

COMPARISON OF THE EFFECTS OF WALKING AND BENCH-STEP EXERCISE ON OSTEOCALCIN AND CTX-1 IN POST-MENOPAUSAL WOMEN WITH OSTEOPENIA

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ABSTRACT

Purpose: Bench-step exercise produces biomechanical movements beneficial to improve bone remodeling. However, no studies have confirmed the effects of bench-step exercise (BE) on bone formation and resorption. The goal of this study is to compare the effects of bench-step and walking exercise (WE) on changes in osteocalcin and CTX-1 levels. **Methods:** Fifty-nine sedentary post-menopausal women with osteopenia (T-score between -1 and -2.5) were randomly divided into two groups: WE group ($n = 29$) and BE group ($n = 30$). Subjects performed 12 weeks of exercise. The osteocalcin and CTX-1 levels were analyzed using enzyme-linked immunosorbent assay. Independent *t*-test or Wilcoxon test was used to analyze the osteocalcin and CTX-1 levels. The difference in changes in the osteocalcin and CTX-1 between the groups before and after was analyzed using Mann-Whitney test. **Results:** The results showed a significant increase in the osteocalcin and CTX-1 levels in both groups ($P < 0.05$). The increased levels of osteocalcin between groups were not statistically different ($P > 0.367$). The increase in CTX-1 resulting from BE was lower than that from WE ($P < 0.048$). **Conclusion:** This study indicates that BE may inhibit resorption stronger than WE.

Keywords: Bone formation; Bone resorption; Mechanical stimuli.

INTRODUCTION

Weight-bearing exercise is an essential part of the non-pharmacological management of low bone mass in osteopenia and osteoporosis.^{9,25,34} Weight-bearing exercise produces ground reaction force (GRF), which involves load and has a substantial impact on bone remodeling.^{13,39} Walking is a common and safe weight-bearing exercise that is recommended to improve bone mass in osteopenia. However, the biomechanical

characteristics of walking with less axial force on the bone may produce less GRF.^{29,30}

Bench-step exercise (BE), another type of weight-bearing exercise, offers more benefits than walking exercise (WE) because it has a greater impact on GRF and the strain on the bone. BE exerts an axial force on the bone and therefore produces greater GRF.^{15,40} Zachazewski *et al.*⁵¹ analyzed the impact of BE on GRF during the stair ascent (SA) and stair descent (SD) phases

in the vertical, anteroposterior and lateral directions. The study showed that GRF response increased because the biomechanical aspects of SA and SD differ significantly from the biomechanical aspects of level WE.

During weight-bearing exercise, mechanical loading produces strains that stimulate bone remodeling. Strain induces shear stress on the lacunar and canaliculi fluid flow. Osteocytes, a type of mechanosensory cells, sense the shear stress and begin to activate a remodeling process characterized by increased osteoblast activity and decreased osteoclast activity.^{3,4,21,23,26,35}

Osteoblast and osteoclast activities can be assessed by examining changes in bone biomarkers. There are several biomarkers commonly used to determine osteoblast and osteoclast activities, such as osteocalcin and bone-specific alkaline phosphatase for osteoblasts and C-telopeptide (CTX-1) and N-telopeptide (NTX) for osteoclasts.^{28,43,48,50} Increased or decreased osteoblast or osteoclast activity will increase or decrease the levels of these biomarkers.

Previous studies showed the effects of exercise training on changes in plasma osteocalcin and CTX-1. The changes in osteocalcin and CTX-1 levels are related to the osteoblast and osteoclast activity. Bone formation and osteocalcin levels increase following increases in osteoblast activity, whereas bone resorption and CTX-1 levels increase following decreases in osteoclast activity. However, the change in osteocalcin is more consistent than the change in CTX-1. Studies by Tartibian *et al.*⁴⁶ and Adami *et al.*¹ on female subjects showed that osteocalcin increased, whereas CTX-1 remained unchanged or decreased after exercise of moderate intensity. A study by Helge *et al.*¹⁸ in some sports found that CTX-1 increased in subjects playing football.

The type of exercise such as BE, as well as WE, is an aerobic exercise recommended to improve cardiorespiratory and muscular fitness.^{17,32}

However, BE is much less popular than WE due to insufficient information on how to perform this exercise correctly to make the most of its benefits. Only slightly more information is available about the effects of bench height and stepping rate on GRF. The study by Fularczuk *et al.*¹⁵ might be the only one to have analyzed the relationship of bench-height and stepping rate with GRF. The results showed that stepping rate and bench height determined the GRF impact.

To the best of our knowledge, there has not been a study comparing the effects of WE and BE on changes in the biomarkers of bone remodeling in osteopenic women. The aim of this study is to compare the effects of WE and BE training on osteocalcin and CTX-1 levels in postmenopausal women with osteopenia. We hypothesized that BE training would increase osteocalcin and decrease CTX-1 more than WE training.

METHODS

This is a randomized clinical trial with pre-post design. The study was conducted at Posyandu (Integrated Health Center (IHC)), a non-profit health service activity managed by trained volunteers (cadres) and is under supervision by School of Medicine, Atma Jaya Catholic University of Indonesia. Subjects were members visiting Posyandu regularly. Sixty postmenopausal women (aged 50–65 years) were eligible for this study. The inclusion criteria were as follows: sedentary or no history of physical exercise during the last 3 months; having a good balance and mobility; having osteopenia (at least on one site: femur and pelvic) confirmed with dual-energy X-ray absorptiometry (DXA) examination (with *T*-score of (–1) to (–2)) and having a body mass index (BMI) of 27 kg/m² or lower and were able to complete bench-step test. Bench-step test was conducted as follows: subjects performing step

up and down the bench at a speed matching the 76 bpm tone of a metronome for 1 min. Subjects were randomly divided into two groups: BE group ($n = 30$) and WE group ($n = 29$). One subject of WE was lost to follow-up.

The exclusion criteria included the following: having treatment with steroids and hormones; tobacco and alcohol use; taking drugs affecting bone mass such as vitamin D and calcium supplements; evidence of a chronic disease; having muscle injury; having a hypertension (systolic pressure greater than 140 mmHg and diastolic pressure greater than 90 mmHg); fail to complete 1 min bench-step test and not completing the exercise program. (Flow of recruitment was shown in Fig. 1).

Written informed consent was obtained from each subject by giving signature. This study was approved by the Research Ethics Committee, Faculty of Medicine, University of Indonesia and Cipto Mangunkusumo Hospital. The identity of

the participants was confidential and used only for research purposes.

Anthropometric Measurements

Anthropometric measurements were performed onsite at Posyandu. Body weight was measured using portable digital scales (SECA, Germany) with an accuracy of 0.1 kg, and the subjects were wearing minimal clothing. Height was measured in the Frankfurt standing position without shoes using a wall-mounted stadiometer with an accuracy of 0.1 cm. BMI was calculated by dividing the weight (in kilograms) with the square of height (in meters).

DXA Examination

DXA scans were used to assess bone mineral density (BMD) in a hospital located in the southern part of Jakarta. BMD was performed using DXA

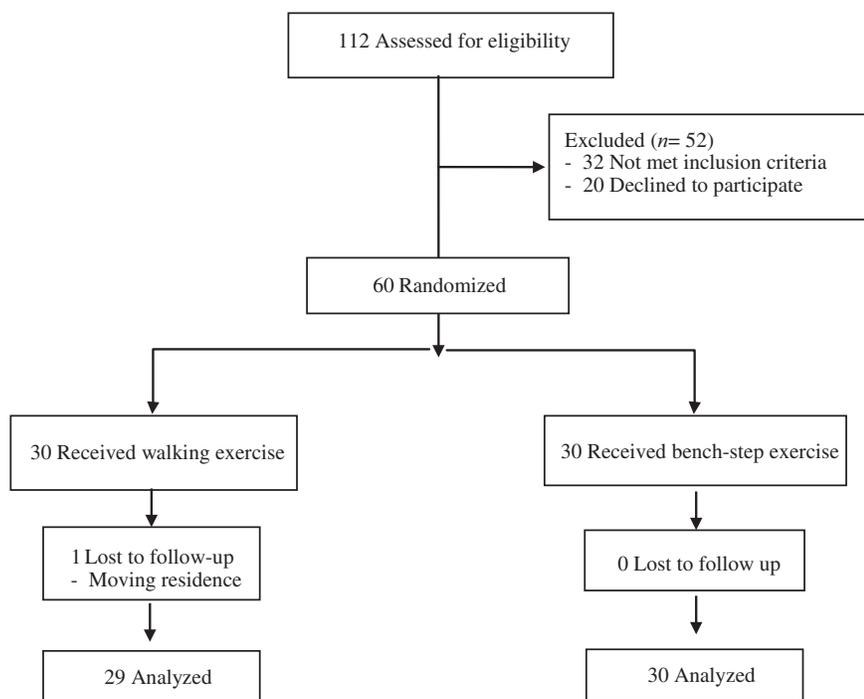


Fig. 1 Flow diagram of subjects recruitment, exercise intervention and follow-up.

(Lunar Prodigy, GE Healthcare, USA). The DXA machine was calibrated daily according to the manufacturer's instructions. Bone sites for scan were selected as recommended by the International Society for Clinical Densitometry. DXA scans were performed on the spine (L1–L4) and left femur (femoral neck, trochanter, femoral shafts, total hip).^{8,12}

Exercise Program

BE

The exercise was performed using a bench made of wood with a height of 20 cm and a surface of 1 m². In each session, BE training was performed for 1 min followed by 30 s of rest interval, for a total exercise time of 15 min. The 30 s of interval was intended for bone sensitivity recovery to mechanical stimuli.^{37,38}

The subjects were instructed to ascend and descend the bench, starting with the first foot landing on the bench, followed by the second foot; then, the first foot landed on the floor, followed by the second foot. The first foot was changed alternately right or left for every training session to obtain balanced stimuli on both legs. Each step was synchronized with a beat of a metronome set at 76 bpm. The speed was in accordance with average physical fitness of the subjects.

WE

The exercise was performed outdoor for a duration of 15 min for each session and at a moderate intensity. The average heart rate was maintained in 64–76% of the heart rate training zone. Heart rate was monitored using a digital heart rate monitor (Polar Heart Rate Monitor).

These exercises were conducted three times a week for 12 weeks. The exercise program was in accordance with the recommendation from American College of Sports Medicine for elderly people with low physical activity and fitness.^{20,36}

Samples and Biochemical Analysis

An aliquot of 10 mL blood samples was drawn from the median cubital vein using vacutainers filled with 5 mL EDTA anticoagulant after 12 h of overnight fasting. Blood samples were taken twice from each subject, before and after 12 weeks of exercise. All blood procedures were done by trained laboratory officers.

Osteocalcin was measured using a human osteocalcin instant enzyme-linked immunosorbent assay (ELISA) produced by eBioscience (California, USA.BMS-2020-INST). The lowest detection limit for sensitivity was 0.2 ng/mL. The calculated overall intra-assay and inter-assay coefficients of variation (CV) were 8.3% and 8.1%, respectively. The range of normal values for osteocalcin was 2.3–3.6 ng/mL.²²

CTX-1 was measured using the human (CTX-1 ELISA) kit produced by Cusabio (Wuhan, China. CSB-E11224h). The lowest measurement sensitivity that could be detected was 0.156 ng/mL. The detection range was between 0.625 ng/mL and 40 ng/mL. The precise intra-assay and inter-assay CV were < 8% and < 10%, respectively. The range of normal CTX-1 levels was 0.5–2 ng/mL.¹⁴

Statistical Analysis

Values of subject's characteristics, BMD and biomarker were expressed as mean and SD. Normality of the data distribution was tested using a Shapiro–Wilk test. Paired *t*-test or Wilcoxon test was used to compare osteocalcin and CTX-1 levels before and after 12 weeks of exercise in both groups. Mann–Whitney test was used to determine the differences of changes in osteocalcin and CTX-1 levels between the BE and WE groups. The level of significance was defined as $P < 0.05$. Statistical analysis was performed using SPSS version 17.0 for Windows (SPSS Inc., Chicago, IL, USA).

RESULTS

Fifty-nine subjects were diagnosed with osteopenia, but two patients were diagnosed with osteoporosis during lumbar BMD scanning and one other patient was diagnosed with osteoporosis upon pelvic BMD scanning. Characteristics of the subjects included all factors that may affect the study results. As shown in Table 1, there are no significant differences between the BE and WE groups for all characteristics included age, age at menopause, duration of menopause, weight, height, BMI and BMD of the several sites (lumbar L1-L4, neck, trochanter, shaft and total femur).

All of the subjects completed the exercise program. Dependent *t*-test or Wilcoxon test showed an increase in the osteocalcin levels of both groups after 36 exercise sessions ($P = 0.000$ for BE and $P = 0.010$ for WE). Osteocalcin increased by 13.3% and 8.8% after the BE and WE, respectively. Wilcoxon test showed that CTX-1 increased by 33.3% and 50% after the BE and WE, respectively ($P = 0.001$ for BE and WE; shown in Table 2). When the groups were compared, we found differences in the increased levels of osteocalcin and CTX-1 between the groups. The BE group showed greater increases in osteocalcin than the WE group but not significant

Table 1 Subjects Characteristics of BE and WE Groups.

	WE (<i>n</i> = 29)	BE (<i>n</i> = 30)	<i>P</i>
Age (years)	57.71 ± 5.31	58.07 ± 5.39	0.79
Age at menopause (years)	53.75 ± 2.69	53.78 ± 2.82	0.96
Duration of menopause (years)	3.96 ± 3.38	4.30 ± 3.72	0.72
Weight (kg)	58.24 ± 9.80	56.68 ± 9.19	0.52
Height (cm)	150.73 ± 5.27	149.24 ± 3.55	0.20
BMI (kg/m ²)	25.61 ± 3.87	25.41 ± 3.81	0.84
Bone mineral density			
Lumbar (L1-L4) (g/cm ²)	0.905 ± 0.16	0.920 ± 0.14	0.71
Neck (g/cm ²)	0.764 ± 0.15	0.740 ± 0.15	0.51
Trochanter (g/cm ²)	0.634 ± 0.11	0.640 ± 0.13	0.85
Shaft (g/cm ²)	0.994 ± 0.17	0.980 ± 0.19	0.75
Total femur (g/cm ²)	0.816 ± 0.13	0.815 ± 0.14	0.98

Notes: BE: bench step exercise; BMI: body mass index; WE: walking exercise.

Table 2 The Changes in Osteocalcin and CTX-1 Levels.

		Pre-exercise	Post-exercise	Δ
Osteocalcin (ng/mL)	BE	3.26 ± 0.82	3.73 ± 1.02	0.47 ± 0.20*
	WE	3.42 ± 0.75	3.92 ± 0.93	0.50 ± 0.11*
CTX-1 (ng/mL)	BE	1.83 ± 0.87	2.32 ± 0.73	0.49 ± 0.78*
	WE	2.16 ± 0.73	3.26 ± 1.98	1.10 ± 1.64*†

Notes: BE: Bench step exercise; CTX-1: C-telopeptide of type 1 collagen; WE: Walking exercise.

*Significant different within group ($p < 0.05$).

†Significant different between groups ($p < 0.05$).

Table 3 The Changes in Osteocalcin and CTX-1 Levels Based on BMD Classification.

		Osteocalcin (ng/ml)			CTX (ng/ml)		
		Pre-exercise	Post-exercise	Δ	Pre-exercise	Post-exercise	Δ
Lumbar DXA	Normal (n = 21)	3.19 ± 0.78	3.64 ± 0.98	0.46 ± 0.19*	2.11 ± 0.91	3.08 ± 2.12	0.96 ± 1.92*
	Low (n = 38)	3.42 ± 0.78	3.93 ± 0.97	0.51 ± 0.19*	1.92 ± 0.93	2.62 ± 1.11	0.70 ± 0.79*
Hip DXA	Normal (n = 24)	3.28 ± 0.80	3.75 ± 1.00	0.48 ± 0.20*	1.97 ± 0.89	2.91 ± 2.02	0.94 ± 1.80*
	Low (n = 35)	3.38 ± 0.78	3.88 ± 0.97	0.50 ± 0.19*	2.00 ± 0.95	2.70 ± 1.12	0.69 ± 0.81*

Notes: CTX-1: C-telopeptide of type 1 collagen ; DXA: Dual-energy X-ray absorptiometry.

*Significant different within group ($p < 0.05$).

($P > 0.367$), whereas the WE group showed greater increases in CTX-1 ($P < 0.048$; Furthermore, the changes of osteocalcin and CTX-1 were analyzed based on the BMD classification. The results are shown in Table 3).

The increase of osteocalcin and CTX-1 within a group were significant. In the contrary, the increase of osteocalcin and CTX-1 between groups were not significant.

DISCUSSION

This study compared the effects of WE and BE on bone remodeling biomarkers. After 12 weeks of exercise, there was a general increase in CTX-1 as well as in osteocalcin. Comparing the results shows no statistically significant difference between the WE and BE groups in terms of the increased osteocalcin. In addition, the increased CTX-1 level resulting from WE was significantly higher than that resulting from BE.

The change in osteocalcin is determined per exercise period or per single bout of exercise or training. The change in osteocalcin after a single bout of exercise is unclear; it could decrease,¹⁹ remain unchanged²⁷ or increase.^{16,42} Conversely, the change in osteocalcin after a period of training is more obvious and tends to increase. After 2–3 months of exercise, the subjects enter an adaptation phase. A study by Adami *et al.*¹ found that training exercise with moderate intensity for

1 month increased osteocalcin by up to 25%. Tartibian *et al.*⁴⁶ found increased osteocalcin in post-menopausal women following 24 weeks of exercise with an intensity of 65% of the maximal heart rate. In this study, osteocalcin increased by 8.8% and 13.3% after WE and BE, respectively. The increased levels of osteocalcin in both groups indicated an effect of training on bone formation. The adaptation process of bone formation occurs when the osteocytes produce mechanical signals and activate bone morphogenetic proteins, WntS, prostaglandin E2 and NO.^{11,24,33} Then, the recruitment, differentiation and activation of osteoblasts are amplified, and bone formation ensues. However, the increased levels of osteocalcin between the groups were not significantly different. This result might be related to the sensitivity of the osteoblasts; osteoblasts have the same degree of sensitivity to any weight-bearing exercise. The GRF produced by axial loading during BE with moderate intensity in this study was not strong enough to induce greater osteoblast and bone formation.

Comparing the increases in osteocalcin between the two groups, bone formation can potentially be improved further by modifying the exercise program, for instance, by increasing the stepping rate as well as extending the duration of exercise and training. BE may potentially improve bone formation further through modifying the exercise program by increasing the

stepping rate and the duration of exercise and training. The bench height used in this study was in the same range as the bench height of previous work and was comfortable for the subjects. The stepping rate, duration of exercise and training in this study were adjusted to the subjects' physical ability and fitness and were lower than those in previous studies.^{15,32,41,44} Modifying the training program to strengthen the effect of BE on mechanical load is still possible but should be performed gradually.

In contrast to the findings for osteocalcin, we found increases in CTX in both groups. This finding was not consistent with our hypothesis. Unlike osteocalcin, the change in CTX-1 after training was unclear. Studies by Alp² on premenopausal women and by Wen *et al.*⁴⁹ on postmenopausal women showed that CTX-1 decreased after 2 months of training with moderate intensity in 60–85% of maximal heart rate, 40 min per session, five times per week and 75–85% of heart rate reserve, 90 min per session, three times per week, respectively. A study by Adami *et al.*¹ found that CTX-1 was unchanged after 1 month of training (90 min/session, 3–4 times/week). Tartibian *et al.*⁴⁶ reported that 3 months of aerobic exercise at light and moderate intensity decreased CTX levels (25–30 min/session, 3–4 times per week, 45–55% of maximal heart rate).¹⁸ Compared with previous studies, the duration of each session in this study was much shorter, which may lead to less suppression of osteoclast activity. In addition, bone resorption and osteoclast activity were greater in post-menopausal subjects, and neither type of exercise training was able to balance the pace and halt the bone resorption. This reason may explain why CTX-1 levels remained high.

The increase in CTX-1 in this study indicated that bone resorption was still ongoing. The mechanism of increased CTX-1 after a period of training might be explained as follows: exercise is

associated with bone remodeling under certain conditions. During exercise, muscle contraction causes changes in the calcium metabolism. Calcium will trigger muscle contraction, but in the absence of calcium, the interaction of actin and myosin will be prevented. Therefore, the calcium intake requirements will increase during exercise, and parathyroid hormone will be stimulated to manage calcium regulation.^{5,6,10,31,45,47}

Further analysis was performed to evaluate the changes in osteocalcin and CTX-1 on different BMD classification. The result showed on both normal and low BMD at lumbar and hip, exercise increased osteocalcin and CTX-1. There were similarity between increased osteocalcin and CTX-1 in both normal BMD and low BMD. Based on the increment it shown that the effect of BMD on bone remodelling is not significant during the low-moderate intensity exercise. This is in accordance with a previous study.⁷ Biomarkers and BMD were not changed significantly between control and exercise group in postmenopausal, whereas increased greater significantly in exercise group than in control in premenopausal women. Allegedly due to little heavier, poorer muscle performance, and lower values for BMD, postmenopausal women have dramatically increased biomarkers regardless of BMD and training status.⁷

This study may be the first to compare these two weight-bearing exercises and their respective effects on biomarkers of bone remodeling. As such, our work provides the most up-to-date information on which exercise is more effective in improving bone mass. The BE protocol in this study could be used as a reference to conduct further research or to provide an exercise prescription for osteopenia.

This study has certain limitations. First, the osteocalcin and CTX-1 levels were only measured twice. The first sample was taken prior to the beginning of exercise training, and the second

was taken 24h after the last session. We suggest taking at least two more samples to obtain patterns of acute and training responses: one post-exercise sample after the first session and one prior to the last exercise session. Second, only one biomarker each for osteoblasts and osteoclasts was analyzed. The effects of the exercises on bone formation and resorption would be clearer and more certain if the evaluation had involved more than one biomarker each for osteoblasts and osteoclasts.

CONCLUSION

Both BE and WE increase osteocalcin and CTX-1 in post-menopausal women with osteopenia. The increase in osteocalcin in all subjects confirmed that both WE and BE exercises resulted the same effects on bone formation. The lower degree of increase in CTX-1 resulting from BE indicated that bone resorption appears to have been inhibited more effectively by BE than by WE. Our finding suggests that BE with a few modifications to the stepping rate and duration of exercise may provide more benefits and can be considered as an exercise program to help improve bone mass.

Duration of exercise in this study might be too short to generate different changes of the biomarkers between groups. We recommend a future study with longer duration of exercise to obtain the different effect of exercise types and significant changes of the biomarkers.

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We represent that this submission is original work, and is not under consideration for publication with any other journal.

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