ABSTRACT

Background and objectives: Bone-related osteokines play an important role in the response of bone metabolism to physical activity. The purpose of this study was to investigate effects of different intensities of resistance training on serum levels of some osteokines associated with the Wnt signaling pathway and receptor activator of nuclear factor kappa-B ligand (RANKL) pathway in old male Wistar rats.

Methods: Twenty-four old (23 months) male Wistar rats (mean weight: 437.93±33 g) were randomly divided into three groups of moderate-intensity resistance training (n=8), high-intensity resistance training (n=8) and control (n=8). Moderate- and high-intensity resistance trainings were performed at 60% and 80% maximal voluntary carrying capacity, respectively. The level of sclerostin, osteoprotegerin (OPG) and RANKL was evaluated by enzyme linked immunosorbent assay. Data were analyzed using one-way analysis of variance and at significance level of ≤0.05.

Results: The results showed that sclerostin (p=0.014), OPG (p=0.049) and RANKL (P=0.034) differed significantly between the study groups. The Tukey post-hoc comparison showed that sclerostin decreased significantly in the high-intensity resistance training group compared to moderate-intensity resistance training group (P=0.048). In addition, OPG decreased significantly in the moderate-intensity resistance training group compared to the control group (P=0.033). Moreover, RANKL decreased significantly in the high-intensity resistance training group compared to the control (P=0.048).

Conclusion: The results showed that resistance training with appropriate repetition and intensity can have positive effects on bone formation signaling pathways.

Keywords: Aging, Resistance training, Rats, Wistar.
INTRODUCTION

Low bone density is one of the changes in old age during which the bones become increasingly fragile, increasing susceptibility to bone fracture (1-2). Aging increases the number of active osteoclasts and decreases osteoblast activity, which reduces bone mass (3). Sclerostin is one of the proteins secreted by osteocytes that exerts anti-anabolic properties during ossification (4) and reduces bone formation by inhibiting the Wnt (Wingless-type mouse mammary tumor virus integration site) signaling pathway (5). Members of the tumor necrosis factor (TNF) receptor superfamily such as the receptor activator of nuclear factor-κB ligand (RANKL) and the osteoprotegerin (OPG) are other molecules present in the bone marrow stromal cell that play an important role in bone regeneration by binding to various cytokines and calcium-dependent hormones (6). The OPG is a trap for RANK that is secreted from osteoclasts and prevents the differentiation and secretion of osteoclasts and increases osteoclasts apoptosis (7). The Wnt pathway stimulates the production and secretion of OPG and is therefore considered a RANK antagonist. The OPG/RANK ratio is a sensitive regulator of osteoclast structure and bone resorption. The RANK is a marker of bone resorption, but its presence alone is insufficient for stimulation of resorption and requires OPG-related reduction (8). Constant physical pressure through osteoblast stimulation causes bone deposition and calcification (9). Optimal stimulation for skeletal development is achieved with weight bearing exercises, which have an osteogenic effect (10, 11). Past research has repeatedly shown that exercise and physical activity increase bone density. Exercise is also an important factor in the prevention of osteoporosis (12). However, the mechanisms by which exercise affects bone metabolism are not yet fully understood. It seems that exercise can increase RANKL levels in osteocytes (13). There are several factors to consider when choosing the best resistance training program for an appropriate bone response, including the intensity and duration of resistance training (14). It has been shown that the exercise-induced osteogenic response is intensity dependent (15-16). Therefore, it is thought that training with different intensities can stimulate different cellular responses and consequently different bone adaptations (17). Exercise intensity can also affect bone density (18). In a study on postmenopausal women, resistance training or aerobic exercise for eight months did not change OPG, RANKL or OPG/RANKL ratio (19). In another study, 32 weeks of combined resistance and balance training in old men and women increased OPG levels in women compared with men but did not change RANKL levels (20). Krug et al. reported OPG and RANKL increase after one training session (5 minutes) of high-intensity training in rats with muscle atrophy, but both markers returned to constant levels after 24 hours (21). It is essential to understand how mechanical stress can control osteoporosis in order to better understand age-related changes in the serum levels of these proteins. Therefore, the purpose of this study was to determine the effect of resistance training on RANKL and sclerostin levels as downregulators and OPG as an upregulator of bone formation. We hypothesized that the stimulation provided by exercise may serve as a useful way to prevent intracellular molecular signaling leading to osteoporosis in old age.

MATERIALS AND METHODS

In the present study, 24 old (23 months) male Wistar rats with an average weight of 438.27 g were purchased from the Pasteur Institute of Iran and transferred to the laboratory of Shahrekord University. The animals were kept at 12/12 h light/dark cycle and 22±3°C, with free access to food and water. All procedures were carried out according to the Guide for the Care and Use of Laboratory Animals. The study was also approved by the Deputy of Research and Graduate Studies of Shahrekord University. After adaption to the environment, the animals were weighed using a digital scale (0.1-2000 g, model SF-400A) and randomly divided into three groups accordingly: control (n=8), moderate-intensity resistance training (MIRT, n=8) and high-intensity resistance training (HIRT, n=8). The control group did not receive any training. The rats in both HIRT and MIRT groups performed a one-week non-weight training climbing ladder to learn how to perform the training protocol. After the last session of adaptation, the animals were tested for maximal voluntary carrying capacity (MVCC). The MVCC was then defined as the highest.
successfully carried load. At the end of the fourth week, the MVCC test was performed again and the intensity of animal training was determined based on the new test (22,23). The resistance training protocol included climbing a special training ladder (length of 110 cm, tilt of 80 degrees) five days a week at high and moderate intensities for eight weeks (22,23).

To determine the MVCC, 75% of the animals’ body weight was attached to their tails, and the animals began to climb the ladder carrying this load. Then, for each successful repetition, 30 g were added to the previous training load. This procedure was repeated until the rat was able to climb the entire length of the ladder in three consecutive attempts. The MVCC was measured at the beginning of the first and fourth weeks and at the end of the eighth week (22).

The rats were anesthetized by intraperitoneal injection of ketamine (30 to 50 mg/kg) and xylazine (10 mg/kg) 72 hours after the last injection of ketamine (30 to 50 mg/kg) and xylazine (10 mg/kg) 72 hours after the last training session. Then, 8 cc of blood samples were added to the previous training load. This procedure was repeated until the rat was able to climb the entire length of the ladder in three consecutive attempts. The MVCC was measured at the beginning of the first and fourth weeks and at the end of the eighth week (22).

The rats were anesthetized by intraperitoneal injection of ketamine (30 to 50 mg/kg) and xylazine (10 mg/kg) 72 hours after the last training session. Then, 8 cc of blood samples from each rat were collected directly from the heart and poured into normal tubes. Serum was separated by centrifugation at 3000 rpm for 15 minutes and stored at -80 °C. The level of sclerostin, OPG and RANKL was evaluated by enzyme linked immunosorbent assay. The Levene’s test was used to evaluate equality of variances and the Shapiro-Wilk test was used to check normality of data distribution. The one-way ANOVA test and Tukey post-hoc test were used to evaluate the effectiveness of the interventions. All statistical analyses were carried out in SPSS 21 and at significance level of P≤0.05.

RESULTS

Table 1 shows the changes in weight and MVCC of the old rats in different study groups.

The level of sclerostin (p=0.014), OPG (p=0.049) and RANKL (p=0.034) differed significantly between the study groups (Tables 2).

Sclerostin decreased significantly in the HIRT group compared to the MIRT group (p=0.048). In addition, OPG decreased significantly in the MIRT group compared to the control group (p=0.033). Moreover, RANKL level decreased significantly in the HIRT group compared to the control group (p=0.048) (Table 3).

Table 1- Mean changes in weight (g) and MVCC (g)

<table>
<thead>
<tr>
<th>Variables</th>
<th>Groups</th>
<th>At baseline</th>
<th>After four weeks</th>
<th>After eight weeks</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weight (g)</td>
<td>HIRT</td>
<td>434.1 ± 37.8</td>
<td>434.37 ± 39.2</td>
<td>424.6 ± 35.2</td>
</tr>
<tr>
<td></td>
<td>MIRT</td>
<td>434.2 ± 37.9</td>
<td>425 ± 38.9</td>
<td>426 ± 38.7</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>445.50 ± 24.07</td>
<td>452 ± 30.93</td>
<td>441.87 ± 27.95</td>
</tr>
<tr>
<td>MVCC (g)</td>
<td>HIRT</td>
<td>0.251 ± 0.15</td>
<td>0.4355 ± 0.20</td>
<td>4.4567 ± 1.50</td>
</tr>
<tr>
<td></td>
<td>MIRT</td>
<td>0.2579 ± 0.17</td>
<td>0.5872 ± 0.31</td>
<td>1.2847 ± 0.59</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>0.9932 ± 0.31</td>
<td>1.0735 ± 0.44</td>
<td>1.1082 ± 0.55</td>
</tr>
</tbody>
</table>

Table 2- Comparison of sclerostin, OPG and RANKL levels between the study groups after eight weeks of resistance training

<table>
<thead>
<tr>
<th>Variables</th>
<th>Groups</th>
<th>Mean ± standard deviation</th>
<th>F</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sclerostin (ng/ml)</td>
<td>HIRT</td>
<td>13.101±2.181</td>
<td>5.278</td>
<td>0.014</td>
</tr>
<tr>
<td></td>
<td>MIRT</td>
<td>17.208±3.061</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>16.565±2.895</td>
<td></td>
<td></td>
</tr>
<tr>
<td>OPG (ng/ml)</td>
<td>HIRT</td>
<td>2.912±0.0404</td>
<td>3.482</td>
<td>0.049</td>
</tr>
<tr>
<td></td>
<td>MIRT</td>
<td>2.536±0.781</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>3.322±0.470</td>
<td></td>
<td></td>
</tr>
<tr>
<td>RANKL (pg/ml)</td>
<td>HIRT</td>
<td>72.982±14.296</td>
<td>3.990</td>
<td>0.034</td>
</tr>
<tr>
<td></td>
<td>MIRT</td>
<td>96.978±14.296</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>101.042±27.011</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 3- Result of the Tukey post-hoc test for comparisons of sclerostin, OPG and RANKL levels between the study groups after eight weeks of resistance training

<table>
<thead>
<tr>
<th>Variable</th>
<th>Group comparison</th>
<th>Mean difference</th>
<th>Standard error</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sclerostin (ng/ml)</td>
<td>HIRT/MIRT</td>
<td>-4.107</td>
<td>1.420</td>
<td>0.048*</td>
</tr>
<tr>
<td></td>
<td>HIRT/C</td>
<td>-3.464</td>
<td>1.512</td>
<td>0.017</td>
</tr>
<tr>
<td></td>
<td>MIRT/C</td>
<td>0.643</td>
<td>1.472</td>
<td>0.792</td>
</tr>
<tr>
<td>OPG (ng/ml)</td>
<td>HIRT/MIRT</td>
<td>0.378</td>
<td>0.296</td>
<td>0.711</td>
</tr>
<tr>
<td></td>
<td>HIRT/C</td>
<td>-0.410</td>
<td>0.315</td>
<td>0.692</td>
</tr>
<tr>
<td></td>
<td>MIRT/C</td>
<td>-3.786</td>
<td>0.307</td>
<td>0.033*</td>
</tr>
<tr>
<td>RANKL (pg/ml)</td>
<td>HIRT/MIRT</td>
<td>-23.996</td>
<td>9.128</td>
<td>0.086</td>
</tr>
<tr>
<td></td>
<td>HIRT/C</td>
<td>-28.057</td>
<td>9.723</td>
<td>0.048*</td>
</tr>
<tr>
<td></td>
<td>MIRT/C</td>
<td>-4.061</td>
<td>9.467</td>
<td>0.993</td>
</tr>
</tbody>
</table>

*Statistically significant difference.
DISCUSSION

It is essential to understand how bones respond to exercise at various intensities since training intensity plays an important role in the differentiation, development and function of osteoblasts and osteoclasts (23). The OPG/RANKL system has recently been shown to play an important role in bone circulation and regeneration. In recent years, the effects of physical activity on bone-related osteokine have received a great deal of attention. The present study investigated effect of eight weeks of resistance training at moderate and high intensities on sclerostin, OPG and RANKL levels in old male rats in order to generalize the effect of mechanical load on osteogenesis. At the end of the eight-week intervention, serum levels of sclerostin, OPG and RANKL differed significantly between the study groups. Inconsistent with our results, Herrmann et al. found no difference in OPG and RANKL levels between skiers and control subjects (24). Six weeks of aerobic exercise (walking and jogging with 60% VO2max) caused no significant change in RANKL level of postmenopausal women (25). However, our findings were in line with findings of some previous studies on bone-related cytokines in young men (26), OPG in young women (27) and the OPG/RANKL pathway in boys and young men (28). The RANKL is essential for osteoclast activity (29), so the significant reduction observed in RANKL levels in HIRT may explain the positive effects of resistance training on bone formation. Given the high level of RANKL in the elderly with osteoporosis, an exercise effective in reducing RANKL levels could indirectly prevent bone loss or osteoporosis (30, 31). A number of enzymes and proteins are released into the blood during the process of bone formation or regeneration, reflecting the activity of bone-forming cells or osteoblasts. On the other hand, resistance training seems to be a reliable stimulus for bone formation due to the high pressure it exerts on the bones (32). Evidence suggests the physiological role of the OPG/RANK/RANKL pathway in the regulation of bone changes (33). As a bone metabolism regulator that is expressed by endothelial cell osteoblasts in smooth muscle cells and arteries, OPG binds to RANKL (on osteoclasts) and prevents RANKL reaction with RANK, thereby inhibiting differentiation, activation and survival of osteoclasts and ultimately preventing bone resorption (34, 35). Our findings showed that HIRT could significantly alter OPG and RANKL levels. Downregulation of RANKL levels should prevent bone loss. Past studies have shown that resistance training downregulates RANKL (36), which is consistent with the results of the present study. The bone resorption markers such as RANKL are decreased immediately after resistance exercise. This immediate reduction of the Wnt pathway-independent osteoclastogenesis after HIRT indicates greater bone circulation, which may be a prerequisite for the Wnt pathway and a subsequent shift toward ossification through suppression of catabolic osteokines (37). Assessing the RANKL/OPG ratio is critical for explaining the effects of OPG and RANKL on the bone system. Previous studies reported a relationship between this ratio and bone mineral density. Mödder et al. showed a low RANKL/OPG ratio in women taking contraceptives (38). Kim et al. showed an association between decreased RANKL/OPG ratio and bone mineral density (39). However, a study found an inverse correlation between osteocalcin and serum RANKL levels and RANKL/OPG ratio (40). In the present study, serum RANK levels decreased by almost 30% in the HIRT group, suggesting the inhibition of RANKL/RANK. On the other hand, a significant decrease in RANKL after HIRT could increase the OPG/RANKL ratio, which reduces osteoclastosis and leads to ossification by suppression of catabolic osteokines. In this study, sclerostin level increased in the MIRT group and decreased in the HIRT group compared with the control group. Sclerostin level increases with age and might contribute to the age-related bone loss (41). Ardawi et al. found that even a small increase in mechanical load caused a significant decrease in serum sclerostin level and an increase in bone remodeling markers (41). Spatz et al. emphasized that the bone mineral levels decreased significantly which was associated with an increase in sclerostin levels after 90 days of home rest (42). Lombardi et al. found higher levels of sclerostin in female athletes compared to male athletes (43). Sheng et al. reported higher sclerostin levels in postmenopausal women without osteoporosis than those with osteoporosis (44). The age-related increase in sclerostin may be related to
the secretion of sclerostin and binding to LRP5 and LRP6 co-receptors on bone surface, thereby inhibiting the Wnt pathway and reducing osteoclastogenesis and ossification (45). Therefore, it is possible that reduced sclerostin may prevent the inhibition of the Wnt pathway and lead to an increase in osteoclastogenesis and ossification. Therefore, a combination of sclerostin inhibitor therapy and exercise can be useful for treatment of reduced bone mass. In the present study, sclerostin level reduced by about 20% in the HIRT group but increased in the MIRT group. A limitation of this study was not performing bone densitometry. To clarify various aspects of sclerostin, RANKL and OPG, it is recommended to measure bone density. On the other hand, it seems that intensity and duration of moderate-resistance training were not enough to reduce sclerostin. Older people may respond to the training exercise if performed in longer period.

CONCLUSION
The results showed that resistance training with appropriate repetition and intensity can have positive effects on bone formation signaling pathways. Our results are consistent with previous studies showing that muscle-produced forces play an important role in stimulating bone formation or resorption. This is justified by the decrease in serum levels of sclerostin and RANKL after resistance training, especially after HIRT. The obtained results emphasize the role of physical activity as an effective stimulation method of bone formation in old people.

ACKNOWLEDGMENTS
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CONFLICT OF INTEREST
The authors declare that there is no conflict of interest regarding publication of this article.

REFERENCES


18. Aido MIFd. The influence of age and mechanical loading on bone structure and material properties: Technical University Berlin; 2015. [View at Publisher] [Google Scholar]


27. West DW, Burd NA, Staples AW, Phillips SM. Human exercise-mediated skeletal muscle hypertrophy is an intrinsic process. The international journal of biochemistry & cell biology. 2010;42(9):1371-5. [View at Publisher] [DOI:10.1016/j.biocel.2010.05.012] [PubMed] [Google Scholar]


29. Bernstein CN, Sargent M, Leslie WD. Serum osteoprotegerin is increased in Crohn's disease: a population-based case control study. Inflammatory bowel diseases. 2005;11(4):325-30. [View at Publisher] [DOI:10.1097/01.MIB.0000164015.60795.ca] [PubMed] [Google Scholar]


34. Robin J, Murphy TC, Zhu L, Roy E, Nanes MS, Fan X. Mechanical Strain Differentially Regulates Endothelial Nitric-oxide Sythase and Receptor Activator of Nuclear B Ligand Expression via ERK 1/2. MAPK. Journal of Biological Chemistry.278(36), [View at Publisher] [DOI:10.1074/jbc.M302822200] [Google Scholar]
43. Shahbazi and colleagues

35. Dekker J. The Effects of a Single Bout of Plyometric Exercise on Anabolic and Catabolic Osteokines in Girls and Adolescents. Broke University. 2016. [View at Publisher] [Google Scholar]


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