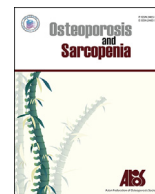




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Original article

Possible effects of whole body vibration on bone properties in growing rats

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ABSTRACT

Objectives: To examine the effects of whole body vibration (WBV) on bone properties in growing rats, and to explore the optimal conditions for enhancing bone properties.**Methods:** Thirty-six 4-week-old male rats were divided into 1 control and 5 experimental groups. Each experimental group underwent WBV at 15, 30, 45, 60, and 90 Hz (0.5 g, 15 min/d, 5 d/wk) for 8 weeks. We measured bone size, muscle weight and bone mechanical strength of the right tibia. Trabecular bone mass and trabecular bone microstructure (TBMS) of the left tibia were analyzed by micro-computed tomography. Serum levels of bone formation/resorption markers were also measured.**Results:** WBV at 45 Hz and 60 Hz tended to enhance trabecular bone mass and TBMS parameters. However, there was no difference in maximum load of tibias among all groups. Serum levels of bone resorption marker were significantly higher in the 45-Hz WBV group than in the control group.**Conclusions:** WBV at 45–60 Hz may offer a potent modality for increasing bone mass during the period of rapid growth. Further studies are needed to explore the optimal WBV conditions for increasing peak bone mass and TBMS parameters. WBV modality may be a potent strategy for primary prevention against osteoporosis.© 2019 The Korean Society of Osteoporosis. Publishing services by Elsevier B.V. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

1. Introduction

Lifestyle of the childhood was reported to influence adult peak bone mass [1], and physical activities and exercise can effectively promote bone health in children and adolescents [1–3]. Increasing peak bone mass at the earlier age, i.e., in children and adolescents, appears to serve as primary prevention against osteoporosis [4,5]. Therefore, effective interventions, which increase bone mineral density (BMD), are needed as primary prevention measures against osteoporosis, because the low levels of BMD tend to persist in children with low BMD [6,7]. High- and odd-impact, resistant, and weight-bearing exercises are known to effectively improve bone mass in children and adolescents [2,3,8,9]. In recent studies, whole body vibration (WBV) was found to improve bone mass in children with Down's syndrome [10], overweight [11], and disable

conditions [12]. However, little is known about the effects of WBV on bone mass in normal children and adolescents.

In animal studies, WBV was found to increase bone mass and improve structural parameters in young rodents (aged ≤8 weeks) with spinal cord injury, unloading or ovariectomy [13–15]. On the other hand, a few studies examined the potential effects of WBV on bone mass in normal young rodents [16–18]. In 7-week-old male mice, WBV (90 Hz, 2 g, 15 min/d, 5 d/wk) for 3 weeks increased femoral trabecular bone cellular activity, femoral cortical thickness, and cross-sectional area, and subsequently WBV for 9 weeks increased femoral cortical tissue mineral density (TMD) [16]. Furthermore, in 8-week-old female mice, WBV (45 Hz, 0.3 g, 15 min/d) decreased osteoclastic activity in tibial trabecular bone, and increased bone formation rate in the endocortical surface of tibial metaphysis [17]. In addition, WBV (45 Hz, 0.3 g, 15 min/d, 5 d/wk) for 5 weeks increased femoral mechanical strength in 3-week-old wild-type female mice, and also increased the tibial trabecular bone fraction in 3-week-old female mice with osteogenesis imperfecta [19]. However, in 4-week-old female rats, WBV (45 Hz, 0.3 g, 15 min/d, 7 d/wk) for 12 weeks did not affect femoral bone mass, structure or mechanical strength [18].

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Thus, the effects of WBV on bone properties in young rodents still remain controversial. Rodent bone tissue is known to grow rapidly from around 4 weeks of age through 12 weeks of age [20]. Rapidly growing bone tissue may be much sensitive to WBV. This study aimed to examine whether WBV has positive effects on bone properties in growing rats or not, and further to explore the optimal WBV conditions for enhancing bone properties when that effect is positive.

2. Methods

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).

2.1. Animals care and experimental protocol

Thirty-six 3-week-old 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°C–24°C; lighting, 12:12-h light-dark cycle). We preferred using male rats, whose bone response to loading/bone fatigue resistance are higher than those of female rats [21,22], to female rats because of the following 2 reasons; (1) so far, there are few WBV studies using male rats, and (2) since male rats have much lower levels of estrogens responsible for maintenance of BMD compared with female rats, there are few or no influences of estrogens on bone maturation in growing male rats, probably resulting in a smaller individual difference in bone properties. Rats were fed standard rodent chow (CE-2; CLEA Japan Inc., Tokyo, Japan) and water *ad libitum* throughout the experimental period. After 1 week of acclimatization, rats were divided into 1 control (CON) and 5 experimental groups ($n = 6$ each). Since the Committee of Research Facilities of Laboratory Animal Science in the Kio University restricted the number of experimental animals based on 3R principles (reduction, replacement, and refinement), 6 rats per group were used in this study. Using a vibration device system (Big Wave G-MasterPRO; Asahi Seisakusho Co. Ltd., Tokyo, Japan), rats in all experimental groups underwent WBV (vertical direction vibration, acceleration of 0.5 g, 15 min/d, 5 d/wk) at 15, 30, 45, 60, and 90 Hz of frequency for 8 weeks.

Blood samples, muscles and tibias were collected from all rats at the end of WBV intervention period. Serum samples, obtained from blood centrifugation at 1,000 g for 30 min, were stored at -80°C until biochemical analyzes and enzyme-linked immunosorbent assays (ELISA). Bilateral soleus and extensor digitorum longus (EDL) muscles were harvested and weighed. After harvesting bilateral tibias, we removed soft tissues and measured wet weight and length of each tibia. Right and left tibias were stored in saline and 70% ethanol, respectively, until analyzed.

2.2. Analyzes of bone mass and trabecular bone microstructure

Analyzes of bone mass and trabecular bone microstructure (TBMS) were performed as previously reported [13]. Using an X-ray micro-computed tomography device (Micro-CT; Hitachi Medical Corp., Tokyo, Japan), the left proximal tibia was scanned at 70 kV, 90 μA , with a voxel size of 19.2 μm in the high-definition mode for TBMS analysis. The region of interest (ROI) for TBMS of the proximal tibia was a 2-mm length portion of the tibia metaphysis, and the first slice was scanned 1 mm proximal from the physal-metaphyseal demarcation. Scanned data were transmitted to a personal computer, and TBMS of the ROI was analyzed using bone

analysis software (TRI BON 3D; Ratoc System Engineering Co. Ltd., Tokyo, Japan). Bone volume (BV), bone volume fraction (BV/tissue volume [TV]), trabecular thickness (Tb.Th), trabecular number (Tb.N), trabecular separation (Tb.Sp), and connectivity density (Conn.D) were assessed as TBMS parameters in the tibia metaphysis. In addition, a BMD phantom was simultaneously scanned under the same scanning conditions to obtain TMD, bone mineral content (BMC) and volume BMD (vBMD; BMC/TV).

2.3. Measurements of bone mechanical strength

Maximum load of the right tibia was measured by a 3-point bending strength test using a Universal Testing Machine (Auto-graph AGS; Shimadzu Corp., Kyoto, Japan). Bones were supported by 2 fulcrums (5 mm in diameter). The distance between both fulcrums was half of the bone length. Downward pressure was applied to the center of the bone at a fixed speed of 1 mm/min.

2.4. Dry bone and ash weight measurements

After the tibia was used for the measurements of TBMS parameters, bones were dehydrated in 100% ethanol for 48 h and then heated at 100°C for 24 h in a drying machine (Yamato Kagaku, Tokyo, Japan) to obtain dry bone weight. Finally, the bones were burned to ash at 600°C for 24 h with an electric furnace (Nitto Kagaku Co. Ltd., Nagoya, Japan), and the ash content was weighed.

2.5. Serum biochemical analyzes and ELISAs

Serum samples were analyzed for calcium, inorganic phosphorus, total protein, triglycerides, total cholesterol and alkaline phosphatase (ALP). In addition, serum osteocalcin (OC) and tartrate-resistant acid phosphatase-5b (TRACP-5b) concentrations were determined with commercially available ELISA kits for OC (Immunodiagnostic Systems Ltd., Boldon, UK) and TRACP-5b (Immunodiagnostic Systems Ltd., Boldon, UK).

2.6. Statistical analysis

All values are expressed as mean $\pm$ standard deviation. First, normal distribution of each group data was examined by Shapiro-Wilk normality test. In the presence of normal distribution, differences in the effects of WBV frequency on measured parameters between the CON group and each experimental group were examined using Dunnett's test. The overall difference among experimental groups was determined by one-way analysis of variance and differences between individual groups were examined using Bonferroni *post hoc* test. In the absence of normal distribution, differences in the effects of WBV frequency on measured parameters between the CON group and each experimental group were examined using Steel's multiple comparison test. The overall difference among experimental groups was determined using Kruskal-Wallis test and differences between individual groups were examined using Steel-Dwass *post hoc* test. The data with normal distribution were bone weight (BW), dry BW, ash weight, BMC, vBMD, BV, BV/TV, Tb.Th, Tb.N, OC, TRACP-5b, Ca, and triglycerides. All statistical analyzes were performed using Excel Statistics software (BellCurve for Excel version 3.10 for Windows; Social Survey Research Information Co., Ltd., Tokyo, Japan). $P < 0.05$ was considered statistically significant.

3. Results

3.1. Body weight, food intake, muscle weight, and bone size

Table 1 summarizes the body weight, food intake, muscle weight, and tibia bone size (bone length, wet/dry BW, and ash weight) in all groups. There was no difference in final body weight, food intake, EDL weight, and tibia bone size among all groups. However, soleus weight was significantly heavier in the 15 Hz-, 30 Hz- and 90-Hz WBV groups than in the CON group.

3.2. Bone mass parameters

Bone mass parameters of trabecular bone did not significantly differ among all groups (Table 2). However, the 45- and 60-Hz WBV groups showed the prominent values of BMC and vBMD.

3.3. TBMS parameters

Fig. 1 shows the results of tibia TBMS parameters in all groups. All groups showed similar values of TBMS parameters. However, compared with the CON group, the 45- and 60-Hz WBV groups showed an increasing trend in Tb.N, BV, BV/TV and Conn.D, but, in reverse, a decreasing trend in Tb.Sp.

3.4. Bone mechanical strength

There was no difference in maximum load and break point of tibias among all groups (Fig. 2).

3.5. Biochemical analyzes

The 30-Hz WBV group showed an increase in serum inorganic phosphorus compared with the CON group (Table 3). However, the 90-Hz WBV group showed a decrease in serum total protein concentration compared with the CON group (Table 3). There was no difference in serum calcium, triglyceride, total cholesterol and ALP concentrations among all groups. Likewise, there was no difference in serum concentrations of bone formation marker OC among all

groups (Fig. 3A). On the other hand, serum concentrations of bone resorption marker TRACP-5b were significantly higher in the 45-Hz WBV group than in the CON group (Fig. 3B).

4. Discussion

In the present study, 4-week-old male rats, which underwent WBV at frequencies of 45 Hz and 60 Hz for 8 weeks, tended to show higher values of trabecular bone mass (TBM, BMC, and vBMD) and TBMS parameters (BV, BV/TV, Tb.N, and Conn.D). The effects of WBV on bone properties may be influenced by not only the WBV-starting age but also bone condition, i.e., normal health or osteopenia, in young rats. However, WBV was reported to improve bone mass and structure by increasing bone formation or decreasing bone resorption in both normal and osteopenia rodents older than 4 weeks [13–17]. On the other hand, the effects of WBV on bone properties were inconsistent among young rodents under the age of 4 weeks [18,19]. One study previously reported that femoral stiffness and yield load are significantly increased by 45-Hz WBV (0.3 g, 15 min/d, 5 d/wk) for 5 weeks in wild-type female mice compared with those of wild-type female mice without WBV [19]. In contrast, another study previously reported that BMD and BV/TV of the femoral head and BMD of the femoral cortical bone are decreased by 45-Hz WBV (0.3 g, 15 min/d, 7 d/wk) for 12 weeks in 4-week-old normal female rats [18]. It should be noted that the WBV conditions excluding the intervention period and intervention days per week were the same in these 2 studies. Therefore, such inconsistency in the effects of WBV on bone properties appears to be caused by the different period of WBV intervention, the number of intervention days per week and/or species differences between the animals used in the respective studies. In the current study, 45- to 60-Hz WBV (5 d/wk) for 8 weeks tended to increase trabecular bone mass and TBMS parameters. Likewise, positive effects on bone mechanical properties were observed in rats which underwent WBV (5 d/wk) for 5 weeks [19]. On the contrary, daily WBV (7 d/wk) for 12 weeks negatively affected bone mass and TBMS parameters [18]. In that particular study, the negative effects of WBV were probably caused by an excess of bone resorption over bone formation, which was evidenced by the decreased ALP and increased

Table 1
Body weight, food intake, muscle weight, and tibial bone size.

Variable	CON	15 Hz	30 Hz	45 Hz	60 Hz	90 Hz
Final body weight, g	306.1 ± 17.2	312.7 ± 13.1	312.7 ± 9.7	312.3 ± 15.9	311.4 ± 13.0	311.0 ± 13.6
Food intake, g/d	18.2 ± 2.4	17.9 ± 2.3	18.2 ± 2.3	18.2 ± 2.3	18.0 ± 2.2	18.1 ± 2.1
Muscle weight, mg						
Soleus	106.3 ± 6.4	112.3 ± 4.2*	113.8 ± 7.2*	109.9 ± 7.7	109.8 ± 6.5	114.0 ± 9.6*
EDL	126.1 ± 6.0	127.4 ± 6.4	129.3 ± 4.3	126.2 ± 7.8	127.3 ± 4.5	127.1 ± 6.4
Bone length, mm	38.6 ± 0.6	38.9 ± 0.5	38.9 ± 0.5	38.9 ± 0.6	38.8 ± 0.5	38.9 ± 0.4
Wet bone weight, mg	649.8 ± 32.1	648.3 ± 25.5	646.8 ± 25.1	657.3 ± 39.2	641.2 ± 17.6	668.3 ± 40.5
Dry bone weight, mg	420.8 ± 22.2	417.2 ± 15.8	419.9 ± 11.5	431.3 ± 29.9	417.8 ± 12.0	430.1 ± 16.1
Ash weight, mg	255.1 ± 12.9	251.5 ± 10.5	254.4 ± 6.9	261.4 ± 16.8	252.8 ± 6.6	258.3 ± 11.0

Values are presented as mean ± standard deviation.

EDL, extensor digitorum longus.

*P < 0.05, significantly different from the CON group.

Table 2
Bone mass parameters of trabecular bone in tibia.

Variable	CON	15 Hz	30 Hz	45 Hz	60 Hz	90 Hz
TMD, mg/cm ³	347.1 ± 24.4	357.7 ± 14.8	353.2 ± 11.1	358.8 ± 17.7	363.3 ± 12.2	355.0 ± 17.9
BMC, mg	0.131 ± 0.059	0.147 ± 0.027	0.133 ± 0.050	0.184 ± 0.060	0.182 ± 0.040	0.146 ± 0.054
vBMD, mg/cm ³	12.5 ± 5.9	13.7 ± 2.9	12.5 ± 4.4	16.7 ± 5.2	16.9 ± 3.7	13.6 ± 4.9

Values are presented as mean ± standard deviation.

TMD, tissue mineral density; BMC, bone mineral content; vBMD, volume bone mineral density.

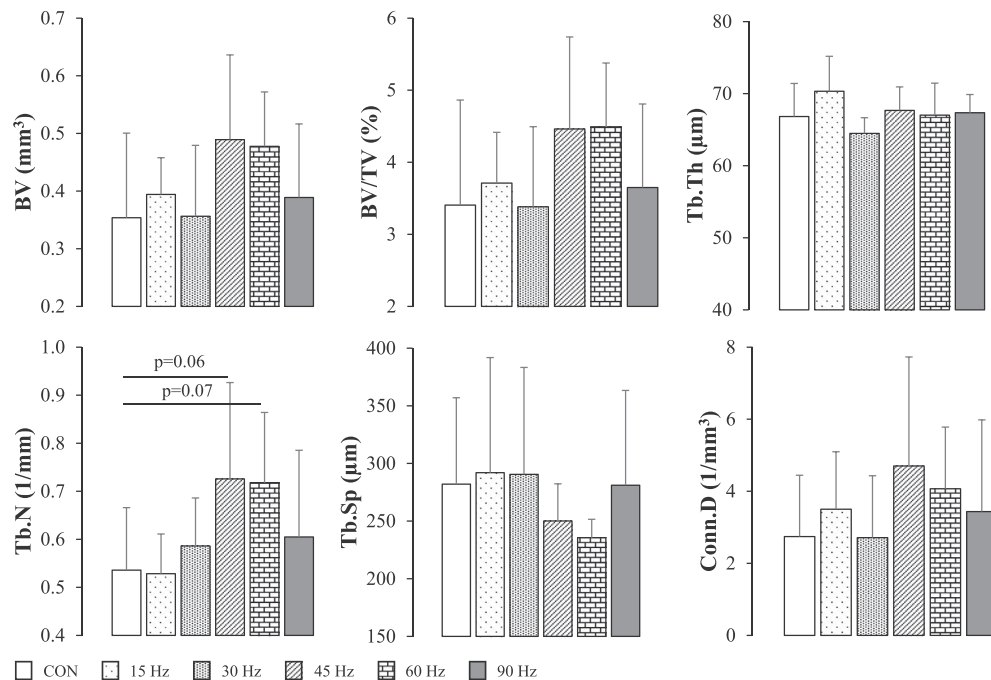


Fig. 1. Trabecular bone microstructure parameters of tibial metaphysis. BV, bone volume; BV/TV, bone volume ratio; Tb.Th, trabecular thickness; Tb.N, trabecular number; Tb.Sp, trabecular separation; Conn.D, connectivity density. The bar indicates standard deviation.

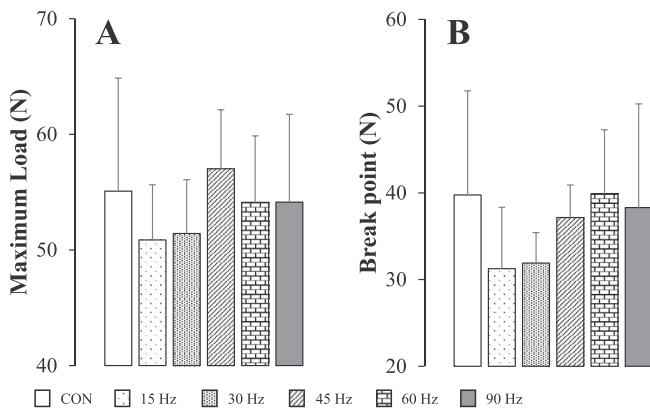


Fig. 2. Maximum load (A) and break point (B) of tibia in all groups. Bar indicates standard deviation.

TRACP-5b levels. Such negative effects of daily WBV were prominent in combination with weight bearing [18], suggesting that the bone response to loading may be invalidated by overloading. In order to ensure the most effective bone strain, a rest period may be needed during the WBV intervention period [23]. As compared with daily WBV for 8 weeks, 8-week WBV intervention with rest days without WBV enhanced TBMS parameters, mechanical strength, mineral apposition rate and bone formation rate of the femoral head in unloaded model rats [24]. Such conflicting effects of WBV on bone properties in young rodents may be explainable not only by, as mentioned above, the number of intervention days per week/different periods of WBV intervention but also by the complexity of the process in which rapidly growing bones respond to mechanical stress. In fact, the trabecular bone's adaptive response to mechanical loading was reported to be greater in young mice than in adult or old mice [21,25,26], and the mechanical loading could increase bone mass most effectively in rapidly

growing bones [21].

Our WBV at 45 Hz and 60 Hz had the potential to increase trabecular bone mass and TBMS parameters. The previous studies found that WBV at high frequencies and low magnitude positively acts on bone properties [27,28]. Pasqualini et al. [29] found that the effects of 4 week-WBV intervention (magnitude 0.7 g, 10 min/d, 5 d/wk) on bone properties differ by frequency in mature rats. WBV at a higher frequency exerted beneficial effects on trabecular and cortical bones, while WBV at a lower frequency (<10 Hz) had harmful effects [29]. Moreover, in ovariectomized rats, strain magnitude and strain rate were higher in 45-Hz WBV intervention (magnitude 0.15 g, 10 min/d, 5 d/wk) for 4 weeks than in 90-Hz WBV intervention, although bone formation rate was significantly higher in 90-Hz WBV than 45-Hz WBV [30]. From these results, it seems likely that WBV at 45–60 Hz improves bone properties more effectively compared with WBV at other frequencies examined in the present study.

WBV may influence muscular function, i.e., muscle contraction and relaxation, as well as bone properties, because WBV of 50–60 Hz was reported to strengthen extensors and flexors of the knee [31,32]. Therefore, in this study, it seems likely that WBV at 45 and 60 Hz induce an increase in strain magnitude/rate and muscle contraction cycles, and thereby positively influence trabecular bone mass and TBMS parameters. Further studies are required to explore the WBV conditions optimal in the earlier life stages.

During the growth period from the age of 8 weeks until the age of 16 weeks in Sprague-Dawley rats, serum OC levels declined with age, while serum TRACP-5b levels remained unchanged [20]. Thus, in growing rats aged over 8 weeks, osteoblastic bone formation decreased with increasing age. On the other hand, WBV was reported to reduce the osteoclastic activity in trabecular bones, and consequently attenuate the decline in bone formation in young rodents [17]. However, our 45- and 60-Hz WBV groups have shown a slightly upward trend in serum OC levels compared with the CON group. On the other hand, our 45-Hz WBV group showed a significant increase in serum TRACP-5b levels compared with the CON

Table 3
Biochemical analysis of serum.

Variable	CON	15 Hz	30 Hz	45 Hz	60 Hz	90 Hz
Ca, mg/dL	10.6 ± 0.2	10.7 ± 0.2	10.7 ± 0.2	10.9 ± 0.2	10.8 ± 0.3	10.7 ± 0.2
IP, mg/dL	6.5 ± 0.4	6.7 ± 0.5	7.1 ± 0.2*	6.9 ± 0.3	7.2 ± 0.6	7.1 ± 0.7
TP, g/dL	6.0 ± 0.1	5.8 ± 0.2	6.0 ± 0.1	5.9 ± 0.1	5.8 ± 0.2	5.7 ± 0.2*
TG, mg/dL	193.8 ± 43.4	164.7 ± 40.8	208.2 ± 35.0	187.8 ± 30.9	188.2 ± 39.2	194.0 ± 44.6
TC, mg/dL	56.8 ± 6.4	58.5 ± 4.6	67.0 ± 7.6	60.2 ± 6.6	62.5 ± 3.5	58.0 ± 6.2
ALP, mg/dL	1223.0 ± 53.4	1240.3 ± 98.0	1170.8 ± 64.2	1197.2 ± 82.4	1159.2 ± 63.7	1147.0 ± 89.1

Values are presented as mean ± standard deviation.

Ca, calcium; IP, inorganic phosphorus; TP, total protein; TG, triglyceride; TC, total cholesterol; ALP, alkaline phosphatase.

*P < 0.05, significantly different from the CON group.

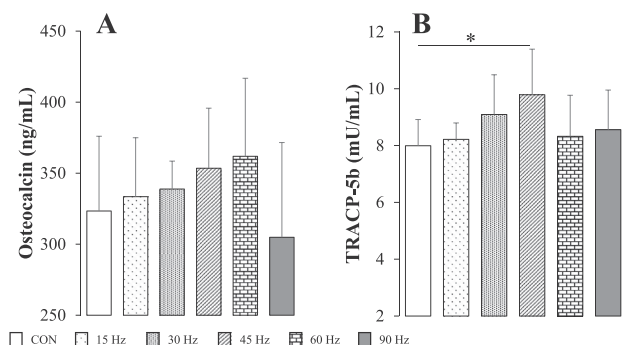


Fig. 3. Serum levels of osteocalcin (A) and tartrate-resistant acid phosphatase-5b (B) in all groups. TRACP-5b, tartrate-resistant acid phosphatase-5b. The bar indicates standard deviation. *P < 0.05, significantly different from the CON group.

group. As mentioned above, the WBV effects on bone mass and microstructure were induced by increased bone formation or decreased bone resorption in the rodents [13–17]. Although the reason why serum TRACP-5b levels were increased in our 45-Hz WBV group still remains to be resolved, such positive effects of 45-Hz WBV would have resulted from an excess of bone formation over bone resorption. Such bone formation/resorption activation which is induced by WBV, i.e., bone remodeling, may be influenced by bone-related hormones/cytokines [33,34] and/or receptor activator of nuclear factor kappa-B ligand (RANKL) [35]. Taken together, in our 45- and 60-Hz WBV groups, the enhancing trend in trabecular bone mass parameters and TBMS parameters may be attributable to activated metabolic turnover of bones.

This study has the following 5 limitations. First, only the fixed magnitude (0.5 g) was used for WBV intervention, while various frequencies were used. This paper is the first to report the effects of WBV at various frequencies on bone properties in 4-week-old rats. Further studies are needed to explore the optimal WBV conditions for enhancing bone mass and microstructure. Second, we did not measure dynamic strain in the tibia. Such a strain could have been estimated by measuring the strain magnitude or strain rate in the tibia [17,21,23,25,26,28,29,31]. Third, we have examined TBMS parameters, but not cortical bone geometry. Cortical bone analysis is needed to clarify the effects of WBV on transverse bone growth, even though bone mechanical strength measured by a 3-point bending test is thought to reflect cortical bone geometry. Fourth, circulating bone formation/resorption markers were analyzed, but hormones/substances related to bone growth, e.g., calcitonin, parathormone, estrogen, growth hormone, RANKL, vitamin D, vitamin K₂ etc., were not. Finally, generalizing the positive effects of 45- to 60-Hz WBV on bone may be limited because of modest sample sizes. Therefore, studies with large sample sizes are required to further confirm our findings. In addition, further studies are needed to reveal not only the effects of WBV on hormone/

cytokine secretion but also their influence on bone properties.

5. Conclusions

We found that WBV at 45 Hz and 60 Hz for 8 weeks from 4 weeks of age tended to enhance trabecular bone mass (TBM, BMC, and vBMD) and TBMS parameters (BV, BV/TV, Tb.N, and Conn.D) in normal rats. Thus, WBV at 45–60 Hz may offer a potent modality for increasing bone mass during the period of rapid growth. Further studies are needed to explore the optimal WBV conditions for increasing peak bone mass and TBMS parameters. In the clinic, WBV modality may be a potent strategy for primary prevention against osteoporosis.

Conflicts of interest

No potential conflict of interest relevant to this article was reported.

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