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Original Article

Effects of whole-body vibration on bone properties in aged rats

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Abstract

Objective: This study aimed to explore optimal conditions of whole-body vibration (WBV) for improving bone properties in aged rats. **Methods:** Eighty-week-old rats were divided into baseline control (BC), age-matched control (CON) and experimental groups, which underwent WBV (0.5 *g*) at various frequencies (15, 30, 45, 60 or 90 Hz) or WBV (45 Hz) with various magnitudes (0.3, 0.5, 0.7 or 1.0 *g*) for 7 weeks. After interventions, femur bone size, bone mechanical strength and circulating bone formation/resorption markers were measured, and trabecular bone microstructure (TBMS) and cortical bone geometry (CBG) of femurs were analyzed by micro-CT. **Results:** Several TBMS parameters and trabecular bone mineral content were significantly lower in the 15 Hz WBV (0.5 *g*) group than in the CON group, suggesting damage to trabecular bone. On the other hand, although frequency/magnitude of WBV did not influence any CBG parameters, the 0.7 *g* and 1.0 *g* WBV (45 Hz) group showed an increase in tissue mineral density of cortical bone compared with the BC and CON groups, suggesting the possibility of improving cortical bone properties. **Conclusion:** Based on these findings, it should be noted that WBV conditions are carefully considered when applied to elderly people.

Keywords: Aged Rats, Bone Mechanical Strength, Cortical Bone Geometry, Trabecular Bone Microstructure, Whole-Body Vibration

Introduction

Osteoporosis is a major public health issue worldwide. In 2010, 49 million individuals developed osteoporosis in North America, Europe, Japan and Australia[1]. The risk of fragility fractures is high in individuals aged 50 years and over, posing a major economic burden on individuals and society[2-4]. In older people, therefore, it is important to prevent fractures by both maintaining bone mass/quality and reducing the risk of falls. As part of fall prevention strategies, exercise aimed at improving bone properties and balance function was recommended for elderly people[5] and was reported to increase/maintain bone mass and decrease bone loss[6-8], and also to reduce the risk of falls[8-10]. However, safety precautions are necessary for exercise to decrease the risk of falls and fractures[11].

Whole-body vibration (WBV) can potentially reduce fracture risk by improving bone mineral density (BMD) and/or balance functions[12-16]. WBV, which is a safe and non-invasive method, requires no vigorous physical activity, and therefore WBV is considered a unique modality suitable for preventing fractures in elderly people. However, the effects of WBV on bone properties in elderly people were inconsistent among the previous studies. Some reported positive effects of WBV on BMD[13-16], while others found no such effects[17-21]. This discrepancy may be ascribable to differences in not only subject characteristics (e.g., species, age, sex and bone conditions) but also WBV conditions (e.g., frequency, magnitude, duration and starting time). Positive effects of WBV on BMD was reported to be dose-dependent (e.g., longer exposure and higher compliance)[22]. In animal studies, WBV at a high frequency (>30 Hz) or with a low magnitude (<1 *g*) showed beneficial effects on bone properties in young and adult rodents[23-25]. However, in aged rodents, only a few animal studies investigated effects of WBV on bone properties[26,27]. In 22-month-old male mice, tibia bone properties were not

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influenced by WBV (90 Hz, 15 min/day, 5 days/week) with two different magnitudes (0.3 and 1.0 *g*) for 5 weeks[26]. In 18-month-old male mice, although WBV (32 Hz, 30 min/day, 5 days/week) with two different magnitudes (0.5 and 1.5 *g*) for 12 weeks had no effects on femoral bone morphology obtained by micro-CT, both low- and high-magnitude WBV significantly decreased pyridinoline crosslinks and increased mineralizing surface, and also high-magnitude WBV increased the number of osteoclasts[27]. These observations may be attributable to a blunted response to vibration in aging bone.

Bone properties may deteriorate with age. Rats aged 9 months or over showed a decrease in cancellous bone mineral content (BMC) and BMD of proximal tibia and femoral neck[28]. Moreover, aged rats showed a decrease in trabecular number and an increase in tibial trabecular separation[29]. Therefore, the present study aimed to examine the optimal frequency and magnitude of WBV for enhancing bone properties in 80-week-old rats with bone loss. This study may lead to a possible modality for improving bone health in elderly people, especially those experiencing difficulties in physical activity.

Materials and Methods

Animals care and experimental protocol

Male Wistar rats were purchased from Japan SLC, Inc. (Hamamatsu, Japan), and housed in standard cages in an animal facility where the room temperature and lighting were controlled (temperature, 22-24°C; lighting, 12:12-h light-dark cycle). Rats were fed a standard rodent chow (CE-2; CLEA Japan Inc., Tokyo, Japan) and water *ad libitum* during the study period. The present study consisted of two experiments: *Experiment I*: Forty-eight 80-week-old male Wistar rats divided into the baseline control (BC, n=6), age-matched control (CON, n=7) and five experimental groups (n=7 each). In the experimental groups, using a vibration device system (Big Wave G-MasterPRO; Asahi Seisakusho Co. Ltd., Tokyo, Japan), WBV (vertical direction vibration, magnitude 0.5 *g* (acceleration 4.9 m/s^2), 15 min/day, 5 days/week) was performed at five different frequencies (15, 30, 45, 60 and 90 Hz) for seven weeks; *Experiment II*: Three experimental WBV groups (n=6 each) were added to the 0.5 *g* WBV (45 Hz) group of Experiment I. Eighteen 80-week-old male Wistar rats divided into three experimental groups. Each group underwent WBV (vertical direction vibration, frequency of 45 Hz, 15 min/day, 5 days/week) with 0.3 , 0.7 or 1.0 *g* of magnitude (2.9, 6.9 or 9.8 m/s^2 of acceleration) for seven weeks. The frequency of 45Hz was selected based on results of Experiment I.

After the WBV interventions, blood, muscle and bone samples were collected from all rats. Serum samples were stored at -80°C until biochemical analysis and ELISA. Bilateral soleus (SOL) and extensor digitorum longus (EDL) muscles were weighed. Bilateral femurs were used for measurements of wet weight and length. Right and left femurs were stored in saline and 70% ethanol, respectively, until analyzed.

This study was approved by the Committee of Research Facilities of Laboratory Animal Science, Kio University, and was performed in accordance with the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication No.85-23, revised in 1996).

Biochemical analyses and ELISA

Serum samples were analyzed for calcium and inorganic phosphorus. In addition, serum levels of osteocalcin (OC) as bone formation marker and tartrate-resistant acid phosphatase-5b (TRACP-5b) as bone resorption marker were determined by commercially available ELISA kits (Immunodiagnostic Systems Ltd., Boldon, UK).

Analyses of bone mass, trabecular bone microstructure (TBMS) and cortical bone geometry (CBG)

Using an x-ray micro-computed tomography system (Micro-CT; Yamato Scientific Co. Ltd., Tokyo, Japan), the left distal femur was scanned at 60 kV, 60 μA, with a voxel size of 9.6-9.7 μm for TBMS analysis. The region of interest (ROI) for TBMS of distal femur was a 1.5 mm-length portion of the femur metaphysis, and the first slice was scanned 0.5 mm proximal from the physeal-metaphyseal demarcation. Likewise, in CBG analysis, diaphysis of the femur was simultaneously scanned under the same scanning conditions using the Micro-CT. The ROI for CBG was a 0.5 mm-length portion of center of femur diaphysis. Scanned data were transmitted to a personal computer, and TBMS and CBG of the ROI were analyzed using the bone analysis software (TRI BON 3D; Ratoc System Engineering Co. Ltd., Tokyo, Japan). Tissue volume (TV), bone volume (BV), bone volume fraction (BV/TV), trabecular bone thickness (Tb.Th), trabecular bone number (Tb.N), trabecular bone separation (Tb.Sp), trabecular bone width (Tb.W), connectivity density (Conn.D) and trabecular bone pattern factor (TBPf) were assessed as TBMS parameters. Cortical bone volume (CV), all bone volume (AV), medullary volume (MV), cortical bone volume fraction (CV/AV), cortical bone thickness (Ct.Th), cortical bone sectional area (Ct.Ar), periosteal perimeter (Ps.Pm), endocortical perimeter (Ec.Pm) and cortical porosity (Ct.Po) were assessed as CBG parameters. Moreover, a BMD phantom was simultaneously scanned under the same scanning conditions to obtain tissue mineral density (TMD), bone mineral content (BMC) and volume BMD (vBMD; BMC/TV) of the femoral trabecular and cortical bones.

Measurement of bone mechanical strength

The maximum load and break point of the right femurs were measured by a 3-point bending strength test using a Universal Testing Machine (Autograph AGS; Shimadzu Corp., Kyoto, Japan), and absorption energy of all area was calculated. Each bone was supported by 2 fulcrums (5 mm-diameter) separated by a distance half the length of the bone, and downward force was applied to the midpoint of the bone at a speed of 1 mm/min.

Table 1. Body weight, food intake, muscle weight, bone length, bone weight and ash weight of femurs in all groups of Experiments I and II.

			Muscle weight (mg/BW)			Bone weight (mg/BW)		
		Final BW (g)	Soleus	EDL	Bone length (mm)	Wet	Dry	% Ash weight
Experiment I	Baseline	500.3±49.1	0.301±0.034	0.340±0.023	41.5±1.3	2.61±0.10	1.82±0.06	63.3±1.9
	CON	493.8±22.5	0.310±0.020	0.341±0.016	41.2±0.8	2.54±0.14	1.79±0.09	62.5±2.3
	15 Hz	469.8±31.9	0.317±0.021	0.356±0.024	41.4±1.4	2.58±0.09	1.79±0.06	61.5±2.0
	30 Hz	471.6±22.0	0.306±0.022	0.335±0.022	41.1±1.4	2.57±0.10	1.82±0.13	62.1ω1.8
	45 Hz	473.1±33.0	0.311±0.019	0.340ω0.015	40.9±0.7	2.60±0.18	1.77±0.12	63.9±1.8
	60 Hz	469.2±48.5	0.302±0.024	0.343±0.028	40.9±1.5	2.62±0.35	1.85±0.21	63.1±2.4
	90 Hz	466.0±39.8	0.325±0.019	0.351±0.015	41.4±1.2	2.66±0.12	1.84±0.10	62.8±2.2
Experiment II	0.3 *g* (2.9m/s²)	479.9±22.4	0.291±0.033	0.340±0.021	41.1±0.7	2.66±0.15	1.92±0.14	61.3±1.6
	0.5 *g* (4.9m/s²)	473.1±33.0	0.311±0.019	0.340±0.015	40.9±0.7	2.60±0.18	1.76±0.12	63.9±1.8
	0.7 *g* (6.9m/s²)	488.7±35.9	0.311±0.021	0.350±0.018	41.3±1.2	2.63±0.16	1.93±0.17	62.2±3.1
	1.0 *g* (9.8m/s²)	468.7±21.1	0.325±0.025[a]	0.353±0.013	41.7±0.5[b]	2.80±0.16[c]	1.90±0.14	62.9±1.9

[a]Significantly different from the 0.3 g WBV group (p<0.05). [b]Significantly different from the 0.5 g WBV group (p<0.05). [c]Significantly different from the baseline, CON and 0.5 g WBV groups (p<0.05). Data are expressed as mean ±SD. Each group is comprised of 5-7 animals. CON, age-matched control; BW, body weight; EDL, extensor digitorum longus.

Table 2. Trabecular and cortical bone mineral parameters of femurs in all groups of Experiments I and II.

		Trabecular bone			Cortical bone	
		TMD (mg/cm³)	BMC (mg)	vBMD (mg/cm³)	TMD (mg/cm³)	BMC (mg)
Experiment I	Baseline	1018.2±39.2	4.96±2.50	220.2±80.4	1568.5±10.0	11.4±1.0
	CON	1038.1±24.3	3.28±0.32	172.7±21.3	1557.4±15.9	10.9±0.6
	15 Hz	1023.0±33.7	2.53±0.34[a]	140.3±25.9	1587.3±18.3[a]	10.6±0.5
	30 Hz	1033.6±52.3	2.81±1.01	159.5±60.3	1569.4±10.7	10.7±0.5
	45 Hz	1037.6±27.2	3.24±0.97	175.6±52.6	1558.4±15.2	10.8±0.6
	60 Hz	1043.7±15.0	2.97±0.88	164.9±27.7	1558.8±17.5	10.9±1.0
	90 Hz	1036.9±18.7	2.75±0.66	154.8±32.0	1567.4±17.3	10.7±0.6
Experiment II	0.3 *g* (2.9m/s²)	1028.8±15.1	3.54±1.10	176.0±35.8	1568.8±22.9	11.3±1.0
	0.5 *g* (4.9m/s²)	1037.6±27.2	3.24±0.97	175.6±52.6	1558.4±15.2	10.8±0.6
	0.7 *g* (6.9m/s²)	1030.6±20.4	3.20±0.54	171.3±25.7	1592.8±4.5[b]	12.0±1.0
	1.0 *g* (9.8m/s²)	1021.0±38.2	3.13±0.68	160.8±37.9	1600.3±10.1[b]	11.5±0.6

[a]Significantly different from the CON group (p<0.05). [b]Significantly different from the baseline, CON and 0.5 g WBV groups (p<0.05). Data are expressed as mean±SD. Each group is comprised of 5-7 animals. CON, age-matched control; TMD, tissue mineral density; BMC, bone mineral content; vBMD (=BMC/tissue volume), volume bone mineral density.

Table 3. Cortical bone geometry of femurs in all groups of Experiments I and II.

		CV (mm³)	MV (mm³)	CV/AV (%)	Ct.Th (mm)	Cr.Ar (mm²)	Ps.Pm (mm)	Ec.Pm (mm)	Ct.Po (%)
	Baseline	7.13±0.68	6.14±0.64	53.6±2.3	0.63±0.05	7.13±0.68	13.3±0.5	10.4±0.6	0.44±0.15
Experiment I	CON	6.86±0.40	6.28±1.01	52.3±4.8	0.59±0.05	6.87±0.40	13.4±0.4	11.1±0.8	0.53±0.19
	15 Hz	6.58±0.35	6.24±0.98	51.4±3.5	0.58±0.04	6.58±0.34	13.2±0.6	10.7±1.3	0.44±0.09
	30 Hz	6.68±0.34	6.47±0.99	50.9±4.3	0.58±0.05	6.69±0.34	13.3±0.4	11.0±1.0	0.48±0.15
	45 Hz	6.82±0.33	5.91±0.67	53.5±3.4	0.61±0.04	6.83±0.34	13.2±0.3	10.6±0.8	0.54±0.08
	60 Hz	6.82±0.51	6.16±0.52	52.4±2.6	0.60±0.04	6.82±0.52	13.2±0.4	10.9±1.0	0.52±0.06
	90 Hz	6.68±0.32	6.44±0.72	50.9±2.9	0.58±0.03	6.69±0.32	13.4±0.5	10.9±0.8	0.47±0.05
Experiment II	0.3 *g* (2.9m/s²)	7.10±0.62	6.29±0.77	53.0±4.4	0.61±0.04	7.11±0.62	13.5±0.7	11.0±1.1	0.41±0.16
	0.5 *g* (4.9m/s²)	6.82±0.33	5.91±0.67	53.5±3.4	0.61±0.39	6.83±0.34	13.2±0.3	10.6±0.8	0.54±0.08
	0.7 *g* (6.9m/s²)	7.43±0.67	5.62±0.97	56.9±4.9	0.67±0.06	7.44±0.68	13.3±0.6	10.3±1.2	0.50±0.17
	1.0 *g* (9.8m/s²)	7.07±0.44	6.91±0.87	50.6±4.4	0.60±0.06	7.09±0.44	13.7±0.3	11.3±1.1	0.38±0.09

Data are expressed as mean ±SD. Each group is comprised of 5-7 animals. CON, age-matched control; CV, Cortical bone volume; MV, Medullary volume; CV/AV, Cortical bone volume fraction; Cr.Th, Cortical bone thickness; Cr.Ar, Cortical bone sectional area; Ps.Pm, Periostea perimeter; Ec.Pm, Endocortical perimeter; Ct.Po, Cortical porosity.

Table 4. Serum concentrations of calcium and inorganic phosphorus in all groups of Experiments I and II.

		Ca (mg/dL)	IP (mg/dL)
Experiment I	Baseline	10.4±0.2	4.0±0.4
	CON	10.4±0.2	4.3±0.2
	15 Hz	10.6±0.4	4.8±0.9
	30 Hz	10.3±0.2	4.1±0.3
	45 Hz	10.3±0.2	4.3±0.3
	60 Hz	10.4±0.2	4.3±0.5
	90 Hz	10.4±0.4	4.1±0.7
Experiment II	0.3 *g* (2.9m/s^2)	10.4±0.1	4.1±0.1
	0.5 *g* (4.9m/s^2)	10.3±0.2	4.3±0.3
	0.7 *g* (6.9m/s^2)	10.5±0.2	4.1±0.2
	1.0 *g* (9.8m/s^2)	10.4±0.2	4.0±0.3

Data are expressed as mean±SD. Each group is comprised of 5-7 animals. CON, age-matched control; Ca, calcium; IP, inorganic phosphorus.

Dry bone and ash weight measurements

After the measurements of TBMS/CBG parameters, femurs were dehydrated in 100% ethanol for 48 hours, heated at 100°C for 24 hours to obtain dry bone weight and burned to ash at 600°C for 24 hours in an electric furnace (Nitto Kagaku Co. Ltd., Nagoya, Japan) to obtain ash weight. Dry bone weight was corrected by BW, and ash weight was corrected by dry bone weight (% ash weight).

Statistical analysis

All values were expressed as mean ± SD. Differences in effects of WBV frequency or magnitude on measured parameters between the BC and other groups, and between the CON and the experimental groups were examined using Steel's test. The overall difference among experimental groups and differences between two groups were examined by the Kruskal-Wallis test and Steel-Dwass test, respectively. All statistical analyses were performed using the Excel Statistics software (BellCurve for Excel version 3.20 for Windows; Social Survey Research Information Co. Ltd., Tokyo, Japan). *P* value less than 0.05 was considered statistically significant.

Results

Final body weight (BW), muscle weight and bone size

Table 1 shows the results of final BW, BW-corrected muscle weight and bone size (bone length, BW-corrected wet and dry bone weights, and % ash weight) of femurs in all groups. Final BW did not differ significantly among the groups in both Experiments I and II. In Experiment II; 1) BW-corrected SOL weight was significantly heavier in the 1.0 *g* WBV than in the 0.3 *g* WBV group; 2) Bone length was significantly longer in the 1.0 *g* WBV than in the 0.5 *g* WBV group; 3) BW-corrected wet bone weight of the femur was significantly heavier in the 1.0 *g* WBV than in the BC, CON and 0.5 *g* WBV groups.

Bone mineral parameters

Table 2 shows that in Experiment I; 1) BMC and vBMD of the femoral trabecular bone were lower in the 15 Hz WBV than in the CON group, with significant difference for BMC; 2) TMD of the femoral cortical bone was significantly higher in the 15 Hz WBV than in the CON group, and in Experiment II; cortical bone TMD was significantly higher in the 0.7 and 1.0 *g* WBV than in the BC, CON and 0.5 *g* WBV groups.

TBMS and CBG parameters

In all groups, several TBMS parameters (BV, BV/TV, Tb.N, Tb.W, Conn D and TBPf) showed that femoral trabecular bone tends to deteriorate with age. Furthermore, BV, BV/TV and Tb.W were significantly lower in the 15 Hz WBV than in the CON group, and Tb.Th was significantly lower in the 15 Hz WBV than in the 60 and 90 Hz WBV groups in Experiment I (Figure 1). As to magnitudes in Experiment II, on the other hand, TBMS parameters did not significantly differ among groups (Figure 1). All CBG parameters also did not significantly differ among groups in both Experiments I and II (Table 3).

Bone mechanical strength

In Experiment I, the break point of the femur was significantly lower in the 15 Hz WBV than in the BC group, and absorption energy was significantly lower in the 90 Hz WBV than in the CON group (Figure 2).

Biochemical analyses

Serum concentrations of calcium and inorganic phosphorus did not significantly differ among groups in Experiments I and II (Table 4). In addition, serum levels of OC and TRACP-5b did not significantly differ among the groups in Experiments I and II (Figures 3A and 3B). Although several groups (1.0 *g* WBV group for OC; BC, 45 Hz, 0.5 *g* and 0.7 *g* WBV groups for TRACP-5b) showed an increased SD bar that reflects an increased individual difference, every group except the 1.0 *g* WBV group showed a relatively small SD bar in OC/TRACP-5b ratio (Figure 3C), suggesting small individual difference in the balance of bone formation and bone resorption. The 1.0 *g* WBV group indicated the highest mean value of OC/TRACP-5b ratio.

Discussion

This is the first to report the effects of different frequencies/magnitudes of WBV in aged rats. In this study, we found that WBV at a low frequency has an impact on trabecular bone. Decreases in BMC and TBMS parameters (i.e., BV, BV/TV and Tb.W) were observed in the 15 Hz WBV group compared with

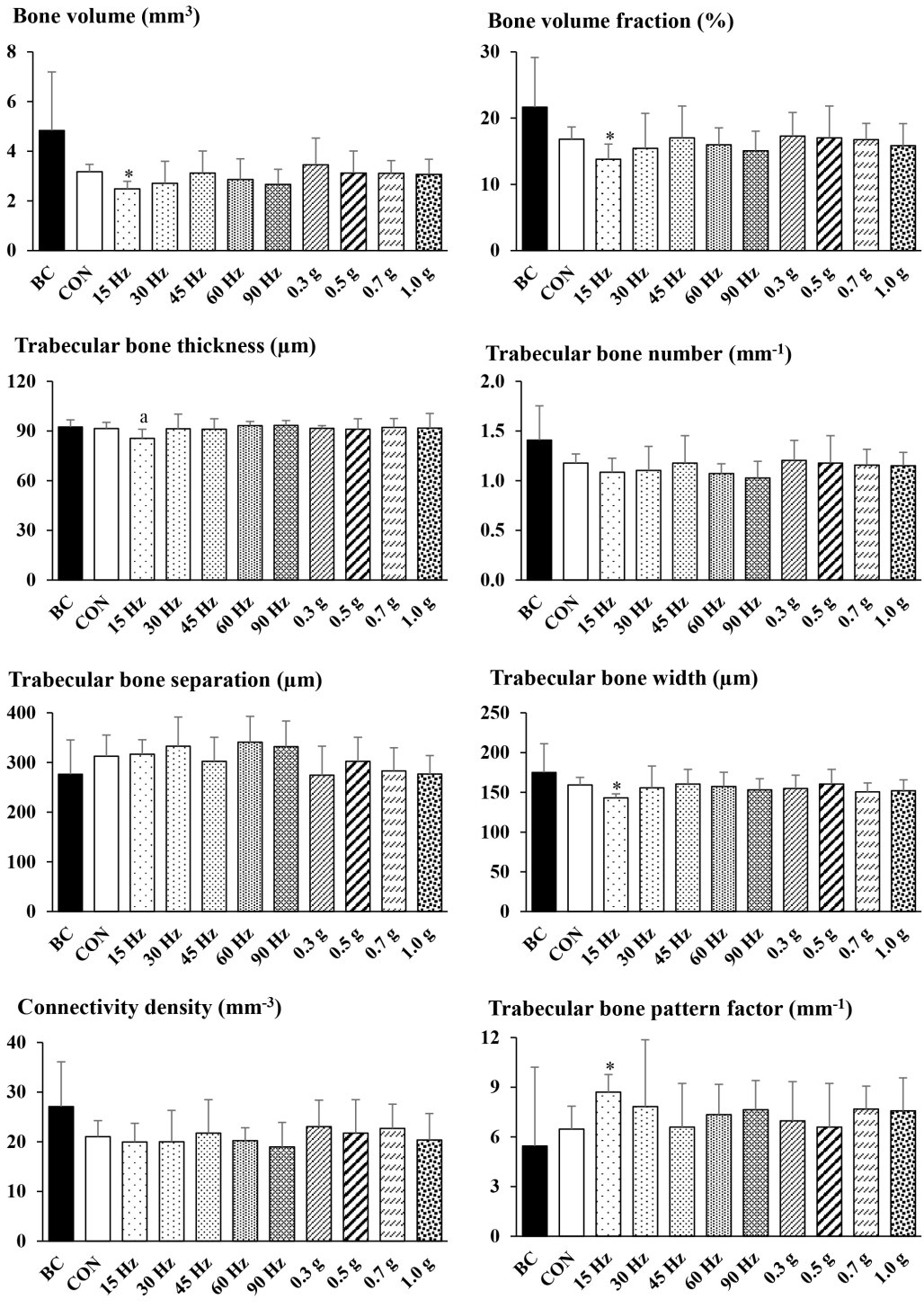

Figure 1. Trabecular bone microstructure of femurs in all groups. **Significantly different from the CON group (p<0.05). [a]Significantly different from the 60 Hz and 90 Hz WBV groups (p<0.05). Bars indicate SD. BC, baseline control; CON, age-matched control. n=5-7.*

the CON group. In addition, TBPf was higher in the 15 Hz WBV than in the CON group. TBPf is defined as the ratio of change in trabecular bone surface area to change in trabecular bone surface volume, and a higher TBPf value means a rod-like trabecular structure. Likewise, the 15 Hz WBV group showed a decrease in bone mechanical strength (i.e., break point) compared with the BC group. Thus, we found negative effects of 15 Hz WBV on BMC, several TBMS parameters and bone break point in aged rats, while WBV at other frequencies had no such effects. The previous studies in aged rats showed no

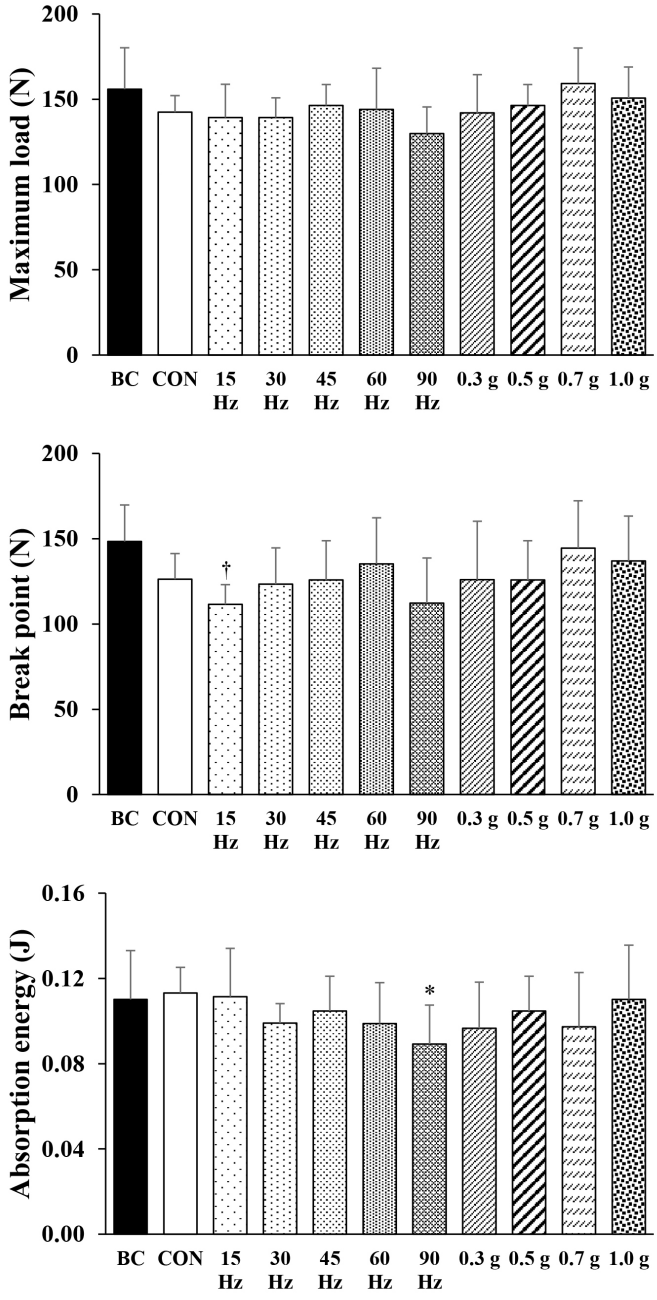

Figure 2. Maximum load, break point and absorption energy in femurs of all groups. *†Significantly different from the BC group (p<0.05). *Significantly different from the CON group (p<0.05). Bars indicate SD. BC, baseline control; CON, age-matched control. n=5-7.*

effects of WBV on bone properties[26,27]. On the other hand, in ovariectomized rats, 90 Hz WBV had beneficial effects on bone properties[25,30,31]. For instance, 90 Hz WBV was found to more effectively improve BV, Tb.Th and bone formation compared with 45 Hz WBV[31]. However, 90 Hz WBV, albeit effective, was not necessarily most effective on bone[32]. Also, WBV superior to 90 Hz influenced cortical bone, but not trabecular bone[33]. To the contrary, 90 Hz WBV was reported to have no effects on tibial structural parameters and mechanical strength in ovariectomized rats[34]. Although WBV appears to be promising in terms of improving bone properties, its effects may differ depending on not only subject's conditions (e.g., species, age, sex, surgery) but also WBV variables (e.g., magnitude, duration, starting time) including frequency which may play a pivotal role in WBV action on bone.

Similar to the present results, WBV at lower frequency was reported to cause a deterioration in bone properties[24].

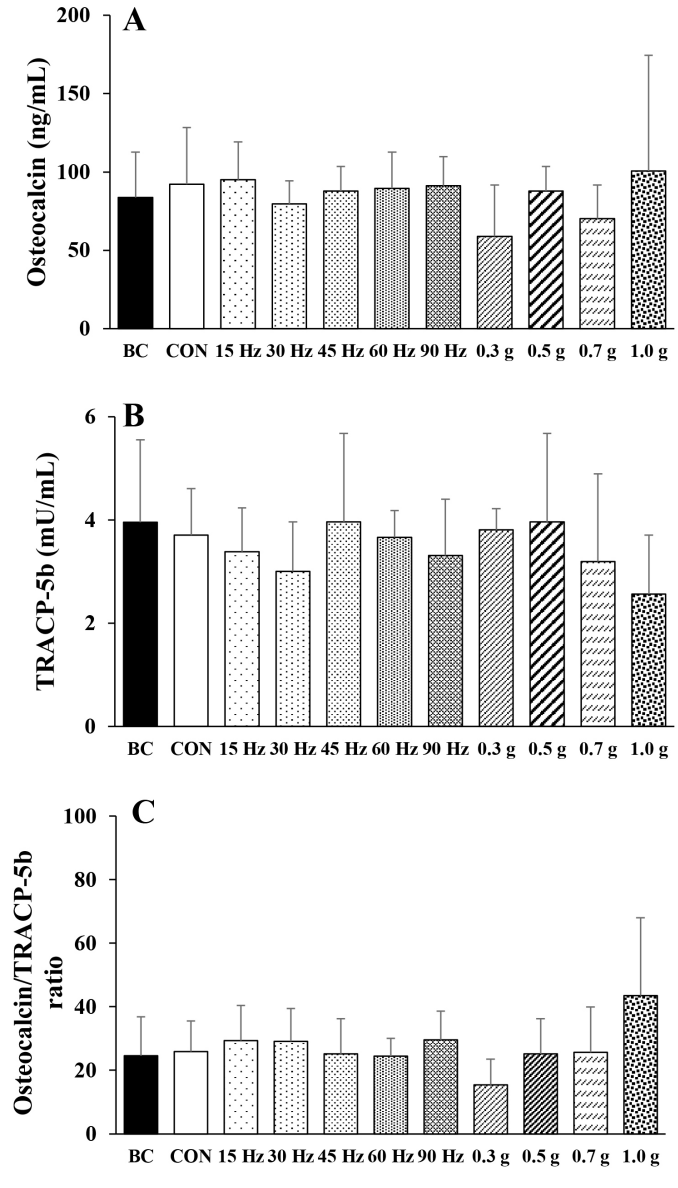

Figure 3. Serum levels of osteocalcin and TRACP-5b, and osteocalcin/TRACP-5b ratio in all groups. *A, serum levels of osteocalcin; B, serum levels of TRACP-5b; C, ratio of osteocalcin to TRACP-5b. Bars indicate SD. TRACP-5b, tartrate-resistant acid phosphatase-5b; BC, baseline control; CON, age-matched control. n=5-7.*

Pasqualini et al. investigated the effects of WBV at 8, 52 and 90 Hz (0.7 *g* of magnitude, 10 min/day, 5 day/week, 28 days) on bone in mature rats, and found that 8 Hz WBV leads to a reduction in trabecular BMD, which is probably caused by an impairment in osteoid maturation and/or an excess of bone resorption over bone formation, whereas 90 Hz WBV has beneficial effects on trabecular and cortical bones[24]. Bone strain and muscle contraction are considered to affect WBV action on bone[35]. Bone strain decreased with increasing frequency of WBV, while bone response to muscle contraction was induced by continuous low-strain, high-frequency stimuli[36]. In addition, bone properties could be maintained or increased by numerous loaded cycles, even though bone strain was small[36]. The above-mentioned negative action of 8 Hz WBV on trabecular BMD seemed to result from a non-optimal combination of generated strain and the number of loaded cycles[24]. In the present study, circulating bone formation/resorption makers did not differ among groups. In addition, trabecular bone deterioration was not observed in the experimental groups except for the 15 Hz WBV group. One possible explanation for the negative effects of 15 Hz WBV would be given by a non-optimal combination of strain

and the number of loaded cycles, same as that of 8 Hz WBV. Although bone response to treadmill running or resistance training in aged rodents was similar to, or more sensitive than that in young rodents[37-39], bone response to loaded cycles was less sensitive in aged rats[40]. Such differences in bone response by age may be associated with the type of stimulus. As for the WBV stimulus, bone response was lower in aged rats than in young rats[26]. In view of these results, WBV at a low frequency, such as 8 Hz or 15 Hz, appear to be stimuli inadequate for aged rats with blunted bone response.

WBV with higher magnitude may influence cortical bone TMD in aged rats. In the current study, although WBV with 0.3-1.0 *g* of magnitude had no effects on both TBMS and CBG parameters, 0.7 or 1.0 *g* WBV significantly increased cortical bone TMD. In adult mice, 0.1-1.0 *g* WBV for 5 weeks increased BV/TV of the proximal tibia and distal femur metaphysis in a magnitude-dependent manner[41]. On the other hand, WBV with higher magnitude (3.0 *g*) effectively promoted cortical bone formation and resorption of tibia diaphysis in 3-month-old ovariectomized rats[42,43]. Thus, bone distressed with estrogen deficiency could respond to WBV with higher magnitude. Exercise (e.g., treadmill running, resistance training) was found to effectively improve trabecular and cortical bone properties in aged rodents[37-39]. However, WBV had no effects on tibia proximal metaphysis and diaphysis, regardless of magnitudes, in 22-month-old mice[26]. Such bone responses in aged rodents may differ by the form of biophysical stimuli. For instance, bone response to loading was less sensitive in aged rats than in young rats[40]. The present results of TBMS and CBG parameters are almost in line with the previous studies[26,27], in which WBV had no effects on bone properties in aged mice. In our aged rats, however, although there was no difference in cortical bone BMC among groups, cortical bone TMD was significantly higher in the 0.7 and 1.0 *g* WBV than in other groups. Bone mineral was found to be related to the magnitude of bone strain[36]. If the frequency of WBV is the same, WBV with higher magnitude induces an increase in the strain to lower limb bone[42]. Therefore, WBV with higher magnitude influenced bone properties at the cortical periosteal and endosteal regions of the diaphysis[42,43]. Likewise, in this study, higher-magnitude WBV (0.7 and 1.0 *g*) might have induced the increased bone strain, and thereby cortical bone TMD has very likely been augmented; nevertheless, femoral CBG parameters have not been improved. The cortical bone formation increased by mechanical stimulation was the only effect of loading in aged rodents, whereas the cortical bone resorption was independent of loading[44]. However, the present increased TMD of cortical bone might have been induced by the decreased cortical bone resorption, which has been suggested by somewhat lower, but not significant, levels of TRACP-5b in the 0.7 and 1.0 *g* WBV groups. Also, this may be supported by the OC/TRACP-5b ratio increased with 1.0 *g* WBV.

In the present study, mean values of cortical/trabecular BMC, trabecular vBMD and several CBG/TBMS parameters were lower in 87-week-old rats of the CON than in 80-week-old rats of the BC group, as previously reported in aged rodent bones[26-29]. In addition to such an age-related bone deterioration, a reduction in osteocyte and/or lacunar density with aging was found in human and rodent bones[45-49]. Osteocytes are well-known to be the principal mechanosensory cells. Osteocytes, embedded inside the bone tissue, are surrounded by fluid filled space, lacunae. Long dendritic processes of osteocytes, surrounded by canalicular wall, form an osteocyte lacuno-canalicular network (OLCN)[45-49]. It is widely accepted that osteocytes are responsive to strain-driven interstitial fluid flow through the OLCN. Mechanical signals sensed by osteocytes are converted to chemical signals, such as nitric oxide (NO) and prostaglandin (PG)[50]. In fact, NO and PGs were required in mechanically induced bone formation in rats[51]. In cultured osteocytes/osteoblasts, moreover, the production of NO and/or PG E2 were stimulated by fluid flow-derived wall shear stress[52] or dynamic mechanical strain[53]. Other possible signaling pathways involved in the cellular mechanotransduction process are described elsewhere[54]. Thus, the OLCN plays a pivotal role in conveying these signals to osteoblasts, osteoclasts and bone-lining cells, leading to bone remodeling through regulation of both osteoclast and osteoblast activity. The periosteal and endocortical surfaces of the tibiae of old rats (19-month-old) had a higher mechanical loading threshold for bone formation compared with younger adult rats (9-month-old)[40]. Besides the aging-induced reduction in osteocyte density and lacunar density, there are several aging-induced changes in osteocyte and lacunar morphology underlying such an altered bone mechanoresponsiveness to mechanical loading with aging. First, osteocytes and lacunae became smaller and more spherical with advancing age[46-49]. Round MLO-Y4 osteocytes could release NO in response to lower mechanical force (~5pN) than the stimulation force threshold for flat osteocytes, indicating that round osteocytes are more mechanosensitive than flat cells[55]. Morphological change from flat to round osteocytes may be a compensative response to the reduced osteocyte density with aging. On the other hand, the local perilacunar bone matrix strains were altered by changes in lacunar morphology[56]. In addition, changes in lacunar fluid volume due to shrinkage of the lacunar and/or osteocyte cell bodies could induce alterations in fluid flow shear stress that could impact mechanotransduction[49]. Second, aged mice showed a dramatic decline in the dendrite number per osteocyte, which correlated positively with cortical thickness and negatively with cortical perimeter, indicating that the maintenance of connectivity of the OLCN may be important in preventing age-related bone loss[47,49]. The glycocalyx of osteocyte dendritic process is required for forming strong integrin attachments, which probably serve as the mechanotransducers that transmit the mechanical signals to the cell body, leading to the opening of hemichannels[57]. The bone modulators released/taken by hemichannels are essential for bone modeling/remodeling process. Thus, although aging-derived round osteocytes are more

mechanosensitive, several degenerative changes that occur in the OLCN with aging appear to cause deterioration in the mechanotransduction, resulting in age-related bone loss.

The present WBV had vague effects on the CBG and TBMS parameters in aged rats, similar to the previous studies[26,27]. Considering that aging reduces bone mechanoresponsiveness to mechanical loading as mentioned above, WBV with higher magnitudes may bring forth positive effects on bone mass and structure in aged rodents. WBV could maintain the osteocyte density in femur cortical bone of growing mice[58] and rats[59]. Furthermore, mechanical stimulation prevented apoptosis of cultured murine osteocytes by activating of an integrin/cytoskeleton/ Src/ERK signaling pathway[60]. In contrast, reduced mechanical forces in a tail-suspension unloading murine model increased the prevalence of osteocyte apoptosis followed by osteoclast recruitment, leading to the increased bone resorption and bone loss[61]. In addition, the length of osteocyte dendritic processes was increased in response to fluid flow shear stress in cultured MLO-Y4 osteocytes[62]. In view of these facts, it seems likely that WBV with higher magnitudes maintains the number of mechanosensitive osteocytes by preventing age-induced apoptosis of osteocytes and restores the OLCN in aged rodent bone. In fact, our 1.0 *g* WBV group showed a prominent OC/ TRACP-5b ratio that suggests an excess of bone formation over bone resorption, and consequently would have shown the increase in cortical bone TMD, femur bone length and wet bone weight. In order to clarify how WBV influences the OLCN in aged bone, 3D morphometric analysis of aged bone, using confocal laser scanning microscopy imaging, are required.

This study has some limitations. First, sample size was small because the Kio University restricted the number of experimental animals based on 3 R principles (reduction, replacement and refinement). Therefore, generalizing our data may be limited, and studies with a larger sample size are required to further confirm our findings. Second, we selected five frequencies (15-90 Hz) for one fixed magnitude (0.5 *g*), and four magnitudes (0.3-1.0 *g*) for one fixed frequency (45 Hz), to investigate the effects of WBV on bone properties. However, other combinations of frequency and magnitude still remain to be examined for exploring every possibility of improving bone properties. Finally, dynamic femoral strain was not measured in this study. Since the magnitude of strain may differ between the femur and tibia even under the same WBV conditions, a further study will need to address this issue.

In our aged rats, 15 Hz WBV showed a decrease in BMC of trabecular bone and a decrease in femoral break point. In addition, 15 Hz WBV decreased several TBMS parameters (BV, BV/TV, Tb.Th and Tb.W), suggesting damage to trabecular bone. On the other hand, although frequency/magnitude of WBV did not influence any CBG parameters, 0.7 and 1.0 *g* WBV increased cortical bone TMD compared with the BC and CON groups, suggesting the possibility of improving cortical bone properties. Based on these findings, it should be noted that WBV conditions are carefully considered when applied to elderly people.

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