Research Article

The effect of exercise program combined with electrical stimulation on bone mass density (BMD) and bone turnover markers (BTMs) in postmenopausal women with osteopenia

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Abstract

The aim of this study was to investigate the effects of an exercise program (EP) in combination with functional electrical stimulation (FES) on bone mass density (BMD) and bone turnover markers (BTMs) in osteopenic postmenopausal women. In this semi-experimental study, 45 women aged 61.25 ± 4.1 years who were classified as osteopenic (-2.5 < T-score < -1) were divided into three groups: 1) EP + FES, 2) EP and 3) control. The EP + FES and EP groups participated in a combined aerobic (45-60% HRR) and strength program for 90 minutes three times a week for 12 weeks. In the EP + FES group, FES with a frequency of 45 Hz and a pulse width of 300 microseconds was applied to the lumbar and hip area. Blood samples were taken at the beginning of the study and again after 12 weeks to determine BMDs. BMD was measured using dual-energy X-ray absorptiometry (DXA). After 12 weeks, cross-linked type 1 collagen C-telopeptide (sCTX) (p=0.002) and pyridinoline (PYD) (p=0.001) levels decreased significantly, while vitamin D (p=0.002), PINP/PYD ratio (p=0,032), the ALP/PYD ratio (p=0.004) and the ALP/CTX-1 ratio (p=0.010) increased significantly in both the EP+FES and EP groups compared to the control group. The EP+FES group showed a significant increase in lumbar (p=0.048) and hip BMD (p=0.038) compared to the control group. Therefore, an exercise programme in combination with FES is recommended as the preferred intervention to maintain or improve bone formation, as FES has a synergistic effect on bone health in patients with osteopenia.

Key Words: Bone turnovers, BMD, Electric stimulation therapy, Exercise therapy, Osteopenia, Vitamin D

Introduction

Osteopenia (OS) is a bone metabolism disorder that precedes osteoporosis (OP) and is characterized by T-score values between -1.0 and -2.5. The prevalence of OP is higher in Asian women, and was estimated at 25-38% compared to 9-16% in Western populations. OS mainly affects postmenopausal women and increases their susceptibility to fractures (Riaz, Shakil Ur Rehman, Hassan, & Hafeez, 2024). Studies suggest that the incidence of fractures in people with OS is similar or even higher than in people with OP (Shin S, 2024). In postmenopausal women, estrogen deficiency leads to decreased bone mineral density (BMD) and increased bone fragility, which affects their social and physical health. About half of older women suffer from pain and disability, affecting their quality of life and health-related functions (Riaz et al., 2024). In addition, aging and menopause lead to an imbalance between bone resorption and formation (Sözen, Özışık, & Başaran, 2017). In postmenopausal women, bone turnover has a significant influence on bone mass, with high turnover being associated with low bone mass. Consequently, the determination of bone marker levels can help to assess the risk of osteoporosis (Garnero, Sornay-Rendu, Chapuy, Delmas, & research, 1996). Biomolecules released during bone resorption and formation are called bone turnover markers (BTMs). These markers are valuable tools for assessing the dynamics of bone remodelling, particularly with regard to bone formation and resorption. The availability of reliable, inexpensive, sensitive and specific assays for BTMs could improve the management of OP by complementing BMD measurements. BTMs are usually measured in blood and urine and are categorized into two types: Markers of bone formation and markers of bone resorption. The International Osteoporosis Foundation and the International Federation of Clinical Chemistry and Laboratory Medicine recommend the use of cross-linked type 1 collagen C-telopeptide (CTX-1) and N-terminal pro-collagen type 1 peptide (P1NP) in serum as reference markers for bone resorption and formation, respectively, to assess fracture risk and monitor ther-

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-rapy in the clinical setting (Schini, Vilaca, Gossiel, Salam, & Eastell, 2023; Shetty, Kapoor, Bondu, Thomas, & Paul, 2016). Serum alkaline phosphatase (ALP) is an important marker for bone formation, which is essential for osteoid formation and mineralization. Changes in ALP levels can indicate bone growth, repair and regeneration. It is the most important biomarker used in both clinical and research settings, and monitoring ALP levels can aid in the early detection of bone metabolic disorders such as OP (Cheng & Zhao, 2023; Shetty et al., 2016). CTX-1 makes up over 90% of the bone matrix. Analysis of collagen cross-linking molecules such as pyridinoline (PYD) provides valuable insight into bone resorption (Ureña et al., 1995). The most reliable markers of bone resorption are collagen degradation products. PYD, a by-product of collagen degradation, has been validated as an effective marker of bone resorption (Camacho & Lopez, 2008).

The prevention and treatment of OP usually involves both pharmacological and non-pharmacological approaches. Regular physical activity is an effective, safe, and inexpensive way to delay the onset of OP. Medication does not affect key fracture risk factors such as muscle strength, power, dynamic balance, coordination and overall functional performance, all of which contribute to an increased risk of falls and fractures. For older adults with OP, multicomponent training— that includes progressive resistance, weight-bearing impact training and balance exercises is recommended (Mineiro et al., 2024). A systematic review and meta-analysis found that multicomponent training that combines two to four types of exercise such as strength, aerobic, balance, flexibility and functional fitness improves strength, flexibility, quality of life, bone mineral density, balance and functional readiness and reduces the risk of falls in older women with osteoporosis (Linhares et al., 2022). Results suggest that a six-month program of aerobic, resistance, and balance training has a positive effect on bone density by altering serum bone metabolism, even in middle-aged adults (Kim, Lee, Shim, & Choi, 2022). Various studies have investigated the effects of exercise on bone formation and have come to different conclusions. More recently, the effects of electrical stimulation on bone formation and improvement in BMD have also been investigated (Guo et al., 2022; Lai et al., 2010b; W. Zhang et al., 2023). Electrical stimulation, which promotes bone growth through various currents such as direct current, biphasic current and alternating current induces osteogenesis via mechanisms such as the inverse piezoelectric effect, Up-regulating calcium level, regulation of growth factors and changing local microenvironment, which ultimately promotes ossification (Shuai et al., 2018). Functional electrical stimulation (FES) is a technique that uses weak electrical currents to activate nerve fibers that cause muscle contractions and stimulate movement (Armengol et al., 2022). The effect of FES on BMD has been investigated (Armengol et al., 2022; BeDell, Scremin, Perell, Kunkel, & rehabilitation, 1996); but, evidence for the ability of FES to alter markers of bone turnover is still limited.

Intensive exercise training to stimulate BTMs in older women with OP poses a problem of safety and feasibility. Therefore, the combination of low to moderate intensity exercise and FES may promote bone formation in this population. This method offers an effective and non-invasive treatment for OP. However, despite numerous studies highlighting the benefits of exercise and FEM for bone health, the optimal treatment parameters such as the frequency and duration of FEM and the intensity and type of exercise remain unclear and require further investigation. Therefore, this study aims to investigate the effects of exercise alone and in combination with FES on OP.

Materials and methods

Study design and participants

This quasi-experimental study used a pre-test-post-test design with three groups and was conducted from 2023 to 2024. The study population included postmenopausal women with reduced bone mass (osteopenia), defined by a BMD-T score for the hip and lumbar spine between -2.5 and -1, who were referred to the Imam Reza Rehabilitation Center in Tabriz, Iran. The sample size was calculated using GPower3.1 software for F-tests, ANOVA and repeated measures, aiming for a statistical power of 0.80, an alpha level of 0.05, an effect size of 0.25, three groups and two measurements. This resulted in a required sample size of 42. 45 subjects were selected and randomly assigned to three groups using a simple lottery procedure in order to take the attrition rate into account: Functional Electrical stimulation plus combined exercise training programme (FES+EP, 15 subjects), combined exercise training programme (EP, 15 subjects) and a control group (15 subjects). In the lottery, the names of the 45 test subjects were placed in a bag. The first 15 subjects selected were assigned to the ECT group, the next 15 to the CT group and the remaining 15 to the control group. The groups were homogeneous in terms of age, BMI and BMD (Table 1). The data analyst and lab technicians have been blinded to the group allocations, which are coded as 1, 2, and 3.

The inclusion criteria for the study included: confirmed osteopenia by dual-energy X-ray absorptiometry (DXA) according to World Health Organization (WHO) criteria, an age between 55 and 65 years, at least two years post menopause and the absence of secondary osteopenia as well as the absence of certain diseases such as chronic kidney disease, Cushing's syndrome, hyperparathyroidism, hyperthyroidism, hypogonadism, diabetes, cancer, rheumatoid arthritis and the use of medication such as corticosteroids or thyroid hormones. Participants also had to be free from alcohol or tobacco use,

severe systemic or cardiovascular disease, significant mental disorders, sensory impairment, depressive or emotional problems, pain or mobility problems in the hip and lower back, and must not have exercised or dieted regularly in the previous six months (Dizdar, Irdesel, Dizdar, & Topsaç, 2018).

Exclusion criteria included irregular adherence to the exercise protocol and voluntary withdrawal from the study, as well as any changes in diet or medication during the study period. Women who were taking medications to treat low bone density, such as bisphosphonates, alendronate, calcium and vitamin D3, as well as blood pressure medications such as losartan and metoprolol. Patients were instructed to adhere to their diet during the three-month study and report any changes in their medication type or dosage. Their dietary intake was monitored with a three-day food diary.

Data collection

At the participants' first visit to the laboratory, demographic data were collected, including age, history of OP, other medical conditions, participation in physical activities, medication use, sun exposure, and anthropometric measurements. BMI (kg/m2) was measured using the Omron BF511 body composition scale. At the second visit, the participants fasted for 12 hours and did not take any medication that affects BTMs for 24 hours. Blood was taken between 8 and 9 am. A total of 10 cc of blood was taken from the brachial vein and placed in tubes containing 3-4 mg of ethylenediaminetetraacetic acid (EDTA) as an anticoagulant. The samples were then centrifuged to separate the plasma, which was subsequently frozen at -70°C for future analysis. BMD in the lumbar spine and hip was determined in the radiology department of Sina Hospital in Tabriz using dual-energy X-ray absorptiometry (DXA). The training protocol began 48 hours after blood collection, and the posttest evaluation was performed 72 hours after completion of the 12-week training program, using the same procedures as the pretest.

Biochemical variables

PINP levels were measured using the Human Total Procollagen Type I Intact N-terminal Kit from ZellBio GmbH (Germany), catalog number ZB-12129C-H9648, based on a dual antibody sandwich assay technology. The sensitivity of this kit for the detection of PINP was 9 nanomoles per liter, with a detection range of 75 to 2400 nanomoles per liter. ALP was measured using the commercial kit ALP p-nitrophenyl phosphate, DGKC from Delta Darman Part-Iran, by an enzymatic calorimetric method. This kit has a measuring range of 5 to 1000 international units per liter. For samples with ALP values greater than 1000 international units per liter, the sample was diluted 1:9 with physiological saline and the test result was multiplied by 10.

PYD was quantified using the commercial kit from ZellBio GmbH (Germany), which uses a dual-antibody sandwich technology to measure human PYD. The sensitivity of this kit is 0.75 nanomoles per liter, with a detection range of 6.25 to 200 nanomoles per liter. CTX-1 was also measured using the kit from ZellBio GmbH (Germany), which requires a dual antibody sandwich ELISA based on biotin technology. This kit has a measurement range of 5 to 160 nanograms per milliliter and a sensitivity of 0.6 nanograms per milliliter.

The calcium content was determined using the Arsenazo III kit from Delta Darman Part-Iran. The measurement was performed at a wavelength of 660 nanometers by a one-point calorimetric method with a range of 1 to 20 milligrams per deciliter. If the calcium level exceeded 20 milligrams per deciliter, the sample was diluted 1:4 with saline and the result was multiplied by 5. Vitamin D3 was measured with the 25 (OH) D vitamin D kit from Pars Pyvand-Iran using the ELISA method. The ratios of ALP and PINP (anabolic indicators) to CTX1 and PYD (catabolic indicators) were calculated to investigate the possible influence of the intervention of exercise training and electrical stimulation on the state of bone metabolism.

Intervention program

The FES+ EP and EP groups participated in a 12-week program and exercised three days a week for 60 to 90 minutes per session. The FES+ EP group received 12 sessions of electrical stimulation in addition to the EP intervention. The control group did not participate in any of the interventions and went about their normal daily activities and did not participate in any regular exercise program.

Combined exercise training program

In this study, the exercises were performed in accordance with the American College of Sports Medicine (ACSM) guidelines for patients with OP. Although there are currently no established guidelines regarding contraindications to exercise in people with OP, the following special considerations have been taken into account:

• Moderate intensity exercises were performed to avoid aggravation of pain. Explosive movements, exercises with heavy loads and activities requiring twisting, bending or excessive pressure on the spine were avoided.

• Exercises requiring forward flexion of the spine, such as rowing, were avoided.

• Ballistic movements were excluded from the programmed.

• Musculoskeletal risks such as acute or chronic back pain, rheumatoid arthritis and inflammation/pain were monitored and

participants were referred to a physician if needed.

• Participants were trained to use the Rate of Perceived Exertion (RPE) scale and other appropriate subjective rating scales, such as for breathlessness, pain, limping and angina, to report any problems during exercise.

• Because most people with OP are older and sedentary, they are generally at moderate to high risk for atherosclerotic disease; therefore, cardiovascular health assessments were prioritized before beginning the exercise program.

• The initial training sessions emphasized proper exercise techniques, particularly those involving equipment, as well as rehabilitation exercises directed by the physiotherapist.

Based on the research guidelines on the effects of different types of exercise on bone mass and metabolic markers, the following exercises were combined in this study:

Aerobic exercise: After a 10-minute warm-up consisting of slow and brisk walking combined with stretching exercises, participants completed 45 minutes of aerobic exercise at low to moderate intensity (45-60% HRR, 11-13 RPE). Aerobic training started with 30 minutes in the first week and was gradually increased by adding 5 minutes every three weeks and increasing the intensity by 5% every three weeks (Behrens et al., 2017; Wen, Huang, Li, Chong, & Ang, 2017). The aerobic training program is divided into three main parts:

1) Aerobics (marching in place, walking forward, walking backward, walking sideways, V-steps, touch steps).

2) Step board exercises (alternate basic step up, mountain climbers, alternate lateral step up, glute bridges, burpees)

3) Mini trampoline exercises (Aragão, Karamanidis, Vaz, Arampatzis, & kinesiology, 2011).

The Karvonen formula, also known as the heart rate reserve (HRR) method, is used to determine the target heart rate zones for exercise (THR), taking into account the resting heart rate (RHR) and maximum heart rate (MHR).

To calculate the MHR: MHR = 220 - age

Then calculate the HRR: HRR = MHR - RHR

Finally, determine the THR: THR = (HRR × Intensity %) + RHR

Resistance exercises: The resistance training consisted of 30 minutes of exercises targeting the core muscles (Elnaggar, Mahmoud, Moawd, & Azab, 2021; Stunes et al., 2022; Zhong, 2022) and the back extensors (Çergel, Topuz, Alkan, Sarsan, & Sabir Akkoyunlu, 2019). This type of training has been shown to affect markers of bone metabolism (Gombos et al., 2016).

The exercises consisted of several stations using the patient's body weight, a physioball and a yellow resistance band. Participants completed three sets of 8-15 repetitions per set, with a 30-second break between sets. The training load was increased over several weeks by increasing the number of repetitions from 8 to 15 and the number of stations from 5 to 10, adapted to the progress and abilities of each individual patient. The selected resistance exercises included Mountain Climber, Dead Bug, Side Plank, Bird Dog Plank, Hand-to-Foot Stability Ball Pass, Superman, Single-Leg Bridge, Cycle Crunches with a Resistance Band, Deadlift with a Resistance Band, Cobra Stretch, Butterfly Stretch with a Resistance Band, and Breathing Cat-Camel.

Electrical stimulation (ES)

The FES+ EP group participated in a three-day exercise training program supplemented with ES in weeks 7 to 10. This included three sessions per week led by a rehabilitation physician. FES was performed for 20-30 minutes at a frequency of 45 Hz, Current Amplitude 20 -40 mA, and a pulse width of 300 microseconds using an available dual-channel STIMULATOR 620 P device (NOVIN). The stimulation targeted the paraspinal muscles of the lumbar spine and the hip area. The duration of stimulation in each area was increased from ten minutes in the first two weeks to fifteen minutes in the last two weeks. The current amplitude was gradually increased for each subject to induce muscle contraction and then maintained at a constant level during stimulation. In this study, the intervention dose was determined and administered according to the prescription of the physician at the Research Center for Physical Medicine and Rehabilitation.

During the treatment, the patient lay face down on the bed. The electrodes were covered with four German-made 10x10 cm sponge pads moistened with water. In addition, a heating pad was heated with an electric device and placed over the electrodes. The electric current was then gradually introduced. Electrodes were attached to both sides of the lumbar spine for the lumbar paraspinal muscles. For the pelvic muscles, one electrode was placed on the prominence of the gluteal muscle and the other on the greater trochanter of the thigh on each side.

Statistical analysis

After confirming that the data followed a normal distribution using the Shapiro-Wilk test, we compared the percentage changes between the three groups using a one-way analysis of variance and Tukey's follow-up test to identify significant differences. To calculate the percentage change for each variable, we subtracted the pre-test value from the post-test value, divided the result by the pre-test value, and then multiplied by 100 (Rezaei, Torkaman, MOVASSEGHE, Hedayati, & Bayat, 2012). A significance level of $p < 0.05$ was considered. Comparison of percentage change

between groups showed acceptable results compared to other techniques, such as mixed ANOVA.

Results

For this study, 45 patients were selected after screening. The data analysis included 41 patients (13 in the FES+ EP group, 14 in the EP group and 14 in the control group), as 4 patients withdrew (3 from the intervention groups and 1 from the control group). In the intervention groups, the reason for withdrawal was irregular participation (less than 32 training sessions), while in the control group the reason was non-participation in the post-test evaluations. Under baseline conditions, the results of the ANOVA test showed that age, BMI and BMD were similar in all groups (p>0.05) (Table 1).

Table 1. Basic characteristics of the study participants.

Parameter	FES+ EP (n = 13)	EP (n = 14)	Control (n = 14)	P-value
	$\bar{x}$ ±SD		$\bar{x}$ ±SD	
Age (years)	62.78 ± 3.62	60.62 ± 4.21	61.57 ± 4.0	0.119
Body mass index (Kg/M^2)	29.15 ± 3.25	30.15 ± 3.54	29.13 ± 4.65	0.513
BMD Hip (g/cm^2)	0.706 ± 0.110	0.685 ± 0.119	0.664 ± 0.118	0.719
BMD Lumbar (g/cm^2)	0.790 ± 0.058	0.801 ± 0.093	0.820 ± 0.092	0.723

Data expressed in means ± SD, * ANOVA test, P<0.05

Table 2. The mean of markers of bone metabolism, calcium and vitamin D before (week0) and after the 12-week study period.

Variables		FES+ EP (n=13)		EP (n=14)		Control (n=14)	
		Week 0	Week 12	Week 0	Week 12	Week 0	Week 12
Bone Turnover Markers	PINP (ng/mL)	269.23±23.3	268.95±26.1	281.30±25.4	278.96±27.4	250.43±38.6	247.64±36.1
	ALP (u/l)	177.50±45.2	172.10±46.4	188.38±9.27	187.09±9.93	166.56±19.5	159.64±21.9
	CTX-1 (nmol/l)	26.45±2.6	24.39±2.6	27±3.1	25.26±2.9	27.28±4.3	28.82±3.4
	PYD (nmol/l)	33.90±4.1	27.92±2.4	30.91±2.5	27.26±4.5	30.37±4.7	31.37±4.5
Calcium (mg/dl)		9.28±0.42	9.39±0.22	9.38±0.27	9.41±0.20	9.15±0.21	9.11±0.31
vitamin D (ng/ml)		41.80±7.60	49.91±6.16	43.80±2.67	48.88±3.63	41.92±8.17	39.26±7.46
BMD Lumbar (g/cm^2)		0.7908±0.05	0.8597±0.02	0.8016±0.09	0.8078±0.10	0.8206±0.09	0.8135±0.07
BMD Hip (g/cm^2)		0.6515±0.05	0.7066 ± 0.11	0.6671±0.10	0.6854±0.11	0.6983±0.16	0.6642±0.11

Data expressed in Mean ±SD

Table 3. Comparison of changes in markers of bone metabolism, calcium and vitamin D for the FES+ EP, EP, and control groups following 12 weeks of exercise training with electrical stimulation program

Variables (Δ %)	Groups			*P-value	P-value+ (group competition, Tukey)
	FES+ EP	EP	Control		
	Δ %means ± SD	Δ %means ± SD	Δ %means ± SD		
PINP (ng/mL)	0.676±13.58	-0.833±3.65	0.172±16.64	0.324	-
ALP (u/l)	-2.85±11.07	-0.46±7.31	-3.26±14.76	0.786	-
CTX-1 (nmol/l)	-7.39±9019	-7.78±10.46	7.49±14.42	0.002 *	FES+ EP VS control =0.007* EP VS control = 0.005* FES+ EP VS EP = 0.996
PYD (nmol/L)	-16.68±11.09	-10.98±18.72	3.90±10.04	0.001 *	FES+ EP VS control =0.001* EP VS control = 0.020* FES+ EP VS EP = 0.542
PINP/ CTX-1	9.71±19.31	8.77±12.41	-3.89±16.48	0.158	-
PINP/ PYD	22.87±22.76	15.75±23.24	-1.82±22.30	0.032*	FES+ EP VS control =0.031* EP VS control = 0.147 FES+ EP VS EP = 0.726
ALP/ CTX-1	5.98±16.49	9.41±16.05	-8.77±14.59	0.010*	FES+ EP VS control =0.049* EP VS control = 0.011* FES+ EP VS EP = 0.840
ALP/ PYD	17.89±16.10	15.18±18.90	-5.44±20.16	0.004 *	FES+ EP VS control =0.006* EP VS control = 0.015* FES+ EP VS EP = 0.924
Calcium (mg/dl)	1.24±3.72	0.27±2.89	-0.48±3.24	0.235	-
vitamin D (ng/ml)	18.67±7.47	12.39±7.39	-5.93±11.70	0.002 *	FES+ EP VS control = 0.00* EP VS control = 0.020* FES+ EP VS EP = 0.622
Lumbar BMD (g/cm^2)	0.0689±0.068	0.0062±0.054	-0.0071±0.080	0.045*	FES+ EP VS control =0.048* EP VS control = 0.802 FES+ EP VS EP = 0.121
Hip BMD (g/cm^2)	0.0551±0.069	0.0183±0.055	-0.0341±0.098	0.047*	FES+ EP VS control =0.038* EP VS control = 0.291 FES+ EP VS EP = 0.536

Δ % percentage changes: [(posttest value- pretest value)/ pretest value] *100, Data expressed in means ± SD *: P values assessed by one-way ANOVA +: P values assessed by post hoc Tukey, * P<0.05

Table 2 presents the average values of variables across the three study groups. To assess the impact of the exercise protocol combined with electrical stimulation, we calculated and compared the percentage changes of these variables between the groups (Table 2).

Table 3 presents the percentage changes in variables and differences between groups. There were no significant differences in PINP, ALP, PINP/CTX-1, or calcium across the three groups ($p>0.05$). However, significant differences were noted in the levels of CTX-1, PYD, PINP/PYD, ALP/PYD, ALP/CTX-1 and vitamin D among the groups ($p<0.05$). Post hoc Tukey results indicated that both the FES+ EP and EP groups had a significant decrease in CTX-1 and PYD levels compared to the control group ($p<0.05$). Additionally, the ratio of ALP to PYD, ALP/CTX-1 and vitamin D significantly increased in both treatment groups relative to the control. The PINP/PYD ratio and lumbar and Hip BMD significantly increased only in the FES+ EP group compared to the control group ($p<0.05$) (table 3).

Discussion

This study is the first to investigate the effects of EP in combination with FES therapy on BMD and biochemical markers of bone turnover in postmenopausal women with osteopenia. In summary, 12 weeks of EP whether alone or with FES reduced markers of bone resorption but had no effect on circulating biomarkers of bone formation. On the other hand, electrical stimulation had a synergistic effect on increasing BMD.

A longer duration may be required to stimulate bone formation in response to exercise training, although the exact time frame is still unclear. Civil et al (2023) found that in healthy adult men, acute treadmill exercise (60-120 minutes at 65-75% $\dot{V}O_2max$) resulted in an immediate increase in PINP levels, followed by a decline from 1 hour to 4 days after exercise. This transient increase in circulating PINP could be due to its release from connective tissue or to changes in blood flow (Civil et al., 2023). Long-term exercise training can deliver different results compared to acute or short-term training sessions. Almstedt et al (2016) (Almstedt et al., 2016), demonstrated that combined training including cardiovascular, resistance, abdominal and stretching exercises resulted in a significant reduction in PINP levels after 13 weeks and 28% after 26 weeks. Their study found

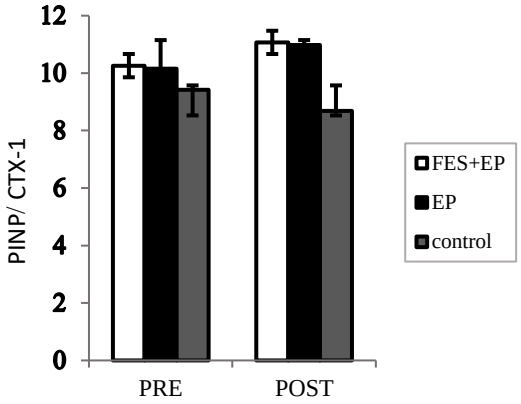

Figure 2. Ratio of procollagen type 1 N-terminal propeptide (PINP) to C-Terminal Crosslinking Telopeptide of Type I Collagen (CTX-1) for the multi-component exercise plus electrical stimulation (FES+ EP), multi-component exercise (EP)and control (CON) groups measured before (PRE) and after (POST) the 12 weeks study period. The values are mean± SE.

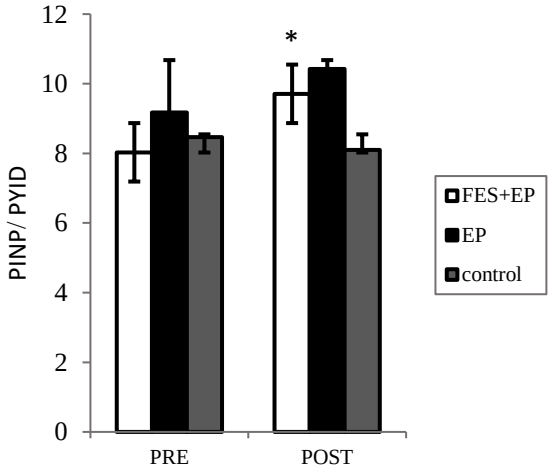

Figure 1. Ratio of procollagen type 1 N-terminal propeptide (PINP) to pyridinoline cross-links (PYD) for the multi-component exercise plus electrical stimulation (FES+EP), multi-component exercise (EP)and control (CON) groups measured before (PRE) and after (POST) the 12 weeks' study period. The values are mean± SE. *P<0.05 vs CON.

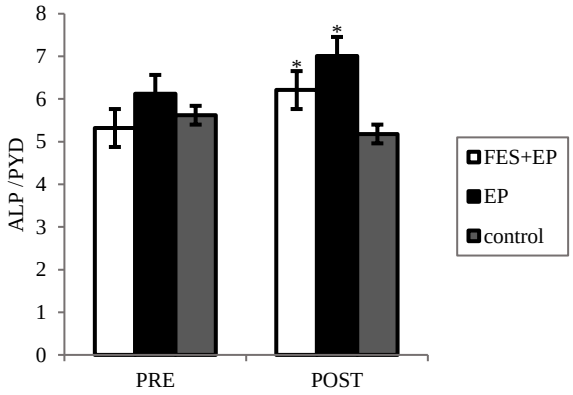

Figure 3. Ratio of alkaline phosphatase (ALP) to pyridinoline cross-links (PYD) for the multi-component exercise plus electrical stimulation (FES+EP), multi-component exercise (EP) and control (CON) groups measured before (PRE) and after (POST) the 12 weeks' study period. The values are mean± SE. *P<0.05 vs CON.

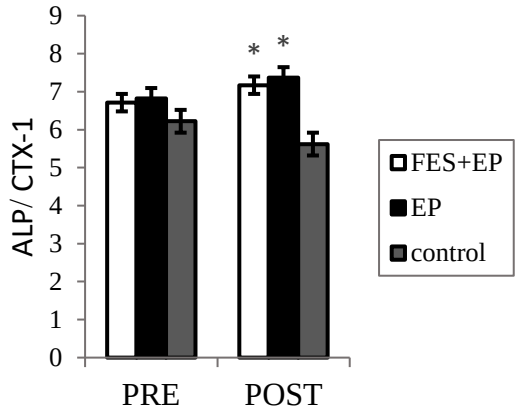

Figure 4. Ratio of alkaline phosphatase (ALP) to C-Terminal Crosslinking Telopeptide of Type I Collagen (CTX-1) for the multi-component exercise plus electrical stimulation (FES+ EP), multi-component exercise (EP) and control (CON) groups measured before (PRE) and after (POST) the 12 weeks study period. The values are mean± SE. *P<0.05 vs CON.

that the increase in BMD was accompanied by a decrease in P1NP levels. They hypothesis that reduced bone resorption, indicated by the increase in BMD with a simultaneous decrease in P1NP and CTX, could improve bone health, particularly in menopausal people. In a parallel study, Chai et al. (2018) (Çay et al., 2018) found no significant changes in PINP levels after 10 weeks of combined aerobic and resistance training. Due to the contradictory results, further research is needed to determine the minimum effective exercise intensity and duration for postmenopausal women, taking into account their age and fitness level.

Bone cells are the main source of ALP. Exercise, which exerts mechanical pressure via cell signaling, appears to increase ALP gene expression in osteoblasts (PeriĆ et al., 2018). However, studies suggest that physical exercise whether through combined endurance and resistance training (Skrypnik et al., 2016) or through flexibility exercise (Uadia, Orumwensodia, Arainru, Agwubike, & Akpata, 2016) does not significantly affect serum ALP levels. Electrical stimulation via the skin alone also showed no significant effect on ALP levels (Guo et al., 2022). In this study, the combination of EP and FES also did not alter serum ALP levels. Several factors could contribute to this lack of effect, including the intensity and duration of exercise and electrical stimulation, as well as changes in BMD (Tariq, Tariq, Lone, & Khaliq, 2019) and fat mass (Skrypnik et al., 2016). ALP is a strong predictor of BMD in osteopenic postmenopausal women, with elevated serum ALP levels indicating a possible decline in BMD (Tariq et al., 2019). In young adults, a negative correlation was observed between serum total ALP and lumbar BMD (Shu, Tan, Li, Huang, & Yang, 2022). In addition, ALP activity tends to increase with abdominal fat mass (Skrypnik et al., 2016).

Therefore, factors such as insufficient intensity and duration of exercise or electrical stimulation as well as changes in body fat mass or BMD may have influenced the results in this study. The increase in BMD in the intervention groups of this study may explain the absence of an increase in P1NP and ALP levels. In this study, BMD increased significantly only in the FES+ EP group compared to the control group, demonstrating the synergistic effect of electrical stimulation on bone mass improvement. Research suggests that FES with a pulse rate of 20 Hz and a pulse duration of 300 µsec for three months (Lai et al., 2010a), FES with a pulse rate of 33.3 Hz and a pulse duration of 350 µsec for 12 weeks (Gorgey et al., 2025), or FES with a pulse rate of 50 Hz and a pulse width of 300-400 µsec for 12 months (Frotzler et al., 2008) can maintain or improve BMD. While several studies have shown the long-term benefits of electrical stimulation for maintaining or increasing bone mass, our results suggest that combining short-term electrical stimulation with exercise training may also promote bone formation and increase BMD. This combined intervention offers a practical advantage over previous studies as it is more accessible and cost-effective for many people in the community, especially considering the difficulties in accessing rehabilitation centres and the need for prolonged electrical stimulation.

Multicomponent exercises that include strength, aerobic, and weight-bearing exercises together with whole-body vibration either alone or in combination may help to increase or at least prevent the decline in bone mass associated with ageing, particularly in postmenopausal women (Gombos et al., 2016; Gómez-Cabello, Ara, González-Agüero, Casajús, & Vicente-Rodriguez, 2012). In the present study, the intervention groups showed a significant reduction in CTX-1 and PYD levels. However, the results on CTX-1 and PYD are contradictory. In line with the present study numerous studies reported a decrease in CTX-1 after one session of strength training (Gombos et al., 2016) and after two months of regular submaximal aerobic training (Alp, 2013). In addition, a significant reduction in serum PYD levels was observed in postmenopausal women after six weeks of aerobic exercise (Ibeneme, Uzoho, Ibeneme, & Nna, 2015). However, some inconsistent studies, have shown no change in CTX-1 after moderate-intensity brisk walking (Gombos et al., 2016), five weeks of endurance training (Banfi, Lombardi, Colombini, & Lippi, 2010), or three months of strength training (Pasqualini et al., 2019). Similarly, no change in serum PYD levels was observed after six months of resistance training (Vincent & Braith, 2002), while an increase was observed after six weeks of resistance training (Shen et al., 2007). A key aspect of the training programme in this study that could effectively address the variables identified in the conflicting research is the unique combination of exercises. Previous studies have shown that exercise duration alone does not lead to significant changes.

Consequently, the discrepancies in results can be attributed to factors such as exercise intensity, type or combination of exercises, gender, age, length of time subjects have been menopausal and different measurement methods.

The mechanisms by which exercise affects CTX and PYD levels include changes in calcium metabolism due to muscle contractions and stimulation of parathyroid hormone, which regulates calcium balance and affects bone resorption markers (Haryono et al., 2017). Another important mechanism could be changes in blood vitamin D levels. Research suggests that improving vitamin D status in postmenopausal women leads to a significant reduction in serum CTX and PINP levels (Nahas-Neto et al., 2018). In this study, vitamin D levels increased significantly in the FES+EP and EP groups compared to the control group. Previous studies have reported that both aerobic and strength training significantly increase vitamin D levels (Çay et al., 2018; Dr. Bell et al., 1988). Observational studies consistently show that people who report higher levels of physical activity have higher circulating vitamin D concentrations. While confounding factors such as increased UV exposure during outdoor activities may influence this relationship, it is noteworthy that increases in serum vitamin D have also been observed without UV exposure, e.g. during indoor exercise or in the winter months when vitamin D synthesis is limited. This suggests that physical activity may have a direct effect on serum vitamin D levels (Ella, 2024). Submaximal aerobic exercise is thought to stimulate the release of vitamin D, which is stored in adipose tissue as a by-product of lipolysis (J. Zhang & Cao, 2022). In addition, research suggests that the lipolytic activity of resistance training prior to endurance training promotes the mobilization of vitamin D from adipose tissue (Elsayyad LK, 2021). Due to its lipophilic properties, vitamin D accumulates to a considerable extent in fatty tissue. In obesity, this accumulation can lead to lower circulating vitamin D levels. However, new evidence suggests that exercise-induced lipolysis may help to mobilize vitamin D from adipose tissue into the bloodstream. Thus, exercise could be an effective strategy to improve vitamin D status (Ella, 2024). Muscle tissue is an important target for vitamin D and an extravascular storage site. The release of 25 (OH) D from skeletal muscle is regulated by the vitamin D receptor, parathyroid hormone (PTH) and vitamin D-binding protein (VDBP). Chronic resistance training can affect serum vitamin D levels by increasing muscle mass and altering VDR, PTH and VDBP levels (J. Zhang & Cao, 2022). Although vitamin D primarily promotes intestinal calcium absorption, this study found that calcium levels did not increase significantly in the FES+ EP and EP groups despite increasing vitamin D levels. Specifically, the percentage changes in calcium levels were 1.24±3.72 for FES+ EP and 0.27±2.89 for EP, compared with -0.48±3.24 in the control group (Table 2). Acute exercise generally lowers serum calcium levels due to increased calcium utilization and shifts in the electrolyte concentrations and acid-base balance (Barry & Kohrt, 2007). Long-term interventions, such as one year of resistance training, did not result in significant changes (Dr. Bell et al., 1988). In addition, transcutaneous electrical stimulation (TEAS) showed a non-significant increase in calcium levels (Guo et al., 2022). Consistent with our results, previous studies have shown that a six-week intervention combining resistance training and pulsed electromagnetic stimulation did not alter blood calcium ion levels in postmenopausal women with early osteoporosis (Rezaei et al., 2012). The small sample size and variations in osteoclast and parathyroid hormone levels (Farajtabar Behrestaq, 2023) may have influenced these results. Future studies with larger samples and additional variables, such as parathyroid hormone levels, could provide more definitive conclusions.

Markers of bone formation and resorption alone may not provide an accurate indication of whether increased turnover leads to anabolism or catabolism of bone during remodeling. In contrast, BMD, which reflects bone strength, together with the ratio of bone formation to resorption (PINP/CTX-1, PINP/PYD, ALP/CTX-1, ALP/PYD, as analyzed in this study) serves as a better indicator of the status of bone turnover (Shen et al., 2007). The reduction in resorption markers in conjunction with stable formation markers (PINP/ALP) indicates a favorable outcome of this study, suggesting a shift towards increased bone formation. The results of our study showed a greater increase in PINP/PYD, ALP/CTX-1 and ALP/PYD ratios from baseline to week 12 in the FES+ EP and EP groups compared to the control group. Bone biomarkers provide valuable insights into bone turnover, particularly in short-term interventions where changes in BMD may not yet be apparent.

The response of BTMs to exercise involves a complex interplay of local responses mediated by cytokines and systemic signals such as hormones. This complexity may be due to the dual role of bone as a structural support and mineral store. In addition, physical activity can affect bone metabolically and mechanically in different ways depending on the type, intensity and duration of activity. Therefore, it is not surprising that different exercise challenges lead to contradictory responses in bone markers (Dror et al., 2021).

Due to the high prevalence of osteoporosis in postmenopausal women in Tabriz, Iran (Hemmati et al., 2021), the implementation of combined exercise programs both independent and with electrical stimulation in health centers could be more effective in the treatment of osteoporosis. It is therefore recommended that these combined exercises be introduced in health centers to rehabilitate people with osteopenia.

Limitations of the study include the difficulty of accounting for seasonal changes and exposure to sunlight. Participants were instructed not to change their sunlight behavior during the study. Another limitation of this study was the choice of a sample size

with a mean effect size of 0.25, which was limited by the nature of the exercise intervention and limited access to a larger sample of participants. This small sample size may affect the significance of differences between groups, so future studies should aim for a larger sample size. In addition, due to the nature and duration of the 12-week intervention, it was not possible to blind the participants.

Conclusion

The present study shows that a 12-week exercise program, both alone and in conjunction with electrical stimulation, significantly reduces biomarkers of bone resorption and increases levels of vitamin D, PINP/PYD, ALP/CTX-1 and the ALP/PYD ratio in postmenopausal women. These results suggest a shift in bone formation versus resorption as FES synergistically improves BMD and overall bone health in patients with osteopenia. Therefore, combining exercise with FES is recommended as an optimal intervention to maintain or improve bone formation.

What is already known on this subject?

Aerobic and resistance training can positively influence bone serum metabolism and density over the long term.

What this study adds?

Short-term (3 months) multi-component exercise training combined with electrical stimulation can impact bone metabolism.

Organ Cross-Talk Tips:

- The exercise program (EP) alone (without FES) induced significant changes in circulating bone turnover markers (BTMs) derived from bone tissue metabolism.
- The combination of EP and FES produced superior bone density outcomes compared to exercise alone. This highlights synergistic crosstalk where systemic signals from whole-body exercise and localized mechanical signals from FES-targeted muscles interact to produce a greater effect on bone tissue than either stimulus alone.

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Data availability

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval Participation in the study was voluntary and participants could withdraw at any time. Written informed consent was obtained and the study complied with the principles of the Declaration of Helsinki and medical ethics regulations. The ethical code IR.AZARUNIV.REC.1402.010 was registered for this study.

Informed consent Participants signed an informed consent form prior to participation in the study

Author contributions

Conceptualization: A.Z., A.F.K., Y.S., A.A., S.E.; Methodology: A.Z., A.F.K., Y.S., A.A., S.E.; Software: A.Z., A.F.K., Y.S., A.A., S.E.; Validation: A.Z., A.F.K., Y.S., A.A., S.E. Formal analysis: A.Z., A.F.K., Y.S., A.A., S.E.; Investigation: A.Z., A.F.K., Y.S., A.A., S.E; Resources: A.Z., A.F.K., Y.S., A.A., S.E.; Data curation: A.Z., A.F.K., Y.S., A.A., S.E.; Writing - original draft: A.Z., A.F.K., Y.S., A.A., S.E.; Writing–review & editing: A.Z., A.F.K., Y.S., A.A., S.E.; Visualization: A.Z., A.F.K., Y.S., A.A., S.E.; Supervision: A.Z.; Project administration: A.Z.; Funding acquisition: A.Z.

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