects seem to be achieved at a specific threshold of plasma concentration. The endurance exercise protocol could not reach this amount, and no significant bone effects were observed, while resistance exercise has shown to have a higher ability to reach this extent.

The exact mechanism underlying the beneficial effects of irisin on bone strength is not well defined, but findings suggest pleiotropic impacts for irisin on osteogenesis. Irisin prevents osteocyte apoptosis through ERK signaling.[28] Its deficiency induces osteoclastogenesis, inhibits osteoblastogenesis, and disturbs bone metabolism.[45]

It positively regulates the differentiation of osteoblast and reverses β-catenin downregulation induced by simulated microgravity.[46] Recombinant irisin increases osteogenic marker gene (alkaline phosphatase, collagen type 1 alpha-1) in primary osteoblasts and promotes calcium deposition.[46]

The immunomodulatory role of irisin is also suggested to involve in osteogenesis through AMPK-mediated macrophage polarization.[47]

The similarities between irisin and mechanical forces of exercise in the beneficial effects on bone might be because of the sensitivity of mechanosensor cells of bone to exercise mimetic myokines.[48]

Conclusion

According to the results of the current study, irisin takes a considerable contribution in beneficial effects of resistance exercise on the bone strength, and these effects are also occurred in the irisin-injected group. These effects are significantly higher in the resistance exercise group. There may be a threshold for plasma irisin level to affect bones which the applied protocols of irisin injection and resistance exercise, but not endurance exercise can reach.

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Conflicts of interest

There are no conflicts of interest.

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