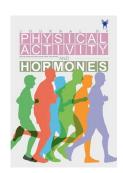


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Effect of combined exercise on bone homeostasis factors in post-menopausal women

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Abstract

Introduction: Women experience specific physiological changes and transformations throughout various life stages, including puberty, menstruation, pregnancy, lactation and menopause, which significantly impact their health and quality of life. This study aims to investigate the effect of a combined exercise program on bone homeostasis in postmenopausal women.

Material & Methods: The methodology of this study was experimental. A statistical participant of 22 postmenopausal women was divided into two groups (EG: Experimental Group; CG: Control Group). The EG engaged in three sessions of aerobic and resistance training, each lasting 90 minutes, over the course of eight weeks. During this period, the CG did not participate in any exercise programmer. Measurements taken included body mass index (BMI), waist-to-hip ratio (WHR), fat percentage, speed, agility, cardiorespiratory fitness, serum alkaline phosphatase, calcium, phosphorus, estradiol, and urinary calcium and phosphorus levels. Data analysis was conducted using paired t-tests for normally distributed data and the Wilcoxon test for non-normally distributed data, with $\alpha < 0.05$ in SPSS version 22.

Results: The results indicated a significant difference in fat percentage, WHR, cardiorespiratory endurance, speed, agility, serum calcium, urinary phosphorus, and estradiol levels (p < 0.05). However, no significant changes were observed in the remaining variables.

Conclusion: In conclusion, the findings suggest that combined training effectively reduced fat percentage and WHR, decreased BMI and serum calcium levels, and increased urinary phosphorus and estradiol levels. Additionally, it improved speed, agility, and cardiorespiratory endurance in the experimental group, while having no significant effect on alkaline phosphatase, urinary calcium, or serum phosphorus levels.

1. Introduction

In the lives of women, there are two pivotal moments: the first is the onset of menstruation, and the second is its cessation. Menopause is defined as the permanent cessation of menstruation due to the lack of ovarian follicle development despite stimulation from the gonadal glands (1). During menopause, hormone levels naturally fluctuate and decline; changes in hormones are a major factor in this transition. Estrogen levels decrease to a point where the endometrial lining is not formed, leading to the cessation of menstruation (2). After menopause, estrogens levels are

approximately 40-60% lower, and progesterone levels are nearly zero. Menopause is an undeniable phase in women's lives. Today, nearly 90% of women are menopausal by the age of 65 (3). The hormonal changes that occur during menopause lead to various symptoms and an increased risk of cardiovascular diseases and osteoporosis in postmenopausal women. According to research findings, these symptoms and diseases are the primary reasons for middle-aged women consulting healthcare professionals (4).

With advancing age, significant changes occur in the functioning of the endocrine glands, and levels of anabolic hormones such as estradiol and testosterone decline (5). The

reduction of these hormones can be a precursor to many changes in body composition that occur with ageing, including an increase in fat mass and a progressive decrease in muscle mass. The prevalence of osteoporosis increases with age, and this condition is most common in women over 50, the age at which postmenopausal women experience bone loss due to decreased estrogen and its protective effects. This disease is recognized as a significant public health issue because it can ultimately lead to bone fractures (5). Various factors, such as genetics, excessive alcohol consumption, nutritional factors, sex, age, hormonal changes, levels of physical activity, prolonged use of certain medications, and others, contribute to the development of osteoporosis. Osteoporosis is a condition in which bones become brittle, increasing the likelihood of fractures from minor impacts. The reduction in bone mass and the gradual deterioration of bone structure are major causes of fractures during menopause (6).

Another important variable indicative of bone synthesis is the total alkaline phosphatase (ALP) enzyme, which can be measured in serum blood levels (7). This enzyme appears to play a role in ossification, newly formed bone, and the production of osteoid (8).

In contrast to factors such as menopause that affect bone mass over a decade, physical activity can have a positive effect on bone mass throughout an individual's life (9). Weight-bearing activities, such as walking a mile and resistance exercises, are recommended for postmenopausal women, as these exercises help maintain bone mineral density (BMD). To date, numerous studies have examined the role of exercise on the bone health of women; however, given the differing impacts of the type and intensity of exercise on bone, this research seeks to answer the question: What is the effect of 8 weeks of combined exercise training on certain anthropometric variables, specific fitness indicators, and some blood and urinary biomarkers of bone homeostasis in postmenopausal women?

2. Methodology

2.1. Materials and methods

This quasi-experimental study employed a pre and post-test design. The statistical population of this research consisted of postmenopausal women in the city of Rasht.

2.2. Participants

Participants volunteered to take part in the study through an open call. Ultimately, 26 postmenopausal women who had no history of medication use or regular exercise and who had also experienced a complete cessation of menstruation for at least one year were selected. The individuals were divided into two groups: (EG: Experimental Group; CG: Control Group). The EG participated in concurrent resistance and endurance training for a duration of eight weeks.

2.3. Measurements

2.3.1. Anthropometry

Body Mass Index (BMI): To obtain the BMI, the height and weight of the participants were measured using a medical scale (SCA, manufactured in Japan) with an accuracy of 0.01 kg. These measurements were then applied in the following BMI formula:

$$BMI = \frac{Weight}{(Height)2}$$

Waist-to-Hip Ratio (WHR): The waist-to-hip ratio was calculated using a tape measure, with the obtained values substituted into the following formula:

$$WHR = \frac{Waist\ Circumference}{Hip\ Circumference}$$

Fat Percentage: Subcutaneous fat was measured using calipers (SAEHAN, manufactured in South Korea) at three sites (the triceps, suprailiac, and thigh). It is important to note that all measurements were taken from the right side of the body and at the same time of day. Subsequently, the subcutaneous fat percentage was calculated using the Jackson-Pollock method (13):

Body Density=1.0994921-(0.0009929×Sum of Skinfolds) + (0.000023×Square of the Sum of Skinfolds) -(0.0001392×Age)

Cardiorespiratory Endurance: This was assessed using the Rockport walking test, which provides a measure of aerobic fitness for individuals aged 30 to 69 years, with validity reported using VO2 Max and a standard error of estimate of 0.325 liters per minute. Participants walked a distance of 1600 metres on a flat surface at their maximum possible speed. Their heart rates were measured immediately after completing the 1600 metres by palpating the pulse over a duration of 10 seconds. The time taken was accurately recorded using a stopwatch, and the maximum oxygen consumption was calculated using the following formula (14):

Agility: Agility was measured using the Illinois test, which involves running in a zigzag pattern along a designated course. The course is 10 meters long and features four cones placed 3 meters apart. The participant starts in a prone position on the ground with their hands beside their shoulders. Upon hearing the signal, the tester initiates the test, and the participant runs the course at maximum speed (15).

Speed: Speed was measured using a 60-metre sprint test, with the time recorded using a Q&Q stopwatch accurate to one-hundredth of a second (16).

2.3.2. Measurement of Hormone

Hormone Estradiol: Estradiol levels were measured using the VIDAS device through a chemiluminescent method (Biomerieux kit; manufactured in France) on 200 cc of serum. The participants were centrifuged, and blood was collected from the antecubital region in a laboratory setting.

Calcium and Phosphorus Tests: Serum calcium was tested using the Elite kit (manufactured in France) via the Endpoint/MTB method; blood phosphorus was tested using the Elite kit (manufactured in France) via the Endpoint/Phosphomolybdate method; and alkaline phosphatase was tested using the Pars Azmoon kit (manufactured in Iran) via the kinetic/dimethanolamin method. All tests were conducted using the Alpha-Classic and Hitachi auto analyzer with Full Automation (manufactured by the Measurement Equipment Company and Hitachi, Japan).

Urine Tests: Urine calcium was tested using the Men kit (manufactured in Iran), and urine phosphorus was tested using the Hayyan kit (manufactured in Iran) on centrifuged urine participants using the classical method.

2.4. Intervention

2.4.1 Exercise program

The exercise protocol employed in this study was derived from the recommendations of the American College of Sports Medicine (ACSM) (10). The exercises were conducted three days a week (on Sunday, Tuesday, and Thursday) for a duration of 90 minutes. Strength training began at 50% of one-repetition maximum (1RM) and increased to 80% of 1RM. Endurance training commenced at 50% of maximum heart rate and progressed to 80%. The endurance exercises were conducted in a progressive manner. Initially, the resting heart rate of the participants was measured, after which the maximum heart rate was calculated using the Karvina formula (age – 220). In the first week, participants began exercising on a treadmill and cycle ergometer at 50% of their maximum heart rate, reaching 80% by the seventh and eighth weeks.

The 1RM was calculated indirectly for eight exercises in the experimental group. Training started at 50% of 1RM and progressed to 80%. The weight was increased by 10% every two weeks. In the first week, all eight exercises were performed with three sets of six repetitions each, and in the following seven weeks, they were performed with three sets of eight repetitions each. A rest period of 30 seconds was allowed between each set, with a rest interval of 1 minute and 30 seconds between each exercise (11).

The exercises for which the 1RM was determined included four upper-body exercises (machine bench press, machine pull-down, bicep curl, and butterfly) and four lower-body exercises (leg press, machine leg curl, machine leg extension, and standing calf raise on a machine) (12).

2.5. Statistical Methods

Data were analysed using SPSS software, version 22. Descriptive and inferential statistics were employed in this study. Descriptive statistics included appropriate indices such as mean, standard deviation, and ratio. For inferential statistics, independent t-tests were used for normally distributed data, while the Mann-Whitney test was applied for non-normally distributed data to assess data homogeneity. Subsequently, paired t-tests were used for normally distributed data and the Wilcoxon test for non-normally distributed data. A significance level of 0.05 was considered for hypothesis testing.

3. Results

The demographic characteristics of the participants are presented in Table 1.

Table 1. Demographic Characteristics of Participants (Mean ± SD)

| 1 4000 | . Demograpi | ite Characteri | sites of 1 cirricip | titis (mean = 5D) |
|--------|-------------|----------------|---------------------|-------------------|
| GP | index | age (year) | weight (kg) | height (meter) |
| EG | Mean±SD | 54.83±4.72 | 76.33±11.05 | 1.59±0.55 |
| CG | Mean±SD | 56.90±4.93 | 71.56±6.90 | 1.58 ± 0.59 |

The results of the Shapiro-Wilk test indicated that the data for the variables BMI, estrogen, speed, agility, and estradiol in the experimental group, as well as agility, speed, body fat percentage, and estradiol in the control group, did

not follow a normal distribution, while the remaining variables did.

The results shown in Figures 1 and 2 revealed significant differences between the pre-test and post-test data for body fat percentage, body mass index (BMI), and waist-to-hip ratio (WHR) in the experimental group. No significant differences were observed in the other data for this group. During this period, no significant differences were observed in the dependent variables in the control group; therefore, there were significant differences between the means for BMI, body fat percentage, and waist-to-hip ratio.

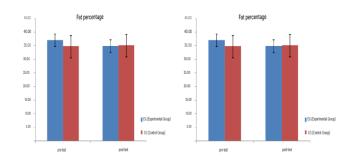


Fig 1. Comparison of average fat percentage in Two group

Fig 2. Comparison of mean WHR in Two group

The results presented in Figure 3 indicated that there was a significant difference in agility between the pre-test and post-test in the experimental group. Significant differences were also found in cardiorespiratory endurance and speed between the two groups, while no significant difference in agility was observed for the control group; thus, significant differences existed between the means for cardiorespiratory endurance, agility, and speed.

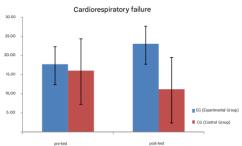


Fig 3. Comparison of average cardiorespiratory endurance two group

The results in Figures 4 and 5 demonstrated significant differences in serum calcium and urinary phosphorus levels in the experimental group when comparing the pre-test and post-test, as well as in urinary phosphorus levels in the control group. No significant differences were observed in the other variables for both groups; therefore, significant differences were noted between the means for the reduction in serum calcium levels, while no significant differences were found for the other variables.

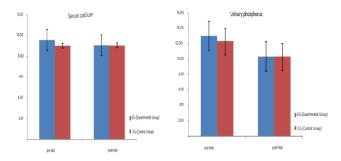


Fig 4. Comparison of serum Fig 5. Comparison of serum virial Fig 5. Comparison virial Fig 5. Co

Fig 5. Comparison of average urinary phosphorus in two group

Finally, it was shown that there were no significant differences between the means for estradiol levels.

Table 2. Summary of Research Variable Results Comparing Pre-Test and Post-Test in the Experimental and Control Groups

| Group | WHR | BMI | Fat Percentage | Speed | Agility | Cardio Respiratory | Calcium Serum | Calcium Urine | Phosphor Serum | Phosphor Urine | Alkaline | Estradiol |
|----------|-----|-----|----------------|-------|---------|--------------------|------------------|---------------|----------------|----------------|----------|-----------|
| EG CG | *↓ | *↓ | *↓ | *↓ | *↓ | *↑ | *↓ | × | × | *↓ | × | *↑ |
| CG | X | × | × | *↑ | × | *↓ | × | × | × | *↓ | × | × |

4. Discussion

The results of the study indicated that there were significant differences in body fat percentage, BMI, and WHR between the experimental group in the pre-test and post-test, one week prior to the commencement of the exercise and after the exercise. Conversely, the results showed that there were no significant differences in body fat percentage, BMI, and WHR in the control group between the pre-test and post-test, one week before the start of the exercise and after the exercise.

Among the studies that align with the present research, one examined the effect of combined exercise on body composition in 29 postmenopausal women with breast cancer, measuring WHR before and after 15 weeks of combined exercise, indicating that combined activity significantly impacted WHR (17). In another study involving 45 healthy postmenopausal women, it was found that both resistance and aerobic training resulted in significant reductions in body fat percentage (18).

In contrast to the current findings, a study on the impact of a period of resistance training accompanied by vitamin D supplementation on serum IGF-1 levels and functional muscle indices in postmenopausal women indicated that resistance training did not have a significant effect on BMI (19). Furthermore, a six-year evaluation of resistance training on body composition in 122 postmenopausal women demonstrated that strength training could significantly increase lean body mass in the experimental group compared to the control group, without affecting the weight and BMI of the women (20).

Combined training, which includes aerobic exercises, has a positive effect on reducing subcutaneous fat and, consequently, WHR and BMI. The amount of body fat accumulation is influenced by age, diet, exercise habits, and

genetics. Although genetics cannot be changed, modifications in fat stores can be achieved through diet and exercise.

The results of the study showed that there were significant differences in cardiorespiratory endurance between the experimental group in the pre-test and post-test, one week before the start of the exercise and after the exercise. On the other hand, the results indicated that there were no significant differences in cardiorespiratory endurance in the control group between the pre-test and post-test, one week before the commencement of the exercise and after the exercise. Moreover, agility and speed did not show significant differences in either group in the pre-test and post-test assessments.

Despite a search for studies on the impact of exercise on speed in postmenopausal women, none were found. Additionally, no opposing research was identified regarding the effects of combined or aerobic training on aerobic fitness. Despite the challenges associated with menopause, studies suggest that a combination of aerobic and strength training has a greater impact on cardiorespiratory endurance and VO2 max.

Given that the participants in this study were nonathletes, the aerobic exercises employed led to improvements in the cardiorespiratory performance of the participants; however, they had minimal effects on speed and agility, potentially due to the participants' unfamiliarity with the 60metre test and the limited testing conditions (restricted to two pre-test and post-test assessments).

In summary, factors such as increased muscle volume and strength, improved cardiac function, enhanced VO2 max, and increased vascular bed can be considered potential mechanisms underlying the effectiveness of combined training on cardiorespiratory endurance. Additionally, endurance training during middle age and beyond can slow the decline in lung and chest wall elasticity, resulting in increased pulmonary ventilation capacity in older adults who engage in endurance training. An increase in weight training may enhance the speed of signal transmission from muscles to neural centers and vice versa, potentially leading to faster transmission times when converting received stimuli into neural impulses and accelerating neural signals through motor neurons to the muscle, thereby reducing reaction times (21).

A study demonstrated that a 12-week programmer of selected aerobic and resistance training in water significantly affected the bone density of the lumbar vertebrae and femur in 20 obese postmenopausal women, indicating a significant difference in calcium levels (22). In another study, it was found that six months of aerobic training did not significantly affect alkaline phosphatase levels. Conversely, another study concluded that regular and prolonged physical activity, particularly exercises involving body weight, could be effective in maintaining calcium levels (23). Additionally, another study on the effects of aerobic exercise on bone metabolism markers in 19 middle-aged women found no significant changes in calcium levels after six months of aerobic training (24).

Considering the findings of this study, the lack of significance in the reduction of alkaline phosphatase may be attributed to the type of exercise applied and various other factors such as the age of the participants, intensity, duration, frequency of activity, as well as genetic, nutritional, and

hormonal factors that mediate the effects of physical activity. Furthermore, the increase in serum phosphorus and the decrease in urinary phosphorus in the control group may be due to their dietary habits prior to blood sampling.

Although resistance training is a significant stimulus for anabolic hormones, it appears that its stimulating effect occurs only momentarily during exercise. Physiologically, it has been established that physical activity and exercise influence the endocrine glands, although the mechanisms of this effect are not yet fully understood. Regarding estradiol, it can be stated that the release of this hormone from the ovaries is influenced by follicle-stimulating hormone, which is secreted by the anterior pituitary gland and enhances the growth of ovarian follicles in women and sperm production in men (25). Follicle-stimulating hormone increases with physical activity, but this increase is not substantial and does not seem to correlate with exercise intensity. Therefore, the significant increase in estradiol observed in the present study may be attributed to changes in the aforementioned mechanisms. Nevertheless, maintaining estradiol levels is essential for bone health (26).

In light of the findings, it is suggested that as women enter menopause and undergo changes in body composition and hormonal levels, regular exercise can help mitigate these changes.

5. Conclusion

The results of the study indicated that combined training leads to the enhancement of endurance adaptations. It also results in improvements in speed and agility. Regarding the impact of combined training on certain body composition factors, based on the obtained results, it can be concluded that combined training reduces body fat percentage, BMI, and WHR, thereby improving body composition in postmenopausal women. Furthermore, combined training increases estradiol and certain bone-forming factors, contributing to improved bone density.

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Conflict of interests: The authors declare that they have no conflict of interest relating to the publication of this manuscript.

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