Effect of Eight Weeks of Aerobic Interval Training on the Serum Concentrations of Alkaline Phosphatase, Osteocalcin and Parathyroid Hormone in Middle-aged Men

Mohsen Bakhtiyari ¹, Mehrdad Fathi ², * and Keyvan Hejazi ³

¹Faculty of Physical Education and Sports Sciences, Islamic Azad University, Bojnourd Branch, Bojnourd, Iran
²Faculty of Sports Sciences, Ferdowsi University of Mashhad, Mashhad, Iran
³Department of Sport Physiology, Hakim Sabzevari University, Sabzevar, Iran

*Corresponding author: Faculty of Sports Sciences, Ferdowsi University of Mashhad, Mashhad, Iran. Email: mfathei@um.ac.ir

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Abstract

Background: Osteoporosis is the most common disorder of bone and mineral metabolism. This disease is associated with decreased bone density and loss of bone microstructure, leading to increased fragility and risk of bone fractures.

Objectives: The aim of this study was to evaluate the effect of eight weeks of aerobic interval training on the serum concentrations of alkaline phosphatase, osteocalcin, and parathyroid hormone in middle-aged men.

Methods: This was a quasi-experimental study of 24 men, who were divided into two groups of control (n = 12) and aerobic interval training (n = 12). Training performed during eight weeks, three times a week, each session for 45 to 60 minutes. Blood samples were analyzed for the concentrations of calcium, phosphorus, alkaline phosphatase, osteocalcin, and parathyroid hormone before and after completion of the training program. Paired samples t-test and analysis of covariance (ANCOVA) were run to analyze the data (P ≤ 0.05).

Results: Weight and body mass index decreased significantly, and the maximum oxygen consumption at the end of the training program increased significantly in the experimental group. Eight weeks of aerobic exercise led to a significant increase in the amounts of calcium (P = 0.001), phosphorus (P = 0.001), alkaline phosphatase (P = 0.001), osteocalcin (P = 0.001), and parathyroid hormone (P = 0.001) in the experimental group.

Conclusions: It can be stated that eight weeks of aerobic interval training may be effective in preventing osteoporosis by reducing body composition indices and increasing calcium, phosphorus, alkaline phosphatase, osteocalcin, and serum parathyroid hormone in inactive men.

Keywords: Osteocalcin, Aerobic Interval Exercise, Alkaline Phosphatase, Overweight Men

1. Background

Osteoporosis is a privilege systemic skeletal disease characterized by severely low general bone mass (bone hollowing). In this condition, bone composition does not change in quality, but it does decrease in quantity (1). The risk of osteoporosis is higher in men during the first 3 to 5 years after inactivity due to increased bone loss rate (2 - 6.5% per year) (2). Fracture caused by osteoporosis occurs in 60% of women and 30% of men aged over 60 years. Approximately one in three women and one in twelve men develops osteoporosis, leading to 10,000 debilitating fractures in patients. However, only a small number of these patients are evaluated (3, 4). Therefore, increased bone resorption with disproportionate formation could result in decreased bone density and osteoporosis (5). The bone regeneration process could be assessed using bone formation and bone resorption biomarkers. These biomarkers include bone matrix components, substances, and enzymes that are released during the regeneration process (6). Alkaline phosphatase, osteocalcin, and parathormone hormone are the main biomarkers of the bone regeneration process (7).

Alkaline phosphatase is a noble indicator of bone formation (8). Alkaline phosphatase is a group of hydrolase enzymes produced by the kidneys, liver, and bones. Alkaline phosphatase is mainly attached to the cell membrane. The role of this enzyme is to remove the phosphate agent from phosphate-containing organic esters and to facilitate the movement of substances from the cell membrane. Hepatocyte cells produce these enzymes, and then...
they attach to the canalicular cell surface. Osteoblasts produce the bone isozymes responsible for breaking down pyrophosphate (an inhibitor of bone mineralization) (9, 10). Osteocalcin is a specific bone protein circulating in the blood (11).

Osteocalcin is characterized by the presence of carboxyglutamic acid residues, which are essential for the binding of osteocalcin to hydroxyapatites and bones (12). Osteocalcin produced by osteoblasts is involved in calcium mineralization and homeostasis. Parathormone, which is secreted by the parathyroid gland, plays an important role in regulating blood calcium and osteocalcin, which are the important biomarkers of bone metabolism (13). The parathyroid hormone is key to calcium homeostasis and bone metabolism (13). This hormone is one of the main components of bone mass in adults and is closely related to bone loss, osteoporosis, and fractures. The secretion of this hormone is affected by calcium (14). Regular and long-term physical activity can affect bone metabolism indices, prevent further bone desorption, reduce the secretion of parathyroid hormone, increase calcitonin, and prevent bone tissue from losing calcium and other minerals (15). A number of studies evaluating bone biochemical markers have addressed the effect of exercise on bone metabolism (16, 17), some of which have shown the anabolic effects of exercise on the bone (17, 18), while others have demonstrated the negative effects of exercise on bone metabolism. Empirical evidence confirms that physical activity has positive effects on skeletal mass (19), and relatively intense exercise lasting more than six months is more effective to increase bone density compared to high-repetition and low-pressure exercises (20).

Despite the importance of alkaline phosphatase, osteocalcin, and parathormone levels in calcium and phosphorus metabolism and the significance of preserving bone salts (i.e., calcium and phosphorus) to prevent osteoporosis in inactive men and considering the possible role of long-term periodic aerobic training in changing these factors and maintain bone mass, the relationship between bone markers and bone density adaptations as an indicator of bone growth is not yet fully understood. Former studies have reported contradictory results regarding the response of bone metabolism markers, and optimal training cannot be suggested for maximum bone formation (21).

2. Objectives

The aim of this study was to evaluate the effect of eight weeks of aerobic interval training on the serum concentrations of alkaline phosphatase, osteocalcin, and parathyroid hormone in middle-aged men.

3. Methods

3.1. Subjects

This was a quasi-experimental study with a pre-test and post-test design. The statistical population of this study included 60 overweight and middle-aged men in residing Bojnourd, Iran. The statistical samples of this study included 24 overweight middle-aged men with an age range of 40 to 47 years, who were selected by the available and purposive sampling methods. Firstly, the study objectives and procedure were explained to the participants, and they signed written informed consent forms. Health was examined based on the health questionnaire. The inclusion criteria comprised of not using any medications, not smoking, and not having participated in any exercise program for at least two months before participating in the exercise program. Subjects were then randomly divided into the two groups of aerobic interval training (n = 12) and control (n = 12).

3.2. Body Composition

To evaluate the body composition, we measured the height of the subjects with Saka caliper (made in Germany) with an accuracy of 5 mm, hip and waist circumference with a tape measure (Mabis, Japan) with an accuracy of 5 mm, and body fat percentage and weight with an accuracy of 100 g. Body mass index was obtained by dividing body weight in kilograms by squared height in meters. All the measurements were performed while the subjects had abstained from eating and drinking for four hours before the test, and their bladder, stomach, and intestines had been emptied as much as possible.

3.3. Maximum Oxygen Consumption

To estimate the maximum oxygen consumption, the Naughton protocol was performed on a treadmill. The Naughton test was performed in 10 two-minute stages, except for the first stage, which was at a speed of one mile per hour; the constant speed at the next stages was two miles per hour. The slope of the device was zero in stages 1 and 00, and as of the third stage the slope increased 3.5% in each stage. The maximum oxygen consumption in the Naughton protocol was calculated using following equation (22).

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\text{Maximum oxygen consumption} \ (\text{ml} / \text{kg} / \text{min}) = 1.61 (\text{time in minutes}) + 3.6
\]

3.4. Blood Samples

Blood samples were collected by an experienced laboratory sciences expert in two stages from the left vein of each case in a sitting position and at rest 48 hours before
the start of the training program and 48 hours after the last training session. Both before and after training, 5 cc of blood was taken from the brachial anticoagulant vein, and the samples were frozen at -48°C. To determine the serum amount of alkaline phosphatase using Pars ALP kit (made in Iran, with a sensitivity of 3 units per liter), the standard synthetic photometric method was used. Also, serum phosphorus and calcium levels were determined using the photometric method (Pars Company Kit, Iran) with a sensitivity of 0.7 and 0.2 mg/100 ml, respectively. The level of osteocalcin was determined by the ELISA method with a standard BIOVENDER kit (made in England). ELISA method and Biosource hPTH commercial kit (Biosource Group, Belgium) were used to measure the parathormone level. The kit’s sensitivity was 0.1 ng/L.

3.5. Training Program

The training program included periodic aerobic exercise for eight weeks, three sessions a week each session for 45 to 60 minutes. The high-intensity training (HIT) program included warm up with a variety of stretching and flexing movements for 10 minutes and then performing intense periodic movements with one active rest minute between each set. The training program was implemented from simple to difficult, taking into account the principle of overload and increasing the training intensity.

The pattern of the intense periodic exercise was as follows: in the first week, three times of 4-minute execution with 90% heartbeat reserve with 2 minutes of active recovery; the second week, 4 times of 4-minute execution with 90% intensity of heartbeat reserve with 2 minutes active recovery; the third week, five times of 4-minute run with 90% heart rate reserve with 2 minutes active recovery; the fourth week, six times of 4-minute execution with 90% heart rate reserve with 2 minutes active recovery; the fifth week, seven times of 4-minute implementation with 90% heart rate reserve with 2 minutes active recovery; the sixth week, eight-time of 4-minute execution with 90% heart rate reserve with 2 minutes active recovery; the seventh week, six times of 4-minute execution with 90% heart rate reserve with 2 minutes active recovery; and the eighth week, five times of 4-minute execution with 90% heart rate reserve with 2 minutes active recovery. The exercise was controlled by a heartbeat monitor (Polar, Finland) (23, 24). The control group was inactive and performed no exercise training during the study.

3.6. Statistical Analysis

The collected data was analyzed using SPSS, version 16. For ensuring the normality of the data, the Shapiro-wilk statistical test was used, and Levene’s test was run to examine the homogeneity of variance. To compare intragroup and intergroup changes, t-test and analysis of covariance (ANCOVA) were run, respectively. A P-value of less than 0.05 was considered significant.

4. Results

The characteristics of the participants are presented in Table 1. Based on Table 2, the eight-week aerobic interval training program led to a significant increase in calcium (P = 0.001), phosphorus (P = 0.001), alkaline phosphatase (P = 0.001), osteocalcin (P = 0.001), and parathyroid hormone (P = 0.001). Moreover, according to this table, the changes in the mean levels of calcium (P = 0.001), phosphorus (P = 0.001), alkaline phosphatase (P = 0.001), osteocalcin (P = 0.001), and parathyroid hormone (P = 0.001) were significantly different between the experimental and control groups.

According to Table 3, eight weeks of aerobic interval training led to a significant decrease in weight (P = 0.004) and body mass index (P = 0.003) and a significant increase in maximum oxygen consumption (P = 0.001) in the experimental group. Finally, the changes in the means of weight (P = 0.02) and maximum oxygen consumption (P = 0.001) were significantly different between the experimental and control groups.

5. Discussion

Eight weeks of aerobic interval training significantly increased the serum level of osteocalcin in men. Osteocalcin is inactively stored in the extracellular matrix of bones as gamma carboxyglutamic acid, and for activating, it is converted to non-carboxylyc osteocalcin in the bone resorption cavity at a pH of 4.5 and released into the bloodstream. Insulin then binds to its receptor in osteoblasts in a feedback axis and promotes bone resorption by inhibiting osteoprotegerin activity. Indeed, insulin signaling in osteoblasts reduces osteoprotein gene expression by inhibiting foxo1 activity, which downregulates bone resorption, promotes osteoclast activity, and releases the active form of osteocalcin (25). One of the main mechanisms through which exercise increases osteocalcin level is increased bone cell activity and response to exercise-induced mechanical stress, which leads to more osteocalcin secretion by these cells, and the second is related to the disruption of energy metabolism homeostasis during physical activity in the body. Recently, bone has been mentioned as an active metabolic tissue during physical activity, and signals from changes in insulin and glucose lead to increased bone cell activity and the stimulation of osteocalcin secretion (26-28).
Table 1. Characteristics of the Participants

| Groups     | Variations (Mean ± SD) |  |
|------------|------------------------|--|
|            | Age (y) | Height (Cm) | Weight (Kg) | BMI (kg/m²) |
| Experimental | 42.75 ± 1.86 | 161.50 ± 2.22 | 74.60 ± 2.56 | 28.62 ± 1.46 |
| Control    | 43.00 ± 2.17 | 159.50 ± 1.05 | 74.90 ± 2.56 | 29.44 ± 0.98 |

Table 2. Variations of Bone Biomarkers and Serum Osteocalcin Levels of Inactive Men

| Variables              | Stages (Mean ± SD) | PValue |
|------------------------|--------------------|--------|
|                        | Pre-test | Post-test | Paired Samples t-test | ANCOVA (Between Group) |
| Calcium (mg/dL)        |          |          |                    | 0.001<sup>a</sup> |
| Experimental           | 9.48 ± 0.12 | 9.75 ± 0.15 | 0.001<sup>a</sup> |
| Control                | 9.46 ± 0.24 | 9.45 ± 0.30 | 0.67 |
| Phosphorus (mg/dL)     |          |          |                    | 0.001<sup>a</sup> |
| Experimental           | 4.15 ± 0.10 | 4.49 ± 0.12 | 0.001<sup>a</sup> |
| Control                | 4.34 ± 0.13 | 4.26 ± 0.20 | 0.15 |
| Alkaline phosphatase (U/L) |     |          |                    | 0.001<sup>a</sup> |
| Experimental           | 208.25 ± 4.86 | 217.16 ± 4.36 | 0.001<sup>a</sup> |
| Control                | 212.00 ± 8.23 | 212.33 ± 11.20 | 0.77 |
| Osteocalcin (ng/mL)    |          |          |                    | 0.001<sup>a</sup> |
| Experimental           | 29.54 ± 0.89 | 31.02 ± 0.89 | 0.001<sup>a</sup> |
| Control                | 29.45 ± 0.64 | 29.66 ± 0.47 | 0.28 |
| Parathormone (pg/mL)   |          |          |                    | 0.001<sup>a</sup> |
| Experimental           | 21.69 ± 1.44 | 23.60 ± 0.96 | 0.001<sup>a</sup> |
| Control                | 21.55 ± 1.55 | 21.55 ± 1.78 | 0.97 |

<sup>a</sup>The mean difference is significant at the 0.05 level.

Table 3. Variations of Bone Biomarkers and Serum Osteocalcin Levels in Inactive Men

| Variables              | Variations |
|------------------------|------------|
|                        | Stages (Mean ± SD) | Paired Samples t-test | ANCOVA (Between Group) |
|                        | Pre-test | Post-test | t | PValue |
| Weight (kg)            |          |          |   |        |
| Experimental           | 74.60 ± 2.56 | 74.03 ± 2.63 | 3.63 | 0.004<sup>a</sup> |
| Control                | 74.90 ± 2.56 | 74.82 ± 2.71 | 0.69 | 0.501 |
| BMI (kg/m²)            |          |          |   |        |
| Experimental           | 28.62 ± 1.46 | 28.40 ± 1.49 | 3.70 | 0.003<sup>a</sup> |
| Control                | 29.44 ± 0.98 | 29.41 ± 1.07 | 0.68 | 0.508 |
| VO₂ max (ml/kg/min)    |          |          |   |        |
| Experimental           | 21.06 ± 1.06 | 22.36 ± 0.70 | 4.26 | 0.005<sup>a</sup> |
| Control                | 20.90 ± 0.76 | 20.60 ± 0.75 | 1.67 | 0.122 |

<sup>a</sup>The mean difference is significant at the 0.05 level.
Based on our results, eight weeks of aerobic interval training significantly increased parathormone, calcium, and phosphorus levels in inactive men, which is consistent with some former findings (29-31). However, contradictory results have been reported by some other studies (16, 32). Participating in aerobic interval training causes high energy consumption. In endurance activities, frequent muscle contractions to maintain activity and release of the required energy are strongly dependent on the calcium presence. On the other hand, during endurance activities lasting a long time, a large number of body salts, including calcium, are excreted through the skin during the sweating process. Therefore, blood calcium level decreases (33). Because blood calcium level must always be constant, they activate blood calcium-sensitive hormone systems. Parathormone, which is secreted by the parathyroid gland, activates osteoclasts and bone catabolism to compensate for the required calcium (34). Besides, other factors such as catecholamine level and acidosis can alter parathyroid hormone secretion (17). The adrenergic system is activated during exercise and is a factor to regulate parathyroid hormone. Lactic acid also affects the density of this hormone and acidosis and stimulates hormone secretion and modulates the adrenergic system (17). This discrepancy between the results of the present study and the findings of the above-mentioned studies can be attributed to differences in participants’ age and sex or duration, intensity, and type of exercise program.

In addition to bone mineral density, numerous factors may contribute to bone strength including bone structure, shape, size, and sex hormone levels, which were not addressed in this evaluation; therefore, future studies on this issue are recommended. Because aerobic exercise can be one of the factors affecting sex hormones and bone biomarkers due to physiological variation, and such variables depend on the intensity, exercise duration, and physical fitness, it could be advised to coaches and exercise practitioners to consider the necessary precautions when designing exercise programs. This study had some limitations, including variable diets of the participants, various adaptive responses to physical activity, a number of subjects withdrew from the study, and individual differences; thus, extrapolation of the findings should be performed with caution.

5.1. Conclusions

In sum, periodic aerobic exercise, by reducing weight, body mass index, elevating calcium, phosphorus, alkaline phosphatase, and osteocalcin levels, and increasing the mechanical load on bone mass, causes changes in energy metabolism and body weight and can be considered as an important factor in preventing osteoporosis. Aerobic training programs are recommended for all people, especially inactive middle-aged individuals, to prevent osteoporosis.

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Footnotes

Authors’ Contribution: Study concept and design: MB, MF, and KH; Acquisition of the data: MB and MF; Analysis and interpretation of the data: KH, Drafting of the manuscript: KH, Critical revision of the manuscript for important intellectual content: MF and KH, Statistical analysis: KH, Administrative, technical, and material support: KH, and Study supervision: MF.

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References

1. Ferron M, Karsenty G. Regulation of energy metabolism by bone-derived hormones. *Principles of bone biology*. Elsevier; 2020. p. 1931-42. doi: 10.1016/b978-0-12-814841-9.00084-1.
2. Patti A, Gennari L, Merlotti D, Dotta F, Nuti R. Endocrine actions of osteocalcin. *Int J Endocrinol*. 2013;2013:846480. doi: 10.1055/s-0033-1346120. [PubMed: 23737779]. [PubMed Central: PMC3653994].
3. WHO. *Scientific group on the assessment of osteoporosis at primary health care level*. Genova: WHO; 2004. Available from: https://www.who.int/chp/topics/Osteoporosis.pdf.
4. Rizzoli R, Akesson K, Bouxsein M, Kanis JA, Napoli N, Papapoulos S, et al. Subtrochanteric fractures after long-term treatment with bisphosphonates: a European Society on Clinical and Economic Aspects of Osteoporosis and Osteoarthritis, and International Osteoporosis Foundation Working Group Report. *Osteoporos Int*. 2011;22(2):373-90. doi: 10.1007/s00198-010-1455-5. [PubMed: 21089935]. [PubMed Central: PMC3020164].
5. Khosla S, Oursler MJ, Monroe DG. Estrogen and the skeleton. *Trends Endocrinol Metab*. 2012;23(1):576-81. doi: 10.1016/j.tem.2012.03.008. [PubMed: 22595550]. [PubMed Central: PMC3424385].
6. Delmas PD, Eastell R, Garnero P, Seibel MJ, Stepán J, Committee of Scientific Advisors of the International Osteoporosis F. The use of biochemical markers of bone turnover in osteoporosis. Committee of Scientific Advisors of the International Osteoporosis Foundation. *Osteoporos Int*. 2006;17 Suppl 6:S2-47. doi: 10.1007/s001980070902. [PubMed: 17132077].
7. Shetty S, Kapoor N, Bondu JD, Thomas N, Paul TV. Bone turnover markers: Emerging tool in the management of osteoporosis. Indian J Endocrinol Metab. 2016;20(6):486–52. doi: 10.4103/2230-8210.392914. [PubMed: 27867890]. [PubMed Central: PMC505571].

8. Tosun A, Bolukbasi N, Cingi E, Beyazova M, Unlu M. Acute effects of a single session of aerobic exercise with or without weight-lifting on bone turnover in healthy young women. Mod Rheumatol. 2006;16(5):300–4. doi: 10.1007/s11305-006-0591-5. [PubMed: 1709391].

9. Garnero P, Delmas PD. Assessment of the serum levels of bone alkaline phosphatase with a new immunoradiometric assay in patients with metabolic bone disease. J Clin Endocrinol Metab. 1993;77(4):1046–51. doi: 10.1203/00005003-199377040-00004. [PubMed: 8034954].

10. Meier C, Seibel MJ, Kraenzlin ME. Use of bone turnover markers in the real world: are we there yet? Bone Miner Res. 2009;24(3):386–8. doi: 10.3399/jbr.0900104. [PubMed: 19608133].

11. Liu JN, Mao YM, Zhao CH, Wang H, Yuan FF, Li XM, et al. Circulating levels of osteoprotegerin, osteocalcin and osteopontin in patients with Rheumatoid Arthritis: A Systematic Review and Meta-Analysis. Immunol Invest. 2019;48(2):107–20. doi: 10.1080/08820139.2018.1509957. [PubMed: 3088228].

12. Bidlingmaier M. Osteocalcin. Lexikon der Medizinischen Laboratoriumsdiagnostik. Springer; 2019. doi: 10.1007/978-3-662-48986-4_2318.

13. Khan M, Sharma S. Physiology, parathyroid hormone (PTH), parathyroid gland and bone disease. Methods Debakey Cardiovasc J. 2017;13(2):3-9. doi: 10.4779/indcjr.13-2-49. [PubMed: 28740581]. [PubMed Central: PMC521678].

14. Brown SJ, Ruppe MD, Tabatabai LS. The parathyroid gland and heart disease. Method Debakey Cardiovasc J. 2017;13:2(9). doi: 10.4779/indcjr.13-2-49. [PubMed: 28740581]. [PubMed Central: PMC521678].

15. Troy KL, Mancuso ME, Butler TA, Johnson JE. Exercise early and often: Effects of physical activity and exercise on women’s bone health. Int J Environ Res Public Health. 2018;15(5). doi: 10.3390/ijerph150508078. [PubMed: 29700710]. [PubMed Central: PMC598897].

16. Takada H, Washino K, Hanai T, Iwata H. Response of parathyroid hormone to exercise and bone mineral density in adolescent female athletes. Environ Health Prev Med. 1998;2(4):261–6. doi: 10.1007/BF02913695. [PubMed: 21423525]. [PubMed Central: PMC272335].

17. Rong H, Ji H, Tai JA, Pernow Y, Buch E. Calcitonin-suppressed expression of parathyroid hormone-related protein in breast cancer cells. Biochem Biophys Res Commun. 1999;265(1):260–4. doi: 10.1006/bbrc.1999.5859. [PubMed: 10548224].

18. Bouassida A, Zalleg D, Zaouali Ajina M, Gharbi N, Duclos M, Richalet JP, et al. Parathyroid hormone concentrations during and after two periods of high intensity exercise with and without an intervening recovery period. Eur J Appl Physiol. 2003;88(4-5):339–44. doi: 10.1007/s00421-002-0721-2. [PubMed: 12579861].

19. Ryan AS, Nicklas BJ, Dennis KE. Aerobic exercise maintains regional bone mineral density during weight loss in postmenopausal women. J Appl Physiol (1985). 1998;84(4):1305–10. doi: 10.1152/jappl.1998.84.4.1305. [PubMed: 950897].

20. Shibata Y, Ohsumi I, Watanabe T, Miura T, Sato Y. Effects of physical training on bone mineral density and bone metabolism. J PhysiolAnthropol Appl Hum Sci. 2003;22(4):205–8. doi: 10.2114/jpa.22.209. [PubMed: 12939536].

21. Maimoun L, Galy O, Manetta J, Coste O, Peruchon E, Micallef JP, et al. Competitive season of triathlon does not alter bone metabolism and bone mineral status in male triathletes. Int J Sports Med. 2004;25(1):230–4. doi: 10.1055/s-2003-4257. [PubMed: 15082449].

22. Acevedo EO, Starks MA. Exercise testing and prescription lab manual. Hum Kinetics; 2003.

23. Heath EC. ACSM’s guidelines for exercise testing and prescription. Lippincott Williams & Wilkins; 2013.

24. Mohammadi R, Farheh M, Hejazi K. Effect of eight-weeks aerobic training on serum levels of nitric oxide and endothelin-1 in overweight elderly men. Salmand. 2018;33(1):74–85. doi: 10.21859/ja3.31174.

25. Mera P, Ferron M, Mosialou I. Regulation of energy metabolism by bone-derived hormones. Cold Spring Harb Perspect Med. 2018;8(6). doi: 10.1101/cshperspect.a031666. [PubMed: 28778968]. [PubMed Central: PMC598359].

26. Garcia-Martin A, Cortes-Berdonces M, Luque-Fernandez I, Rozas-Moreno P, Quesada-Charneco M, Munoz-Torres M. Osteocalcin as a marker of metabolic risk in healthy postmenopausal women. Menopause. 2011;18(5):537–47. doi: 10.1097/gme.0b013e3181f5656e. [PubMed: 21178791].

27. Wei J, Ferron M, Clarke CJ, Hannun YA, Jiang H, Blaner WS, et al. Bone-specific insulin resistance disrupts whole-body glucose homoeostasis via decreased osteocalcin activation. J Clin Invest. 2014;124(4):1–11. doi: 10.1172/JCI72123. [PubMed: 24642469]. [PubMed Central: PMC3870909].

28. Zanatta LC, Boguszewski CL, Borba VZ, Kulak CA. Osteocalcin, energy and glucose metabolism. Arq Bras Endocrinol Metabol. 2010;54(5):444–51. doi: 10.1590/S0004-27302010000500013. [PubMed: 21566034].

29. Aldahr MHS. Bone mineral status response to aerobic versus resistance exercise training in postmenopausal women. World Appl Sci J. 2012;16(5):806-12.

30. Alghadir AH, Aly FA, Gabr SA. Effect of moderate aerobic training on bone metabolism indices among adult humans. Pak J Med Sci. 2014;30(4):840–4. doi: 10.12699/pjms.30.4.4624. [PubMed: 25097528]. [PubMed Central: PMC4127059].

31. Lin LL, Hiess SS. Effects of strength and endurance exercise on calcium-regulating hormones and calcium homoeostasis among men. J Nutr Biochem. 2013;24(2):267-75. doi: 10.1016/j.jnutbio.2012.09.005. [PubMed: 23046146].

32. Townsend R, Elliott-Sale CK, Pinto AJ, Thomas C, Scott JP, Currell K, et al. Parathyroid hormone secretion is controlled by both ionized calcium and phosphate during exercise and recovery in men. J Clin Endocrinol Metab. 2016;101(8):3233-9. doi: 10.1210/jc.2016-1848. [PubMed: 27294328].

33. Bell JW, Gehrig LA. A comparison of bone mineral density in adolescent female swimmers, soccer players, and weight lifters. Pediatr Phys Ther. 2006;18(3):190-22. doi: 10.1097/00006534-200609000-00006. [PubMed: 16508530].

34. Colletti LA, Edwards J, Gordon L, Shary J, Bell NH. The effects of muscle-building exercise on bone mineral density of the radius, spine, and hip in young men. Calcif Tissue Int. 1989;45(1):132-4. doi: 10.1007/BF00255665. [PubMed: 2504457].