Article

Effects of High-Impact Weight-Bearing Exercise on Bone Mineral Density and Bone Metabolism in Middle-Aged Premenopausal Women: A Randomized Controlled Trial

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Abstract: This study examined the effects of high-impact weight-bearing exercise on bone mineral density (BMD) and bone metabolic markers in middle-aged premenopausal women. Forty middle-aged premenopausal women were initially enrolled, but thirty-one participants (40.34 ± 3.69 years) completed in the study. The subjects were randomly divided into two groups including the high-impact weight-bearing exercise group (HWE, n = 14) and control group (CON, n = 17). The HWE group participated in the exercise for 50 min a day, three days per week for four months, while the CON group maintained their regular lifestyle. The HWE program included 10 different high-impact weight-bearing exercises such as jumping and running. BMD was measured using DXA (Hologic, QDR 4500W, Marlborough, MA, USA). The bone metabolic markers including serum 25-(OH) D, intact parathyroid hormone (PTH), osteoprotegerin (OPG), osteopontin (OPN), receptor activator of nuclear factor κB ligand (RANKL), osteocalcin (OC), C-terminal telopeptide of type 1 collagen (CTX), and calcium were analyzed. The results showed that the BMDs of femur, lumbar, and forearm did not significantly change during the intervention period in both the HWE and CON groups. A significant decrease in bone formation markers such as OC ($F = 10.514$, $p = 0.003$, $\eta_p^2 = 0.266$) and an increase in bone resorption marker including CTX ($F = 8.768$, $p = 0.006$, $\eta_p^2 = 0.232$) were found only in the CON group, while these values did not change in the HWE group. There was a significant increase in serum 25-(OH) D ($F = 4.451$, $p = 0.044$, $\eta_p^2 = 0.133$) in the HWE group. Our findings suggest that four months of HWE is not sufficient to improve BMD and bone metabolic markers, but this impact exercise program may prevent the age-associated changes in bone turnover markers in middle-aged premenopausal women.

Keywords: high-impact weight-bearing exercise; bone mineral density; bone metabolic markers; serum 25-(OH) D; middle-aged premenopausal women

1. Introduction

Physical inactivity adversely affects some health conditions including osteoporosis, neurological diseases, type 2 diabetes, obesity, cardiovascular diseases (CVD), and sarcopenia [1]. In particular, osteoporosis has been reported worldwide as a serious public health problem in recent years. It is estimated that over 200 million women suffer from this disease, and there are approximately 75 million people experiencing osteoporosis in the USA, Europe, and Japan [2–6]. Bone loss is commonly accompanied by aging, which slowly increases bone loss after the age of 35 years, and the process of osteoporosis rises at an exponential rate after menopause [7]. Elderly women with hip fractures have a higher

mortality rate of 10 to 20% than their counterparts, and osteoporosis has a much higher prevalence rate in women over 45 years of age than other diseases including breast cancer, diabetes mellitus, myocardial infarction [8,9]. Furthermore, the previous study suggested that the incidence of hip fracture would increase by 310% in men and 240% in women by 2050 due to the growth of the aging population and vulnerability to hip fracture with age [10]. Due to these clinical prescriptions, the diagnosis, consequences, monitoring of treatment, and therapy for osteoporosis are of crucial importance [11].

Analyzing bone turnover markers, an index of bone metabolism, are novel tools that detect bone remodeling dynamics including bone formation and resorption [12]. Bone remodeling is regulated by the activation of osteoclasts and osteoblasts and these biomarkers reflect the current status of bone turnover rate [11]. Bone turnover rate can be examined by analyzing bone formation markers such as bone-specific alkaline phosphatase, procollagen type 1 N propeptide, and osteocalcin (OC) and bone resorption markers including the N-terminal telopeptide of type 1 collagen and C-terminal telopeptide of type 1 collagen (CTX) [13]. It is also actively regulated by various factors such as osteopontin (OPN), osteoprotegerin (OPG), receptor activator of nuclear factor κB ligand (RANKL), parathyroid hormone (PTH), and vitamin D involved in the bone formation process [14–16]. RANKL is produced in osteoblasts and stromal cells and is the critical factor for the activation of mature osteoclasts and differentiation of monocyte-macrophage osteoclasts, which are precursors to multinucleated osteoclasts [10]. In contrast, OPG, one of the primary regulators of osteoclast-mediated bone resorption, is a factor observed in bone tissue [16]. OPN is a glycoprotein of the extracellular matrix of bone tissues that bind to hydroxyapatite and calcium, and it has been proposed as a mediator of atherosclerosis pathogenic pathways [16]. Vitamin D deficiency may be cause hyperthyroidism, resulting in increased bone turnover and bone loss [17,18]. According to the National Academy of Medicine, vitamin D deficient is defined as a level of serum 25(OH)D of 12 ng·mL^{-1}, whereas a level of 12 to <20 ng·mL^{-1} is considered to be inadequate, and a level of ≥20 ng·mL^{-1} defines vitamin D sufficiency [19]. Additionally, the elevated circulatory PTH levels may have negative effects [20]. However, several confounding factors determine the cause of the inverse relationship between serum 25(OH)D and PTH, and consistent results are not found in previous studies [20,21]. Calcium plays an important role as an essential nutrient for bone health [22]. However, previous studies have shown an excessively increased serum calcium relationship with an increased risk of cardiovascular disease [23–25]. These causes require an understanding of the potential impacts of serum calcium on bone health and care for health benefits. Thus, exploring the changes in bone turnover markers in response to exercise is essential for identifying mechanisms of the bone response [26].

The mechanical loading of high-impact physical activities (e.g., jumping and running) has been shown to increase bone mass in humans [27]. Additionally, impact activities such as high-intensity weight-bearing and jumping are more likely to be osteogenic than non-weight-bearing or low-impact activities such as swimming and walking [28]. Meta-analysis studies have shown that dynamic and resistance exercises are appropriate for increasing bone mineral density (BMD) in middle-aged women [29,30]. The majority of female participants that have been studied in impact exercise interventions were post-menopausal women because they represent the most popular group in which osteoporosis is manifested. Therefore, studies on pre-menopausal females are perceived to be in the greatest need [31–35]. However, a relatively small number of studies utilizing impact exercise training have targeted pre-menopausal women with combinations of supervised and home-based exercise sessions, which resulted in small positive effects [29,30,36]. Analyzing the bone turnover rate, an index of bone metabolism, plays an important role in understanding the biochemical activities in the bone and the changes in these markers can increase bone mineral density after weight-bearing exercise. It has been reported that acute, short-, and long-term exercise training increased the regulation bone remodeling markers such as OC, CTX, OPN, OPG, RANKL, PTH, and calcium [37–40]. Scott et al. [39] reported

that acute weight-bearing exercise training increased serum levels of OPG, calcium, and PTH. Lester et al. [40] reported that combined aerobic and resistance exercise training for eight weeks significantly increased serum levels of OC in young women. Nevertheless, impact exercise training has not been explicitly directed at adult pre-menopausal women, despite the significant risk of developing osteoporosis after menopause.

Therefore, our study aimed to examine the effects of four months of high-impact weight-bearing exercise on BMD and bone metabolic markers in middle-aged premenopausal women.

2. Materials and Methods

2.1. Subjects

The power test was performed using G*Power 3.1.9.2 (Franz Faul, University of Kiel, Kiel, Germany) at the effect size of 0.3, the significant level of 0.05 ($\alpha = 0.05$), and the power of 0.8 for all statistical tests. G*Power showed that 24 subjects had sufficient power for this study. Forty middle-aged premenopausal women aged 33–47 years were initially enrolled in the study. The exclusion criteria were specified as follows: (1) participated in regular exercise within the last three months; (2) users of pharmaceutical agents that directly affect bone, hypertension, and hyperlipidemia; and (3) blood pressure $\geq 140/90$ mmHg. The subjects were randomly divided into two groups including HWE and CON. However, nine dropped out due to personal reasons and pregnancy; thus, thirty-one subjects completed the study (HWE: n = 14, CON: n = 17) (Table 1). All proceedings of the study were approved by the Institutional Review Board of Kyung Hee University (KHGIRB-19-226) and were conducted according to the Declaration of Helsinki.

Table 1. Physical characteristics and nutritional intake of subjects.

Variables	HWE		CON		F-Value		
	Pre	Post	Pre	Post	Time	Group	Interaction
Age (yrs)	40.3 ± 4.23	-	40.4 ± 3.31	-			
Body height (cm)	160.5 ± 4.74	-	161.2 ± 5.04	-			
Body weight (kg)	58.8 ± 10.98	57.9 ± 9.43	62.8 ± 11.73	63.3 ± 12.16	0.148	1.377	1.875
BMI (kg·m^{-2})	22.7 ± 3.62	22.3 ± 3.14	24.1 ± 4.14	24.3 ± 4.46	0.277	1.409	2.137
Total caloric intake (Kcal)	2151.20 ± 487.94	2027.71 ± 636.44	2328.61 ± 640.41	2274.09 ± 582.72	0.632	1.353	0.095
Carbohydrate (g)	293.29 ± 79.16	260.95 ± 89.44	297.46 ± 86.66	294.98 ± 85.55	1.409	0.497	1.036
Fat (g)	72.75 ± 19.01	73.82 ± 23.36	83.69 ± 25.82	80.00 ± 26.35	0.065	1.495	0.215
Protein (g)	98.08 ± 29.89	92.35 ± 29.98	113.30 ± 40.46	102.07 ± 31.60	1.665	1.498	0.175
Vitamin D (ug)	5.18 ± 4.47	4.57 ± 2.84	4.76 ± 2.26	6.02 ± 5.30	0.109	0.260	0.900
Calcium (mg)	878.26 ± 242.49	854.96 ± 346.25	1051.38 ± 474.55	995.38 ± 575.03	0.224	1.352	0.038
Magnesium (mg)	163.6 ± 80.95	136.60 ± 53.77	124.24 ± 51.53	134.56 ± 63.81	0.270	1.650	1.350

Values are expressed as mean ± SD. HWE: high-impact weight-bearing exercise group, CON: control group, BMI: body mass index.

2.2. High-Impact Weight-Bearing Exercise Program

The training group performed the HWE program for 50 min a day (10:30 a.m.–11:20 a.m.), three days per week for four months. The training program was composed of 10 min of warm-up, 30 min of HWE, and 10 min of cool-down. The HWE comprised of the clap, walking, jumping burpee, jumping squat, running in place, wall press, bench stepping, jumping lunge, jumping jack, and push up [30]. The exercise intensity including exercise time increased progressively every four week period. The training intensity was set at 60–80% (mean: 136.9 ± 9.03 beat/min, range: 123–180 beat/min) of the heart rate reserve (HRR) and monitored every day by using a potable heart rate monitor (Polar RS400, Polar Electro Oy, Kempele, Finland). The detailed training program is shown in Table 2.

Table 2. High-impact weight-bearing exercise program for the four-months of the study.

Program	Contents	Phase (Weeks)	Set	Duration (min)		FITT
				Exercise Time	Rest Time	
Warm-up	Dynamic stretching (Upper and lower body)			10		
Main exercise	Clap, Walking, Jumping burpee, Jumping squat, Running in place, Wall press, Bench stepping, Jumping lunge, Jumping jack, Push up	1–4 5–8 9–12 13–16	5 6 5 6	12.5 15 17 20	14 15 12 12.5	Frequency: 3 times/wk Intensity: 60–80% HRR Time: 50 min Type: HWE with music (1–8 wks: 15 s for each exercise) (9–16 wks: 20 s for each exercise)
Cool-down	Static stretching (Upper and lower body)			10		

HRR: heart rate reserve, FITT: frequency, intensity, time, type, HWE: high-impact weight-bearing exercise.

2.3. Anthropometric Measurements

The body height and body weight were measured, and body mass index (BMI) was calculated by dividing body weight (kg) by the square of body height (m^2). Body height was measured using a stadiometer to the nearest 0.1 cm (T.K.K. 11253, Takei Scientific Ins Co., Tokyo, Japan). Body weight was measured using a balance beam scale (Seca 700, Seca Co., Hamburg, Germany) to the nearest 0.1 kg.

2.4. Bone Mineral Density

BMD was measured using dual-energy x-ray absorptiometry (DXA), with a Hologic QDR 4500W bone densitometer (Hologic, Marlborough, MA, USA). All participants were scanned at three different sites of the BMD (e.g., femur, lumbar, and forearm). The same technician performed all tests. The intra-class correlation coefficient (ICC) was measured (femur BMD: 0.997; lumbar BMD: 0.990; forearm BMD: 0.987) [41].

2.5. Bone Metabolic Markers

Blood samples were collected before and after the four-month intervention period. Participants arrived at the laboratory in the morning between 08:30 a.m. and 09:30 a.m. after 12 h, overnight fasting, and avoiding severe physical activity or training the night before. Venous blood samples were taken 5 mL at the antecubital vein by a medical laboratory technologist and separated into each serum separate tube. The clotting of blood was separated using centrifugation with 3000 rpm for 15 min. The samples obtained were stored at −80 °C until analysis.

Serum 25-(OH) D was measured by a chemiluminescence microparticle immunoassay (CMIA) method (Architect i2000SR, Abbott, Singapore) with an ARCHITECT 25-(OH) vitamin D kit (Abbott Diagnostics, Lake Forest, IL, USA). The electrochemiluminescence immunoassay (ECLIA) method (E170, Roche, Germany) was used for intact PTH and CTX with an Elecsys PTH kit (Roche, Germany) and β-CrossLaps/serum kit (Roche, Germany). Bone metabolic markers including OPG, RANKL, and OPN were measured (VERSA Max, Molecular Device, Sunnyvale, CA, USA) by an enzyme-linked immunosorbent assay (ELISA) with OPG kit (ICL, Portland, OR, USA), RANKL kit (sRANKL ELISA, Immundiagnostik, Germany), and OPN kit (ICL, Portland, OR, USA). A radioimmunoassay (RIA) method (COBRA 5010 Quantum, USA) was used for analyzing OC, a marker of bone formation, with an OC kit (BRAHMS, Berlin, Germany). Calcium was measured by a homogeneous enzymatic colorimetric assay (HECA) method (c702, Germany) with a Calcium Gen.2 (Roche, Germany). The blood analysis was performed at the expertized

research laboratory (Green Cross LabCell, Korea). The company is certified by the Korean Board for Accreditation and Conformity assessment.

2.6. Nutritional Intake

The dietary intake was assessed by three-day dietary records including twice on weekdays and once at the weekend in the first and the last weeks of the intervention. All data were analyzed by a computerized nutrient-intake assessment software program (CAN-PRO 4.0, Korean Nutrition Society, Seoul, Korea). The participants were instructed to maintain the same macronutrient distribution throughout the study.

2.7. Statistical Analysis

Statistical analyses were performed by using SAS software version 9.4 (SAS Institute, Cary, NC, USA). The mean, standard deviation, and 95% confidence interval (CI) were calculated. The normality of distribution of all dependent variables was verified using the Kolmogorov–Smirnov test. Two-way repeated-measures ANOVA was applied to determine the interaction effect for the group by the time during the intervention. If any significant interaction or main effects were observed, the independent t-test and paired t-test were applied to analyze the significant differences within groups and between groups. The effect size was computed as partial eta-squared values (η_p^2; small: ≥ 0.01, medium: ≥ 0.06, large: ≥ 0.14) [42]. The statistical significance level was set at 0.05.

3. Results

3.1. Bone Mineral Density

There were no significant interaction effects for group by time on femur BMD ($F = 0.458$, $p = 0.504$, $\eta_p^2 = 0.016$), lumbar BMD ($F = 0.009$, $p = 0.925$, $\eta_p^2 = 0.000$), and forearm BMD ($F = 0.048$, $p = 0.827$, $\eta_p^2 = 0.002$) during the intervention period (Table 3).

Table 3. Changes of bone mineral density (BMD) between pre- and post-tests in middle-aged premenopausal women.

Variables	HWE		CON		*F*-Value (η_p^2)		
	Pre (95% CI)	Post (95% CI)	Pre (95% CI)	Post (95% CI)	Time	Group	Interaction
Femur BMD (g/cm^2)	0.891 ± 0.103 (0.838–0.946)	0.895 ± 0.103 (0.842–0.950)	0.898 ± 0.109 (0.846–0.952)	0.898 ± 0.113 (0.846–0.952)	0.515 (0.017)	0.015 (0.001)	0.458 (0.016)
Lumbar BMD (g/cm^2)	1.036 ± 0.158 (0.956–1.122)	1.036 ± 0.167 (0.952–1.126)	1.009 ± 0.111 (0.957–1.062)	1.009 ± 0.112 (0.958–1.064)	0.003 (0.000)	0.290 (0.010)	0.009 (0.000)
Forearm BMD (g/cm^2)	0.563 ± 0.036 (0.544–0.584)	0.560 ± 0.037 (0.541–0.581)	0.579 ± 0.041 (0.560–0.598)	0.576 ± 0.045 (0.555–0.596)	3.413 (0.105)	1.243 (0.041)	0.048 (0.002)

Values are expressed as mean ± SD. 95% CI: 95% confidence interval, BMD: bone mineral density, HWE: high-impact weight-bearing exercise, CON: control.

3.2. Bone Metabolic Markers

There were significant interaction effects for group by time on serum 25-(OH) D ($F = 4.451$, $p = 0.044$, $\eta_p^2 = 0.133$) and OC ($F = 10.514$, $p = 0.003$, $\eta_p^2 = 0.266$) (Figures 1 and 2). The post-test showed that serum 25-(OH) D concentration was increased significantly following the four-months of high impact weight-bearing exercise ($p < 0.01$), while no significant change was found in the CON group. OC level was not changed in the HWE group, but this value decreased significantly in the CON group ($p < 0.05$). There were significant time effects on intact PTH ($F = 4.447$, $p = 0.044$, $\eta_p^2 = 0.133$), OPN ($F = 5.480$, $p = 0.026$, $\eta_p^2 = 0.159$), CTX ($F = 8.768$, $p = 0.006$, $\eta_p^2 = 0.232$), and calcium ($F = 7.986$, $p = 0.008$, $\eta_p^2 = 0.216$) (Table 4).
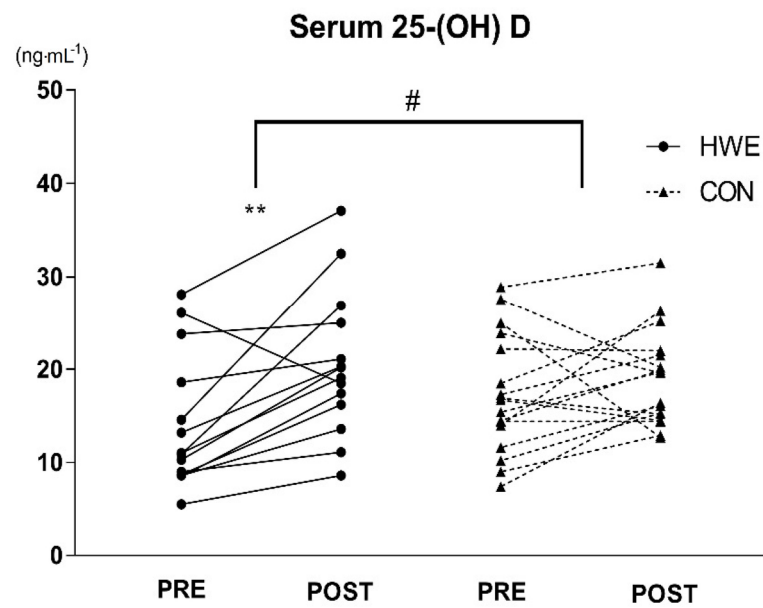
Figure 1. Serum 25-(OH) D before and after the four-month exercise program. For serum 25-(OH) D, statistical analyses revealed an increase between pre- and post-tests in the HWE group. HWE: high-impact weight-bearing exercise, CON: control. Significant difference between pre- and post-tests, ** $p < 0.01$. Significant interaction effect, # $p < 0.05$.
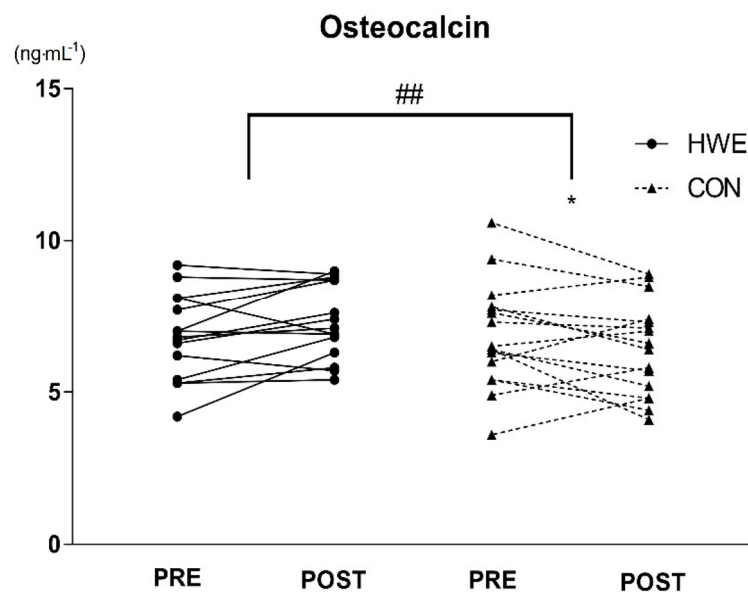

Figure 2. Osteocalcin before and after the four-month exercise program. For osteocalcin, statistical analyses revealed a decrease between pre- and post-tests in the CON group. HWE: high-impact weight-bearing exercise, CON: control. Significant difference between pre- and post-tests, * $p < 0.05$. Significant interaction effect, ## $p < 0.01$.

Table 4. Changes in bone metabolic markers between pre- and post-tests in middle-aged premenopausal women.

Variables	HWE		CON		*F*-value (ηp^2)		
	Pre (95% CI)	Post (95% CI)	Pre (95% CI)	Post (95% CI)	Time	Group	Interaction
25-(OH) D ($ng \cdot mL^{-1}$)	14.1 ± 7.24 (9.9–18.2)	20.5 ± 7.81 ** (16.0–25.1)	17.3 ± 6.37 (13.9–20.5)	19.0 ± 5.17 (16.4–21.7)	13.559 ## (.319)	0.158 (0.005)	4.451 # (0.133)
Intact PTH ($pg \cdot mL^{-1}$)	36.6 ± 9.00 (31.4–41.8)	42.6 ± 8.73 * (37.6–47.7)	36.5 ± 10.18 (31.2–41.7)	38.9 ± 8.85 (34.4–43.5)	4.447 # (0.133)	0.498 (0.017)	0.800 (0.027)
OPG ($pmol \cdot L^{-1}$)	5.7 ± 0.80 (5.2–6.1)	5.1 ± 1.26 (4.4–5.8)	5.6 ± 1.26 (5.0–6.3)	5.3 ± 1.65 (4.4–6.1)	3.911 (0.119)	0.021 (0.001)	0.173 (0.006)
RANKL ($pmol \cdot L^{-1}$)	286.9 ± 178.95 (183.6–390.3)	277.8 ± 186.06 (170.3–325.2)	199.6 ± 108.34 (143.9–255.3)	224.5 ± 124.01 (160.7–288.2)	0.225 (0.008)	1.868 (0.061)	1.062 (0.035)
OPN ($ng \cdot mL^{-1}$)	63.2 ± 31.97 (44.7–81.6)	50.1 ± 37.46 * (28.5–71.8)	51.2 ± 15.56 (43.2–59.2)	47.8 ± 20.78 (37.1–58.5)	5.480 # (0.159)	0.622 (0.021)	1.867 (0.060)
Osteocalcin ($ng \cdot mL^{-1}$)	6.8 ± 1.46 (6.0–7.7)	7.3 ± 1.29 (6.6–8.1)	6.9 ± 1.69 (6.0–7.8)	6.2 ± 1.50 * (5.5–7.0)	0.132 (0.005)	1.068 (0.036)	10.514 ## (0.266)
CTX ($ng \cdot mL^{-1}$)	0.24 ± 0.10 (0.19–0.30)	0.28 ± 0.09 (0.23–0.34)	0.26 ± 0.16 (0.18–0.35)	0.31 ± 0.14 * (0.23–0.38)	8.768 ## (0.232)	0.296 (0.010)	0.018 (0.001)
Calcium ($mg \cdot dL^{-1}$)	9.7 ± 0.46 (9.5–10.0)	9.5 ± 0.40 (9.3–9.8)	9.8 ± 0.29 (9.7–10.0)	9.5 ± 0.29 ** (9.4–9.7)	7.986 ## (0.216)	0.204 (0.007)	0.442 (0.015)

Values are expressed as mean ± SD. 95% CI: 95% confidence interval, HWE: high-impact weight-bearing exercise, CON: control, PTH: parathyroid hormone, OPG: osteoprotegerin, RANKL: receptor activator of nuclear factor kB ligand, OPN: osteopontin, CTX: C-terminal telopeptide of type 1 collagen. Significant interaction or main effect, # $p < 0.05$, ## $p < 0.01$. Significant difference between pre- and post-tests, * $p < 0.05$, ** $p < 0.01$.

4. Discussion

The present study examined the effects of four months of HWE on BMD and bone metabolic markers in middle-aged premenopausal women. Our main findings showed that a significant decrease in OC and an increase in CTX were found only in the CON group while these values did not change in the HWE group. However, four months of HWE were not sufficient to improve BMD in middle-aged premenopausal women.

Substantial empirical evidence has shown that resistance and step exercise have several benefits, which work as non-pharmacological interventions for the improvement of bone health in children/adolescents, adulthood, and the elderly [31,43]. However, the effects of high-impact exercise on BMD in middle-aged pre-menopausal women remain unknown [36]. The present study showed no significant effects on femur, lumbar, and forearm BMD after four-month of high-impact weight-bearing exercise. The International and National Osteoporosis Foundation, and other organizations recommend weight-bearing exercises to prevent osteopenia and osteoporosis [44]. There are different types of body weight-bearing exercises including high-impact exercises such as running and jumping as well as low-impact exercises such as body weight training, water aerobics, and walking [44]. It has been recently reported that trochanter and femoral neck BMD were significantly improved after high-impact weight-bearing exercise in pre-menopausal women [30]. Additionally, high-impact weight-bearing and resistance training either in combination or alone, induced gains in BMD by 1–2% at the femoral neck and lumbar spine with a minimal effect on other body sites in pre-menopausal and post-menopausal women [45]. Similar results in other studies have found that high-impact exercise for 12 months (3 times/week) resulted in a significant improvement in femoral neck BMD by 0.6–1.1% in pre-menopausal women [46,47]. Although the present study did not show a significant change, there was a tendency where the femur BMD increased following four months of HWE (0.47%) without any improvement on other sites of the body. Foster and Armstrong [48] reported that a bone remodeling cycle takes about 3–4 months and a minimum of 6–8 months is required for the bone to reach a new, measurable steady-state bone mass. Therefore, the absence of the significant improvement in BMD is likely due to the insufficient duration of an exercise intervention to promote bone formation in the present study. In fact, combined-

impact exercise protocols lasting more than six months (duration: 30–60 min; frequency: ≥3 days/week) have been recommended for greater bone health benefits [49]. Moreover, adults might benefit from high-impact exercises, but previous studies have suggested that its effect might occur to a lesser extent than in children or adolescents [50]. In this study, the effect of four-months of high-impact weight-bearing exercise appeared to be none or small only in particular sites such as femur BMD in middle-aged premenopausal women.

Biochemical bone metabolic markers indicate changes in bone metabolism more quickly than changes in BMD, which are commonly used to monitor osteoporosis [51]. Additionally, bone metabolic markers could be used to evaluate the antiresorptive agents in the clinical diagnosis of osteoporosis and osteopenia [52]. One intended therapeutic aim of the antiresorptive therapy is to decrease levels of bone metabolic markers below the average reference range for pre-menopausal women [53]. Bone metabolic markers provide important information such as the rate of bone remodeling by changing bone formation and resorption, and the evaluation of these markers has been used in previous intervention studies to identify the effectiveness of exercise [54–56]. The results of our study showed that serum 25-(OH) D (60.22%, $p < 0.01$), and intact PTH (23.86%, $p < 0.05$) were increased significantly in HWE between pre- and post-tests, whereas OPN (-20.45%, $p < 0.05$) was decreased significantly in HWE between pre- and post-tests. Furthermore, OC (-7.89%, $p < 0.05$) and calcium (-2.99%, $p < 0.01$) were decreased significantly in CON between pre- and post-tests, whereas CTX (30.94%, $p < 0.05$) was increased significantly in CON between pre- and post-tests. However, no significant interaction effects were found on other markers including intact PTH, OPG, RANKL, and OPN.

Vitamin D plays a critical role in promoting the intestinal absorption of phosphate and calcium [57], and participating in regular physical activity is a well-known stimulator of serum 25-(OH) D [58]. In accordance with our results, Josse et al. [55] reported that the serum 25-(OH) D increased significantly following 12 weeks of resistance training in young women, but the level of PTH was not significantly changed. It is an interesting result in the present study where the HWE group increased both vitamin D and PTH level after four-months of exercise intervention. Generally, serum calcium level is mediated by vitamin D and PTH [59] and the changes in these levels may increase or decrease the mineralization of bone. It is assumed that an increase in PTH level may help to maintain serum calcium level. In particular, our participants were healthy and had no bone-related disease, thus it is possible that PTH is increased for calcium homeostasis. Moghadasi and Siavashpour [56] reported that resistance circuit training for 12 weeks significantly increased PTH in healthy and sedentary young women, which agrees with the present result. Authors assumed that this increase in PTH after resistance training may promote anabolic activities in bone metabolism. However, it is difficult to explain the direct relations or physiological mechanism between the variables in the present study and conflicting results have been observed in previous studies. Pilch et al. [60] reported that Nordic walking training for six weeks significantly decreased serum 25-(OH) D in post-menopausal women and Evans et al. [54] reported that a recruit training program for four-months significantly decreased serum 25-(OH) D in men, whereas these values remained at baseline levels in women. Vitamin D is an important factor for bone health, and its deficiency leads to osteoporosis and CVD in adults [61,62]. Moreover, physically inactive individuals have two times lower vitamin D levels than active adults [63]. Although it is difficult to determine the direct relations between HWE and increased serum 25-(OH) D, several studies support our results that increase in physical activity is an effective manner to maintain optimal vitamin D level in later life [64,65]. In this study, the serum 25-(OH) D increased from an inadequate level (12–20 ng·mL^{-1}) to a sufficient level (≥20 ng·mL^{-1}) in HWE.

Bone turnover markers provide information on important biochemical indicators such as bone resorption and bone formation in managing osteoporosis [66]. A significant association between bone turnover markers and the risk of fractures has been identified for females and males [67]. Mohr et al. [68] reported that soccer training for 15 weeks

significantly increased plasma OC (37 ± 15%) and CTX (42 ± 18%) in middle-aged premenopausal women. A significant increase in OC has also been observed in untrained young women following sixteen weeks of exercise intervention [69]. Additionally, exercise training caused augmentation in resting plasma OC and CTX in elderly sedentary men with no resistance training [70]. However, CTX has been shown to increase shortly or immediately after impact exercise [26]. Thus, impact exercise may have a high potential to improve bone health in the general population. Therefore, the above intervention studies and our study results indicate that impact exercise describes a feasible intervention modality in middle-aged premenopausal women.

Previous studies by Ferrucci et al. [71] and Fuller et al. [72] emphasized that changes in pro-inflammatory and anti-inflammatory cytokines have profound implications on age-related bone loss. OPN acts through binding to integrin β-3, leading to a decrease in the cytoplasmic calcium concentration associated with osteoclast activation [10]. Humphries et al. [38] reported that participants who underwent whole-body vibration with or without the combination of resistance training for 16 weeks showed a significant decrease in OPN in young healthy women, which is consistent with the present findings. Thus, exercise training induces changes in bone metabolism faster than actual changes in BMD in middle-aged premenopausal women. Therefore, it is important to monitor and stimulate bone metabolic markers to prevent osteoporosis before menopause in women, especially when considering a higher prevalence of osteoporosis after menopause.

5. Limitation of the study

In this study, there are some limitations that need to be considered. Although the present study is well designed with the randomized controlled trials, the relatively short-term intervention period (four-months) may be a limit to confirming the longitudinal effects of high-impact weight-bearing exercise on BMD in middle-aged premenopausal women. However, the positive changes in bone metabolic markers such as OC and vitamin D may provide a potential benefit on bone health. In future study, a longer period of exercise intervention (more than six months) is needed to examine how changed bone metabolic markers are linked to the BMD changes.

6. Conclusions

The present study revealed that four months of HWE is not sufficient to improve BMD and bone metabolic markers, but this exercise program may prevent the age-associated changes in bone turnover markers in middle-aged premenopausal women.

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