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Walking intensity for postmenopausal bone mineral preservation and accrual

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Abstract

Introduction: Mechanical stresses on the bone are an important aspect of physical activity that promotes bone preservation and increases in bone mass. Exercise intensities leading to bone preservation and accrual have not been adequately defined for humans in general, and postmenopausal women in particular.

Materials and methods: To quantify parameters of effective walking intensity for preservation and accrual of bone mineral, healthy postmenopausal women engaged in 30 weeks of supervised walking, 4.8 km per day, 4 days a week at intensities of 102% or 123% of the ventilatory threshold (VT) equivalent to 67% and 86% of maximal effort (VO_2 max). Subjects were matched by age, body mass, hormone replacement status (HRT) and VT. Areal bone mineral density (aBMD) determined by DXA ($n=25$) and bone formation markers osteocalcin (OC), and bone-specific alkaline phosphatase (bALP) ($n=43$), were measured at the outset and at 15-week intervals. Peak vertical forces at corresponding intensities were measured ($n=9$) on a force plate.

Results: aBMD of legs and whole body, but not of other sites, and lean mass of legs, but not of arms, increased after 15 weeks of high intensity, compared to moderate losses for low intensity training. Leg and total body aBMD was preserved and slightly increased with loads greater than 872.3 newtons (N) with a walking intensity above 115% of VT or 74% of VO_2 max, speeds above 6.14 km/h, and heart rates above 82.3% of age-specific maximum. OC and bALP did not correlate with training-induced changes in aBMD.

Conclusions: At exercise intensities above 115% of VT or 74% of VO_2 max, and walking speeds above 6.14 km/h, mechanical loading of 872.3 N or 1.22 times body weight is sufficient for increases in leg muscle mass and preservation of BMD in postmenopausal women.

Keywords: Exercise; Mechanical loading; Osteocalcin; Bone-specific alkaline phosphatase

Introduction

Menopause and an associated precipitous decline in estradiol is accompanied by loss of bone mineral, reduced bone mineral density (BMD), and increased risk of osteopenia and osteoporosis [36]. The decline in mechanical strength and rise in bone fragility increases the risk for bone fractures in response to atraumatic stresses [2]. Currently in the United States osteoporotic fractures occur at a rate of 1.5 million per year (http://www.nof.org/osteoporosis/stats.htm) and are associated with more than 17 billion dollars in health care costs [34]. Genetics, gender, age, body size, and ethnicity are risk factors for osteoporosis that are not amenable to modification. On the other hand, physical activity, hormone replacement therapy (HRT), and a dietary adequacy of calcium and vitamin D3, are lifestyle variables that can be used to preserve or increase BMD [6].

Mechanical loading is presumed to dominate controls of bone mineral balance. Along with some anabolic hormones, mechanical factors probably are crucial in preventing or reversing postmenopausal bone loss [10]. In animal studies, individual bones display increased growth and mineral accretion when they are dynamically loaded, with sufficient strain

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magnitude and novel strain distribution, relatively briefly (for up to 36 loading cycles), and at intervals of 6 to 8 h [42]. Physical activity applies mechanical as well as hormonal influences on bone structure and accumulation of mineral in regions of the bone undergoing loading. In addition, it strengthens muscles and improves balance, thus reducing the overall risk of falls and fractures. Among mechanical influences, high-amplitude, low-frequency physical activities such as weight-bearing activities of rapid locomotion or resistance exercise [6,45,46], and high-frequency, low amplitude forces exerted by muscle fiber contractions during stationary skeletal loading [38,39,44] have been found effective in increasing bone mass and strength. However, the effectiveness of mechanical loading to increase bone mass and/or bone mineral declines with age [37] and thus age needs to be considered when evaluating the effectiveness of any particular exercise. Currently, our understanding of how to use exercise effectively in postmenopausal women in prevention of osteopenia and osteoporosis is incomplete [24] and has prompted the call by a panel of experts at the American College of Sports Medicine for well-designed studies to identify type, intensities and volumes of effective osteogenic exercise [1].

Postmenopausal women can respond to exercise training with modest increases in BMD through physical activities engaging either joint reaction forces (JRFs) produced by muscle contractions [3,26,33], ground reaction forces (GRFs) caused by weight-bearing physical activities [15,22,29], or a combination of both [25,29]. However, a number of studies engaging either JRFs or GRFs have reported no change in some parameter of bone mass or density in postmenopausal women usually against a loss in the same parameter in the control group (for recent reviews, see [6,45,46]). It is unclear which aspects of exercise type, intensity, loading pattern, and timing were critical in causing differences in BMD improvement in these studies.

We chose walking, a form of exercise that is preferred by older women [18,30], to quantify the effects of walking intensity on bone mineral preservation and accrual in postmenopausal women. We focused on exercise intensity, as increases in BMD were more often reported when walking, jogging or running was carried out at, or above, 90% of maximal heart rate (HR_{max}) or 85% of heart rate reserve (percent of the difference between resting and maximal heart rate), rather than at lower exercise intensities [13,15,22,29]. On the other hand, intense jumping training produced no improvements in BMD of postmenopausal women, while slightly less intense jumping, produced about 3% BMD increase in the femoral neck in premenopausal women [4,20] illustrating the importance of taking into account both the age at the time of training [37] and the magnitude of mechanical loading. Few studies were designed to examine the importance of intensity of exercise or mechanical loading on BMD in postmenopausal women, and some did not quantify exercise intensity beyond indicating that the walking pace was brisk [9,17].

The primary goal of this study was, therefore, to define the osteogenic threshold in postmenopausal women in terms that would be useful to different categories of users. Definition of osteogenic threshold in terms of walking speed and heart rate as percent of age-specific maximum would provide convenient exercise prescription for potential users. Identification of this threshold in terms of relative exercise intensity, as percent of VT or of VO_2 max would provide useful exercise parameters for experimental work, and definition of peak mechanical vertical force that increases bone mineral density could be of use in physical rehabilitation. The secondary goal of this study was to determine the usefulness of two markers of bone formation (OC and bALP) in identifying training-induced changes in BMD.

Materials and methods

The training component of this study was a randomized clinical trial that compared the effect of a 30-week walking exercise intervention at two different intensities on BMD and markers of bone formation in early postmenopausal women. The laboratory component of the study determined peak mechanical vertical forces in early postmenopausal women in the range of walking intensities employed in the training study.

Subjects

Healthy postmenopausal women were recruited from the greater Ann Arbor area through newspaper advertisements. Inclusion criteria were age (50 to 65 years), surgical or natural menopause (no menstrual periods during past 1–10 years), body mass index (BMI) less than 39 kg/m^2, non-smoker, absence of metabolic and endocrine disease other than corrected hypothyroidism, absence of musculo-skeletal disabilities that would impair walking, and sedentary status (<60 min of regular exercise per week). Eighty-two women were enrolled in the training trial which was conducted from autumn through early summer during successive years between 1996 and 2002. Subjects were instructed not to alter their customary physical activities or dietary regime while participating in the study. Training started for each subject upon enrollment. Six subjects withdrew during the first 15 weeks of training. Due to funding limitations, only the first 25 enrolled subjects received DXA scans. Another 27 subjects withdrew during the second 15 weeks of training. The reasons given for withdrawal from the studies were inconvenience and lack of time (26 women), family or personal health problems (6 women), and relocation to another city (1 woman). The age and physical characteristics of remaining subjects are shown in Table 1. Bone markers were measured in 19 of the subjects who received DEXA scans and in 24 who underwent training but did not receive the scans. Nine women agreed to measure GRFs in the laboratory at walking intensities in the same range as those used in the training study. This part of the study was conducted in 2003–2004 (Table 1). Women in the entire study were on the average 8 to 10 years post menopause, and approximately 60% of subjects in the training study were on HRT (Table 1). In the DXA-Fast group, two subjects, each, used anti-histamines, antidepressants, and Synthroid to correct hypo-thyroidism. In the DXA-Slow group, medications used included antidepressants (2), antihistamines (2), and medication for fibromyalgia (1).

Study design

All women received a health screen and an aerobic fitness test. All participants signed an informed consent form approved by the University of Michigan Medical School Institutional Review Board. In the training trial, women were assigned to high-intensity ($n=25$) or to low-intensity training ($n=24$) after matching for age, body mass index (BMI), use of hormone replacement therapy (HRT), and ventilatory threshold (VT). Changes in areal BMD (aBMD) of total body, legs, arms, trunk, spine, and pelvis, total body mass, percent body fat, and lean mass of legs and arms in response to high-intensity (DXA-fast) and low-intensity (DXA-slow) training were measured in 25 subjects. Changes in plasma concentrations of two markers of bone formation, osteocalcin (OC) and bone-specific alkaline phosphatase (bALP), were measured in 43 subjects (Table 1). OC is a noncollagenous protein, exclusive to bone, which interacts with hydroxyapatite and appears to regulate both bone mineralization and turnover [21]. It was reported to increase in response to strength training in postmenopausal women [32]. BALP binds to

Table 1
Characteristics of subjects in the three studies

Variable	DXA-Slow	DXA-Fast	OM-Slow	OM-Fast	GRF study
Number	12	13	18	25	9
Age (years)	58.1 (1.09)*	58.9 (1.39)	57.7 (1.09)	58.3 (0.89)	55.22 (1.15)
Body mass (kg)	78.51 (2.96)	76.37 (2.69)	78.09 (2.80)	77.23 (2.97)	71.41 (5.09)
Stature (cm)	162.4 (1.57)	162.1 (1.68)	163.95 (1.04)	163.3 (1.39)	168.0 (2.0)
BMI (kg/m^2)	29.8 (1.22)	29.0 (0.74)	29.11 (1.11)	28.9 (0.76)	25.4 (1.82)
T score	0.59 (0.2)	0.54 (0.4)			
Z score	0.70 (0.3)	0.98 (0.43)			
HRT/no HRT	7/5	8/5	13/5	16/9	
Years PM	7.8 (1.8)	7.7 (1.9)	10.4 (2.5)	9.8 (1.2)	
VO_2 max (ml O_2/kg*min)	22.8 (1.5)	27.0 (0.8)	24.2 (1.9)	26.9 (1.3)	26.9 (2.0)
VT (km/h)	5.40 (0.16)	5.16 (0.20)	5.41 (0.14)	5.16 (0.11)	5.81 (0.14)
VT (%VO_2 max)	63.9 (3.0)	62.5 (3.7)	66.2 (2.6)	62.1 (2.3)	60.4 (3.1)
Speed (km/h)	5.47 (0.12)	6.38 (0.21)#	5.36 (0.11)	6.26 (0.14)#	100%
Speed (% of VT)	102.5 (1.5)	123.0 (2.3)#	100.7 (1.48)	125.1 (2.1)#	
Speed (%VO_2 max)	66.6 (2.7)	86.3 (0.3)#	67.3 (2.5)	88.4 (2.7)#	
Compliance (%)	69.0 (3.7)	76.3 (3.2)	70.1 (3.1)	79.4 (3.0)	

DXA designation is for the groups in study 1, OM (osteogenic markers), for the groups in study 2. GRF is the designation for subjects in study 3. PM = postmenopausal; *Mean (S.E.M.) # $p<0.05$ fast groups vs slow groups.

osteoblasts and facilitates bone mineralization by increasing the calcium-phosphate product. Intense exercise by postmenopausal women has raised B2 and B1 isoforms of bALP [40] while acute unilateral leg exercise increased bALP release by leg muscles in healthy young men [8].

Screening tests

The health screen for all women included a brief health questionnaire, a personal physician's release form, and a brief physical exam for training subjects. The aerobic fitness was assessed through determination of a ventilatory threshold (VT) and maximal aerobic capacity (VO_2 max) both of which increase with aerobic training. Both measures allow assignment of training intensity relative to a subject's individual level of fitness. The fitness test consisted of measurements of rates of ventilation, oxygen uptake and carbon dioxide production with a SensorMedic 2900 metabolic cart (Yorba Linda, CA), and of heart rates with a Polar heart tachometer while subjects walked on a level treadmill. Treadmill speeds ranged from 1.6 to 8 km/h (0.44 and 2.22 m/s) and were increased every 3 min by 0.8 km/h (0.22 m/s) to document a sharp increase in ventilatory rate and to reach a respiratory quotient (ratio of carbon dioxide produced to oxygen consumed) of 1. The sharp upward inflection in the ventilatory rate, determined from the intersection of two linear regressions of the ventilatory rate as a function of oxygen consumption or of walking speed [5], signifies ventilatory compensation for increased blood acidity caused by transition from aerobic to anaerobic metabolism and represents the VT. A respiratory quotient of 1 is a criterion of maximal aerobic capacity.

Exercise training

Exercise training consisted of walking 4.8 km per day, 5 days a week for a period of 30 weeks at a commercial mall, between 6:30 and 7:30 h. The low-intensity assignment was to walk at 95% of subject's VT, equivalent to 62% of VO_2 max, and high-intensity assignment was to walk at 125% of VT, equivalent to 88% of VO_2 max, a near-maximal walking speed sustainable by our subjects for 4.8 km. For walking intensity calculation during the first and second 15 weeks, the initial and 15-week VT values, were used respectively. The speed equivalent of assigned exercise intensity was explained to each subject. Attendance and target speeds at each mile were supervised and recorded by trained personnel. Target speeds were maintained through time-keeping and feedback by supervising staff at 1-mile intervals. The training program started with weekly increases in walking distances of 1.6 km/day until the target exercise volume was attained on week 3. Subjects were allowed to make up for missed days by walking on week-ends. Because almost all training was done under supervision, adherence to the protocol was expressed as percent of total expected kilometers of supervised walking that were actually performed during the 15 and 30 weeks of training.

GRF testing and measurements

Subjects walked at speeds of 1.56, 1.79, and 2.01 m/s (corresponding to 5.6, 6.4, and 7.2 km/h, respectively) over a Bertec force plate embedded in the laboratory floor. Optical sensors placed at the subject's shoulder height allowed photocell detection and computerized recording of walking speeds. GRF measurements were recorded when the speeds fell within 3% of the assigned target speed. The force plate readings obtained with an unloaded plate were subtracted from those obtained during walking tests. Readings obtained with the subject standing stationary on the plate were used to normalize GRF to body weight.

Of the two GRF peaks produced during walking, the first one, corresponding to heel strike, occurs between 12% and 16% of the total gait cycle, while the second represents the push-off and occurs between 40% and 50% of gait cycle. Our outcome measures were the peak vertical force achieved during the gait cycle in newtons (N) or kg-m/s^2, the same measurement normalized by subjects' body weight (N/bw), and the impulse, the area under the GRF curve during the time the foot is in contact with the ground (kg-m/s).

DXA measurements

Whole body scans were performed at 15-week intervals with dual energy X-ray absorptiometry (DXA) scanners (models DPX and DXL, Lunar Radiation Corporation, Madison, WI, software version 1.3y) using the pencil beam mode to determine areal BMD (g/cm^2) of the total body (BMD_{total}), legs ($aBMD_{legs}$), arms ($aBMD_{arms}$), spine ($aBMD_{spine}$), and pelvis ($aBMD_{pelvis}$) and total bone mineral content (BMC_{total}). DXA scans also served for determination of total lean body mass and of total body fat as well as of the masses of the legs (LM_{legs}) and arms (LM_{arms}). Baseline and post-test scans were analyzed by the same experienced technician with the same instrument. Subjects were scanned while lying supine on the table with arms at the side. The CVs for BMD measurements of the separate regions ranged between 1.5% (spine) and 2.9% (arms). The quality control program included daily measurement of an anthropomorphic spine phantom and weekly calibration studies.

Blood collection

A fasting morning blood sample was collected into a heparinized tube containing sodium citrate. Plasma was separated by centrifugation and stored at −70 °C for later bone marker determinations.

Markers of bone formation

OC was measured using an immuno-radiometric (IRMA) assay for active human osteocalcin (DSL-7600, Diagnostic Systems Laboratories, Webster, TX). Sensitivity of this assay is 0.3 ng/ml. The inter- and intra-assay coefficients of variation were, respectively, 6% and 10% at 20 ng/ml, and 4.6% and 8% at 100 ng/ml. BALP was measured with an EIA kit (ALKPhase-B, now METRA-BAP, Quidel, Santa Clara, CA). The assay limit of detection is 0.7 U/l. The inter- and intra-assay coefficients of variation were approximately 5% at both 12 U/l and at 100 to 108 U/l.

Statistical analyses

Data are presented as means and S.E.M.s. Subject characteristics in training studies were compared with 2×2 ANOVA. Intent to treat classification by assigned exercise intensities was used in analysis of changes in BMD and bone markers. Student's *t*-test for independent groups was used for analysis of outcome differences after 15 weeks of training. Repeated measures mixed-model ANOVA was used for analysis of density data, of differences and percent differences in densities, as well as for OC, bALP, and VT measurements after 15 and 30 weeks of training with SAS software version 9.1 [14]. Bonferroni adjustment was used where more than one comparison was made of differences generated by the same group of subjects. Regression analysis was used to establish the relationship between relative exercise intensity expressed as % of VT or % of VO_2 max and the threshold for increases in BMD_{total}.

Results

Effectiveness of training

Subjects assigned to low exercise intensity walked at an average relative intensity of between 101% and 102.5% of VT, equivalent to between 65% and 67% of VO_2 max, and corresponding to average walking speeds of between 5.4 and 5.5 km/h. High intensity exercise was carried out at between 123% and 125.1% of VT, corresponding to 86% and 88% of VO_2 max and average walking speeds of between 6.3 and 6.4 km/h (Table 1). The compliance with the study protocol in terms of the assigned distance walked was between 69% and 71% for the low-intensity group, and between 76% and 79% for the high-intensity group (Table 1). Thus on the average, low-intensity participants walked the target distance on about 3.5 days, and high-intensity subjects on between 3.8 and 4 days per week. After both 15 and 30 weeks of training, aerobic fitness assessed through changes in the VT increased significantly at both training intensities (Tables 2 and 3), with increases ranging between 6.5% for the low intensity and 9.1% for the high intensity subjects.

DXA measurements

Fifteen weeks of high-intensity training increased total bone mineral density by 0.40% (0.005 g/cm^2) compared to a 1.30% (0.010 g/cm^2) $aBMD_{total}$ decline in low-intensity group (Fig. 1). The corresponding changes in aBMD of legs were an 0.08% (0.001 g/cm^2) increase in the high-intensity group and a 1.09% (0.013 g/cm^2) decrease in the low-intensity group (Fig. 1). Significant effect of training intensity on $aBMD_{total}$ ($F(df\ 1, 23)=12.60$, $p=0.0017$) was seen after Bonferroni adjustment in the interaction between training intensity and time in the ANOVA analysis of the 15 week data with 25 subjects. Changes in total body bone density ($t(df\ 1, 23)=3.549$, $p=0.0017$) and percent changes in density ($t(df\ 1, 23)=3.585$, $p=0.0016$) were also significant. Changes in areal BMD_{legs} in each of these analyses nearly reached significance. No other aBMD changes after 15 weeks of training approached significance except for pelvis (Fig. 1).

Significant effects of exercise intensity after 30 weeks of training were evident both when the analysis combined the 25 original subjects who trained 15 weeks with the 16 residual subjects who trained 30 weeks, and when the analysis was restricted to the 16 subjects. In the former analysis, a significant interaction between training intensity and duration of training was present for both $aBMD_{total}$ and for $aBMD_{legs}$ (Table 2). In the latter analysis, $aBMD_{total}$ difference scores ($F(df\ 2,14)=8.66$, $p=0.0107$) and percent difference scores ($F(df\ 2,14)=9.01$, $p=0.0095$) revealed significant interaction between training intensity and duration (Table 3). Training intensity also significantly affected $aBMD_{legs}$ values (Table 3),

Table 2
Effects of exercise on the intensity on the ventilatory threshold (VT), bone mineral density (BMD), bone mineral content (BMC), and lean mass of the limbs (LM) after 15 weeks of training

Variable	DXA-Slow pre-*T*	DXA-Slow post-15 weeks	DXA-Fast pre-*T*	DXA-Fast post 15 weeks	ANOVA F (*df*) *p*
Number	12	12	13	13	
VT (km/h)	5.18 (0.16)	5.62 (0.17)	5.18 (0.18)	5.58 (0.18)	5.42 (2,40) 0.0083^{T}
BMD (g/cm^2)					
Total body	1.172 (0.02)	1.155 (0.02)	1.187 (0.02)	1.185 (0.02)	7.32 (2,34) 0.0023 ^{INT}
Legs	1.192 (0.02)	1.174 (0.02)	1.207 (0.03)	1.202 (0.03)	6.47 (2,34) 0.041 ^{INT}
Arms	0.911 (0.02)	0.900 (0.03)	0.922 (0.03)	0.926 (0.03)	NS
Spine	1.113 (0.05)	1.109 (0.05)	1.173 (0.07)	1.106 (0.04)	NS
Pelvis	1.129 (0.04)	1.098 (0.03)	1.144 (0.03)	1.147 (0.03)	3.08 (2,34) 0.0592 ^{INT}
BMC_{total} (g)	922.67 (38.3)	923.36 (33.1)	970.54 (35.78)	970.0 (36.8)	NS
LM_{legs} (kg)	13.19 (0.48)	13.08 (0.41)	12.96 (0.36)	13.27 (0.397)	4.06 (2,34) 0.026 ^{INT}
LM_{arms} (kg)	3.78 (0.14)	3.98 (0.15)	4.05 (0.16)	4.06 (0.13)	NS
Z score (S.D.)	0.70 (0.28)	0.47 (0.24)	0.98 (0.43)	1.05 (0.25)	5.24 (2,34) 0.010 ^{T} 4.05 (2,34) 0.026 ^{INT}

^{T}=effects of training, ^{INT}=interaction between the effects of training and training intensity. BMCt=total BMC, LM_{legs}=leg lean mass, LM_{arms}=arm lean mass.

Table 3

Effects of exercise intensity on the ventilatory threshold (VT), bone mineral density (BMD), bone mineral content (BMC), and lean mass of the limbs (LM) of subjects who trained 30 weeks

Variable	DXA-Slow pre-*T*	DXA-Slow post-15 weeks	DXA-Slow post-30 weeks	DXA-Fast pre-*T*	DXA-Fast post-15 weeks	DXA-Fast post-30 weeks	ANOVA *F* (*df*) *p*
Number	7	6	7	9	8	9	
VT (km/h)	4.96 (0.19)	5.03 (0.23)	5.17 (0.15)	5.25 (0.20)	5.21 (0.2)	5.91 (0.16)	NS
BMD (g/cm^2)							
Total body	1.160 (0.03)	1.134 (0.03)	1.162 (0.03)	1.183 (0.03)	1.179 (0.03)	1.189 (0.03)	NS
Legs	1.181 (0.02)	1.149 (0.03)	1.179 (0.02)	1.206 (0.04)	1.198 (0.04)	1.194 (0.04)	NS
Arms	0.892 (0.03)	0.896 (0.04)	0.906 (0.04)	0.904 (0.03)	0.925 (0.04)	0.991 (0.05)	NS
Spine	1.073 (0.07)	1.038 (0.05)	1.045 (0.06)	1.198 (0.10)	1.101 (0.05)	1.097 (0.03)	NS
Pelvis	1.084 (0.06)	1.049 (0.05)	1.064 (0.05)	1.158 (0.05)	1.140 (0.04)	1.179 (0.04)	2.65 (1,34)^{INT} 0.112
BMC$_{total}$ (g)	869 (44.0)	867.17 (43.0)	877.0 (41.11)	966.33 (41.4)	960.5 (46.69)	968.33 (40.7)	NS
LM$_{legs}$ (kg)	12.48 (0.52)	12.42 (0.54)	12.22 (0.50)	13.169 (0.29)	13.57 (0.43)	13.55 (0.39)	NS
LM$_{arms}$ (kg)	3.58 (0.16)	3.78 (0.22)	3.64 (0.15)	4.09 (0.19)	4.07 (0.14)	4.33 (0.18)	4.97 (1,34)^{INT} 0.033
Z score (S.D.)	0.787 (0.36)	0.405 (0.33)	0.82 (0.38)	0.969 (0.31)	0.958 (0.34)	1.17 (0.35)	NS

^{INT}=interaction between the effects of training and training intensity. BMC$_{total}$=total BMC, LM$_{legs}$ l=leg lean mass, LM$_{arms}$=arm lean mass.

difference scores (*F*(*df* 2,14)=10.51, *p*=0.0059), and percent difference scores (*F*(*df* 2,14)=10.08, *p*=0.0068).

In addition, lean mass of legs appeared to be influenced by exercise intensity after both 15 and 30 weeks of training. After 15 weeks of high-intensity training, LM$_{legs}$ increased by to 0.3 kg while it decreased by about 0.1 kg after low-intensity training (Table 2). These differences became more pronounced after 30 weeks of training with an 0.4 kg gain in LM$_{legs}$ in high intensity group and an 0.26 kg loss in the low intensity group (Table 3). After Bonferroni adjustment, these group differences were nearly significant when the ANOVA was performed in all subjects for either 15 weeks of training (interaction of intensity and time, *F*(*df* 2, 23)=6.26, *p*=0.0199) or for 30 weeks of training (Table 2). When the analysis was done on only 16 subjects who trained for 30 weeks, LM$_{legs}$ difference after Bonferroni adjustment revealed significant intensity effect (*F*(*df* 2,14)=12.17, *p*=0.0036). A trend for arm lean mass to increase with intense training and decrease with low intensity training only approached significance (Tables 2 and 3).

Weight and percent fat changes in response to training at two intensities were not significant. High-intensity training resulted in an 0.3-kg weight gain after 15 weeks of training and a 1.1 kg weight loss after 30 weeks. The corresponding changes after low-intensity training were 0.6-kg and 1.4-kg weight losses. Respective fat losses were 0.7% and 1.5% in response to high-intensity training, and 1.9% and 1.7% fat losses after low-intensity training.

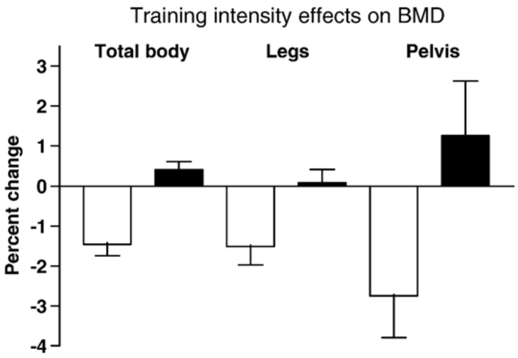

Fig. 1. The effect of 15 weeks of training at different intensities on the total body BMD (BMD$_{total}$), BMD of legs (BMD$_{legs}$), and BMD of pelvis (BMD$_{pelvis}$). Open bars=low-intensity walking, Solid bars=high-intensity walking.

Effect of HRT on BMD and LM changes

There was no effect of HRT on the changes in BMD$_{total}$ (−0.005±0.004 HRT vs 0.002±0.004 g/cm^2 no HRT), BMD$_{legs}$ (−0.006±0.006 HRT vs −0.006±0.005 no HRT), or LM$_{legs}$ (0.08±0.23 HRT vs 0.109±0.19 no HRT).

Markers of bone formation

Pre-training plasma OC concentrations were 15.3±2.5 and 15.5±2.8 ng/ml for the high-intensity and low-intensity groups, respectively. Training did not affect plasma OC concentrations after either 15 weeks (17.5±3.1 and 15.9±3.1 ng/ml, respectively) or 30 weeks of training (9.7±1.9 and 12.2±2.9 ng/ml, respectively). Pre-training plasma bALP concentrations were 12.2±1.1 and 13.4±1.7 ng/ml for the high-intensity and low-intensity groups, respectively. Training did not affect plasma bALP concentrations after either 15 weeks (13.2±1.2 and 13.9±1.7 ng/ml, respectively) or 30 weeks (12.3±1.4 and 14.8±2.6 ng/ml, respectively).

Ground reaction forces

Table 4 shows the effects of three walking speeds, within the range used in the training trial, on the peak vertical force, peak normalized vertical force, and impulse which is the total GRF force exerted during the step. As expected, both measures of peak force tended to increase with increased walking speed, but only the increase in the normalized value was statistically significant (*r*=0.555, *F*=11.14, *df*=1, 25, *p*=0.0026). Impulse tended to decrease with increased speed due to reduced foot contact time with the ground at higher walking speeds, but the change was not statistically significant (*r*=−0.339, *F*=3.255, *df*=1,25, *p*=0.115).

Table 4
Mechanical forces generated by stepping at three speeds

Walking speed	1.57 m/s	1.79 m/s	2.2 m/s
Peak vertical force (N)	865.4 (71.7)	913.1 (76.4)	967.0 (82.1)
Normalized peak vertical force (N/wt)	1.21 (0.03)	1.28 (0.03)	1.36 (0.04)
Impulse kg-m/s	348.2 (24.9)	316.0 (25.0)	288.8 (21.3)

Threshold training intensity for increases in BMD$_{total}$

Fig. 2 shows a significant correlations between changes in aBMD$_{total}$ after 15 weeks of training as a function of individual subjects' training intensities expressed on the left in terms of % VT ($r=0.544$, $F=9.67$, $p=0.005$) and on the right in terms of % VO$_2$ max ($r=0.508$, $F=6.63$, $p=0.019$). Increases in aBMD$_{total}$ were evident above relative exercise intensities of 115% of VT or 74% VO$_2$ max. This threshold corresponds to average walking speeds of 6.14 km/h or 1.71 m/s and a heart rate of 82.3% of age-specific maximum. The threshold exercise intensity for increases in aBMD$_{total}$ corresponds to a peak vertical force of 872.3 N and a peak normalized vertical force of 1.22 times body weight.

Discussion

The present study confirms that fast walking is an effective exercise for bone mineral preservation and accrual in early postmenopausal women. The effective exercise parameters were walking 4.8 km/day 4 days a week at a brisk pace above 6.14 km/h and at heart rates greater than 82.3% of age-specific maximum. This training paradigm, when carried out for 15 weeks, increased leg and total body bone mineral density in early postmenopausal women. Some training studies reporting equal or greater BMD increases in post-menopausal women implemented a combination of different types of locomotor activities such as walking, jogging, and stair climbing [15,29] so that variety and novelty of loading pattern may have contributed to the results [42]. In one of these studies, high intensity was combined with diverse loading exposure in 62-year-old women who were allowed to self select to an exercise group. BMC increased 5.2% after 9 months, and 6.1% above baseline after 22 months. After 13 months of detraining, all BMC increases regressed to baseline [15]. In the other study [29], an 11-month training program emphasizing GRF loading was compared to a program of equal duration emphasizing JRFs. The 2-month taper program and 9-month training program included walking, jogging, and stair climbing for periods of 45 min three times a week with an emphasis on alternating bursts of moderate and higher intensity exercise of up to 85% of maximal heart rate. This program resulted in BMD increases of 2% for the whole body, 1.8% for the spine, and 6.1% for the femur in 65- to 66-year-old postmenopausal women. That positive effects on BMD can be produced in a randomized study with simple walking as a type of exercise, despite its habitual loading pattern and without the need to combine different types of locomotor activities, is useful information, as walking is a preferred form of exercise for older women [18,30].

This study supports the importance of exercise intensity to prevent losses and produce increases in BMD in postmenopausal women. While training at either intensity improved aerobic fitness of subjects in this study, as seen in significant increases in VT in both trained groups, increases in BMD were seen in the legs and total skeleton of high-intensity group only. This study also achieved the primary goal of defining the osteogenic exercise intensity for bone mineral preservation and accrual in early postmenopausal women in terms that are helpful for different constituencies. Definition of osteogenic threshold in terms of walking speeds greater than 6.1 km/h and heart rates at least 82.3% of age-specific maximum is useful information for potential users who wish to prevent or reduce postmenopausal bone mineral loss. Definition of threshold in terms of relative exercise intensity of 115% of VT and 74% of VO$_2$ max provides useful guidelines to experimental scientists interested in further studies. This study has also quantified the osteogenic threshold in biomechanical terms as peak vertical forces greater than 872.3 N, or a force greater than 1.22 times body weight which may have useful application in the fields of physical therapy and rehabilitation.

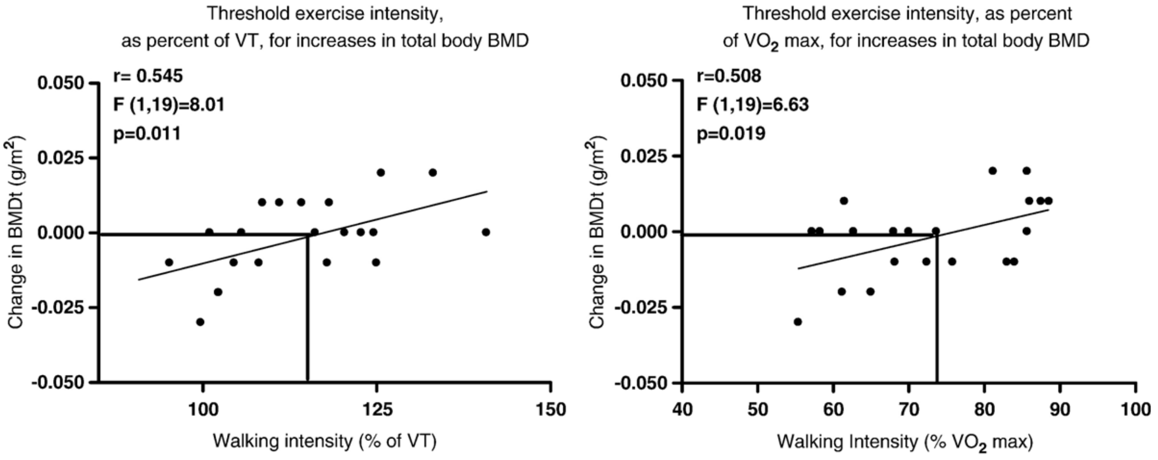

Fig. 2. Threshold walking intensity for increases in total body BMD.

At least one other study has demonstrated the importance of walking exercise intensity on bone mineral preservation and accrual in postmenopausal women. In a 7-month training study with Japanese women of similar age range as in the present study, Hatori reported a 1.1% increase in spinal BMD (the only reported site) in response to walking three times per week at intensities above the anaerobic threshold (corresponding to the VT in the present study), and at average walking speeds of 7.2 km/h, compared to a 1% loss after training at average speeds of 6.2 km/h [22]. Sedentary controls lost 1.7% of spinal bone mineral during the same seven months. Thus while Hatori subjects achieved spinal BMD gains while walking at higher exercise intensities than were generated in the present study, and spinal BMD was lost at walking speeds of between 6.1 and 6.4 km/day in both trials, both studies demonstrate positive and dose-dependent effects of exercise intensity on BMD at different skeletal sites.

A failure to produce increases in spinal trabecular bone density in another walking study by Cavanaugh with 55-year-old postmenopausal women [11] may have resulted from sub-optimal walking intensities. Training was 3 days a week for 52 weeks at the intensity that elicited heart rates in the range of between 60% and 85% of the age-specific heart rate maximum. Using our HR-exercise intensity conversion values, subjects in the Cavanaugh study walked at exercise intensities that ranged from sub-threshold (83.8% of VT) to supra-threshold (118.7% of VT). A failure to produce increases in total BMD in another larger study with 87 walking participants during 12 months of training [13] also was most likely due to their sub-threshold walking intensities of 60% to 75% of HR max. The importance of exercise intensity in BMD accrual in postmenopausal women could be further tested by manipulating the grade of the walking surface. Running at a −9° grade was reported to increase peak vertical forces by about 54%, and raising the grade to 6° to decrease them by 21% [19].

Mechanical loading of bones may not have been the only stimulus contributing to bone mineral accrual in response to high-intensity training in the Hatori study and our own. We recorded nearly significant 0.4 kg increases in the lean mass of the legs of fast walkers, and 0.3 kg loss in the slow walkers, during the 30-week training period. Thus it is probable that accrual of BMD is a combination of greater contractile JRFs, produced by gains in muscle mass and increased GRFs generated by faster walking. The correlation between muscle mass and strength on one hand, and BMD on the other, is well established [10,26,31,35], and high-resistance JRF training results in BMD increases in post-menopausal women [3,26,33]. Lean body mass and BMD losses in women walking up to 16.7 km/week at low intensities were unexpected and may have resulted from the protocol speeds in our study being lower than their habitual and preferred walking intensities.

High-intensity exercise could also have stimulated BMD accretion by eliciting secretion of pulses of osteogenic hormones PTH [7] and GH [43], as their secretion increases in proportion to exercise intensity, and intermittent or pulsatile increases in their concentration have anabolic properties [12,16,23]. It is not clear whether increased secretion of these hormones would have facilitated bone mineral accrual throughout the body, or, as was shown for PTH, enhance it largely at the mechanically stimulated bone sites [41].

Inference about the importance of exercise intensity in bone mineral preservation and accrual in early postmenopausal women generated by the present study should be accepted with caution as one of the study limitations is its relatively small number of participants. However, significant positive dose-dependent effect of exercise intensity on the BMD in the modest number of early postmenopausal women in the present study, combined with similar results of the Hatori study [22], suggest that the preservation and small increases in bone mineral at specific skeletal sites when exercise is performed above the osteogenic threshold intensity may be real. Further larger-scale randomized controlled trials are needed to confirm these results.

An additional limitation of this study is that only two markers of bone formation, and no markers of bone resorption, were included. In this study, markers of bone formation were unaffected by 15 and 30 weeks of training and did not change in parallel to changes in BMD. Thus the usefulness of OC and bALP concentration changes as markers of changes in BMD in response to prolonged training is uncertain, as was also reported by others [9]. It is possible that failure to detect changes in bone formation was a consequence of dissociation between times when rapid changes in BMD were occurring and times selected for documentation of cumulative BMD changes. Increases in bALP, if not OC, occur rapidly in response to acute exercise. They were noted after 40 min of exhaustive jogging [32] and after 60 min of strenuous leg exercise [40]. Increase in bone markers may not be the only means by which exercise protects against bone loss. A recent study reported a decrease in a marker of bone resorption, the n-terminal telopeptide of type I collagen (NTX) that accompanied a modest increase in lumbar BMD in postmenopausal women as a result of 12 months of walking [47]. Therefore, studies should include a wider range of markers of bone turnover when assessing their usefulness as indices of change in bone metabolism during exercise [9,47].

Although animal studies singled out exercise intensity, and de-emphasized the importance of exercise bout duration, as key variables responsible for increases in bone mineral, optimal exercise volumes for human bone mineral accretion also have not been properly defined. Krall and Dawson-Hughes suggest that the threshold exercise volume to produce increases in BMD of whole body, legs, and possibly trunk, is 12.1 km/week [31]. This is less than the 15.6 km/week or 3.1 km/day for DXA-Slow and 18.5 km/week or 3.7 km/day for DXA-Fast carried out in the present study. Threshold exercise volume to increase bone mineral in the calcaneus of late postmenopausal Japanese women, mean age 71, was described as 6000 steps or about 4.8 km per day [27], about 29% greater than in the present study in DXA-Fast subjects, while maximal walking volume above which bone mineral declined, was twice as large at 12,000 steps (or about 9.6 km) per day [28]. However, none of these studies controlled the exercise intensity variable, so the question of threshold walking volume remains unresolved.

Resolution of exercise volume is of particular importance in view of the challenges of motivating and retaining women for a regime of almost daily prolonged bouts of walking over an extended period of time. The drop-out rate was between 7% and 8% during the first 15 weeks of training but increased to between 41% and 47% during the second 15 weeks of training. The latter number is in agreement with reports of others [17]. In addition, compliance to the 5-day a week training protocol was only 69% and 71% in the low-intensity groups and 76% and 79% in the high-intensity groups. It is probable that compliance to osteogenic training protocols could be improved if the duration of sessions and training frequency could be reduced. Thus, greater compliance and lower drop-out rates in training studies with postmenopausal women are likely to be achieved once the least necessary duration, frequency, intensity, and timing of exercise for effective increases in bone mineral are defined with greater precision. In addition, negative results [6,13,45,46] can be avoided if exercise training studies that address duration, frequency, safety, feasibility, and timing of osteogenic exercise implement training at intensities above the thresholds defined in this study.

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