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Original Article

Prevention of bone deterioration by whole-body vibration in a rat model of pre-type 2 diabetes

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Abstract

Objectives: To examine effects of whole-body vibration (WBV) on bone properties in pre-type 2 diabetes mellitus (T2DM) rats. **Methods**: Six-week-old male Hos:ZFDM-*Lepr*^{fa}, *fa/fa* (DM) and Hos:ZFDM-*Lepr*^{fa}, *fa/+* (CON; untreated non-DM) rats were used in the experiments. Half of DM rats were subjected to WBV (45 Hz, 0.5 g, 15 min/day, 5 days/week) for 8 weeks (WBV group), and the other half was not (DM group). **Results**: Bone mass, trabecular bone microstructure (TBMS), and cortical bone geometry (CBG) parameters were worse in the DM and WBV groups compared with the CON group. Maximum load was significantly decreased in the DM group compared with the CON group, and the break point was significantly higher in the WBV group compared with the DM group. Serum levels of bone specific alkaline phosphatase were significantly lower in the WBV group compared with the CON group. Glycemic control was not worse in the WBV group compared with the DM group, but not the same levels as the CON group. **Conclusions**: These findings suggest that WBV can potentially delay the decrease in maximum load, although it does not prevent the deterioration of bone mass, TBMS, and CBG parameters.

Keywords: Bone Mechanical Strength, Cortical Bone Geometry, Diabetic Rat Model, Trabecular Bone Microstructure, Whole-Body Vibration

Introduction

An estimated 374 million people had impaired glucose tolerance (IGT) in 2019, and this number is expected to increase to 454 million by 2030 and 548 million by 2045 worldwide[1]. The lifetime risk to develop diabetes mellitus (DM) from pre-DM was reported to be ≥70% in people aged 45 years[2]. Type 2 DM (T2DM) accounts for approximately 90% of all DM, and the number of people with T2DM is expected to increase together with increases in obesity, physical inactivity, and energy-dense diet[1,3,4]. T2DM results in various complications, one of which is osteoporosis. Fracture risk is increased in people with T2DM relative to healthy people, whereas bone mineral density (BMD) is normal or higher in people with T2DM[5,6]. People with IGT reportedly have a low fracture risk, although people with T2DM have an increased fracture risk despite the higher BMD in both those with IGT and T2DM relative to healthy people[7]. Moreover, hazard risks of hip fracture begin to increase in pre-DM and rapidly increase following the onset of T2DM[8]. Improving IGT and delaying the onset of T2DM can be primary prevention strategies against DM-induced osteoporosis in people with pre-DM, as the risk of DM progression can be reduced by glucose control[9]. Exercise is expected to be useful to this end, particularly weight-bearing exercise from a bone health perspective, for its effect on improving metabolic health in people with IGT or T2DM[10]. However, it is important to note that exercise is performed in various ways with different purposes[11,12].

Voluntary exercise for a long period prevented the onset of T2DM and inhibited bone deterioration in Otsuka Long Evans Tokushima Fatty (OLETF) rats, a T2DM model[13,14]. Glucose control and glycated hemoglobin A1c (HbA1c) were improved, and deterioration of bone structure and mechanical properties were prevented, in these rats. Thus, whole-body exercise such as running is considered to have beneficial effects on preventing both T2DM and bone deterioration. Recently, guidelines about recommendations

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to the use of whole-body vibration (WBV) in human, animal, and cell culture studies were published[15]. Studies using db/db mice have shown that WBV at a frequency of 32 Hz with 0.5 *g* acceleration for 12 weeks attenuated hyperglycemia and insulin resistance in intraperitoneal glucose and insulin tolerance tests and increased circulating osteocalcin levels without improving cortical bone structure[16], and that WBV at a frequency of 45 Hz with 0.5 g acceleration for 12 weeks improved skeletal microstructure and bone mechanical strength[17].

WBV may prevent bone deterioration in T2DM by applying a load on bone and inducing muscle contractions. WBV may also be easier to apply to pre-DM people who are physically inactive. However, only a few studies have simultaneously investigated the effects of WBV on bone properties and metabolic control in T2DM. Against this backdrop, the present study aimed to investigate the effects of WBV on bone properties in T2DM model rats. Male Hos:ZFDM-*Lepr*^{fa} (ZFDM), *fa/fa* rats aged 6 weeks were used in the present study because the ZFDM rat, a novel DM model, exhibits high blood glucose (BG) levels at age 8 weeks and develops DM as early as age 10 weeks, with 100% of rats developing DM by around age 20 weeks[18,19]. To our knowledge, no study has used ZFDM rats to conduct bone research. Therefore, ZFDM rats were used to investigate the preventive effects of WBV on the onset of T2DM and bone fragility.

Materials and Methods

Animal care and experimental protocol

Twenty male ZFDM, *fa/fa* rats for the T2DM model and 10 male ZFDM, *fa/+* rats for controls (CON) were purchased from Japan SLC, Inc. (Hamamatsu, Japan) and housed in standard cages individually in an animal facility under controlled room temperature and lighting (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. Non-fasting BG levels were measured with a BG meter (OneTouch VerioVue®; LifeScan Japan Co. Ltd., Tokyo, Japan) every week during the study period.

The experiment was started at age 6 weeks for the T2DM model and CON (untreated non-DM) rats, with the T2DM model rats divided into control (DM) and experimental (WBV) groups (n=10 each). In the WBV group, WBV (vertical direction vibration, frequency 45 Hz, magnitude 0.5 *g* (acceleration 4.9 m/s^2), 15 min/day, 5 days/week) was applied using a vibration device system (Big Wave G-MasterPRO; Asahi Seisakusho Co. Ltd., Tokyo, Japan) for 8 weeks. After the intervention, blood samples, muscles, and tibias were collected from rats. Serum samples, obtained from centrifugation of blood at 1,000 *g* for 25 minutes, were stored at -80°C until biochemical analyses and enzyme-linked immunosorbent assays (ELISA). Bilateral soleus and extensor digitorum longus (EDL) muscle weights were measured. After harvesting bilateral tibias, wet weight and the length of each tibia were measured. Right and left tibias were stored in saline and 70% ethanol, respectively, until analyzed.

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

The left proximal metaphysis and diaphysis of the tibia were scanned at 60 kV and 60 μA, with a voxel size of 14 μm, for TBMS and CBG analyses using an x-ray micro-computed tomography system (Micro-CT; Yamato Scientific Co. Ltd., Tokyo, Japan). 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 0.5 mm distal from the physeal-metaphyseal demarcation. The ROI for CBG was a 0.5 mm-length portion at the center of the long axis of the tibia. Scanned data were transmitted to a personal computer, and the ROIs for TBMS and CBG were analyzed using TRI/3D-BON software (Ratoc System Engineering Co. Ltd., Tokyo, Japan). Bone volume fraction (bone volume (BV)/tissue volume (TV)), trabecular bone thickness (Tb.Th), trabecular bone number (Tb.N), trabecular bone separation (Tb.Sp), connectivity density (Conn.D), and trabecular bone pattern factor (TBPf) were assessed as TBMS parameters. Cortical bone volume (Ct.V), medullary volume (MV), cortical bone volume fraction (Ct.V/all bone volume (AV)), cortical bone thickness (Ct.Th), cortical bone sectional area (Ct.Ar), periosteal perimeter (Ps.Pm), and endocortical perimeter (Ec.Pm) were assessed as CBG parameters. 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 tibial trabecular and cortical bones.

Measurements of bone mechanical strength

Maximum load and break point of the right tibia were measured by a 3-point bending strength test using a Universal Testing Machine (Autograph 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.

Dry bone weight (DBW) and ash content measurements

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

Serum biochemical analyses and ELISAs

Serum levels of calcium (Ca), inorganic phosphorus (IP), albumin, blood urea nitrogen (BUN), creatinine, total protein

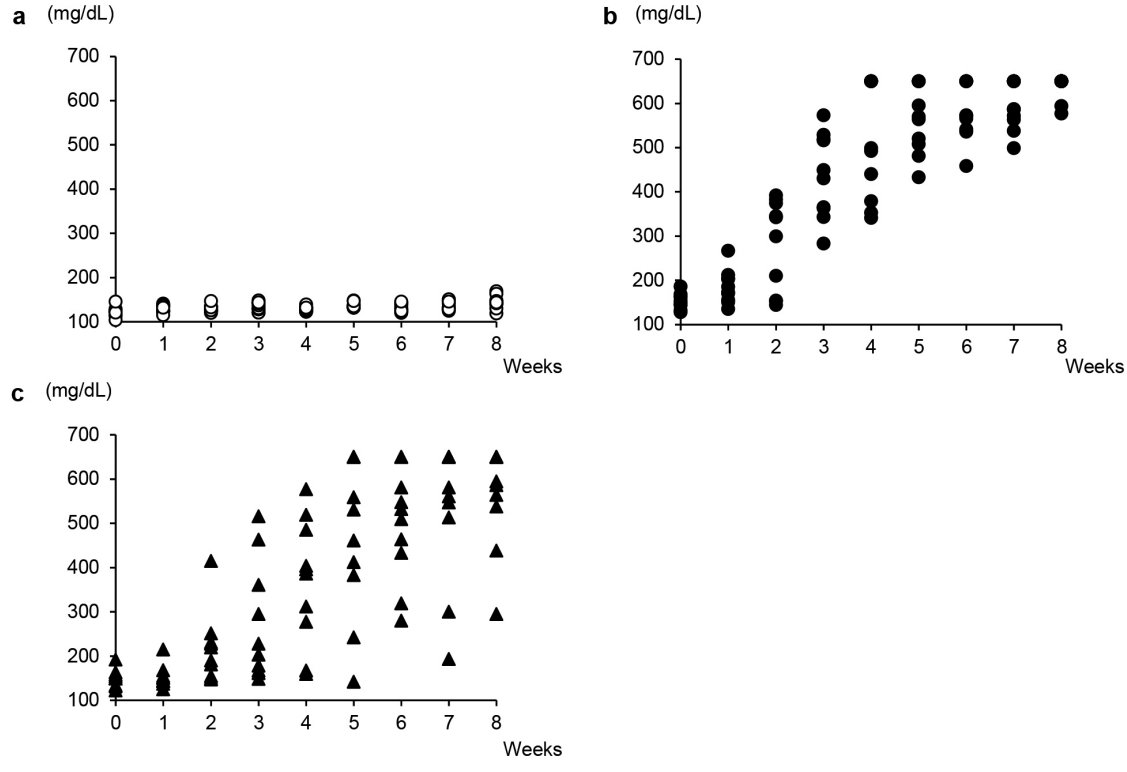

Figure 1. Non-fasting blood glucose levels in the CON (A), DM (B), and WBV (C) groups measured with a blood glucose meter. *Non-fasting blood glucose levels over 600 mg/mL are plotted as 650 mg/mL. CON, age-matched control; DM, diabetic rat model without whole-body vibration; WBV, diabetic rat model with whole-body vibration.*

(TP), total cholesterol (TC), triglyceride (TG), non-esterified fatty acid (NEFA), low density lipoprotein cholesterol (LDL-C), high density lipoprotein cholesterol (HDL-C), glucose, glycoalbumin (GA), and total ketone body (T-KB), and blood levels of HbA1c were determined. Serum levels of bone-specific alkaline phosphatase (BALP), tartrate-resistant acid phosphatase-5b (TRACP-5b), homocysteine, insulin, leptin, and advanced glycation end product (AGE) were analyzed with commercially available ELISA kits for BALP (MyBioSource Inc., San Diego, USA), TRACP-5b (Immunodiagnostic Systems Ltd., Boldon, UK), homocysteine (Cusabio Technology LLC., Wuhan, China), insulin (Mercodia, Uppsala, Sweden), leptin (Yanaihara Institute Inc., Shizuoka, Japan), and AGE (Cell Biolabs Inc., San Diego, USA), respectively.

Statistical analysis

All values are expressed as mean ± standard deviation. The overall difference among groups (CON, DM, and WBV groups) was determined with the Kruskal-Wallis test, and differences between individual groups were examined with the Steel-Dwass test if the Kruskal-Wallis test was significant. Spearman's rank correlation coefficients were determined to examine relationships between maximum load and CBG parameters and DM-related biochemical indices. All statistical analyses were performed using Excel Statistics software (BellCurve for Excel version 4.02 for Windows; Social Survey Research Information Co., Ltd., Tokyo, Japan), and $p<0.05$ was considered statistically significant.

Results

Body mass, food intake (FI), muscle weight, and bone size

Body mass and daily FI were significantly higher in the DM and WBV groups compared with the CON group ($p<0.01$), and daily FI was significantly higher in the DM group compared with the WBV group ($p<0.001$) (Table 1). Body mass-corrected soleus and EDL muscle weights were significantly lower in the DM and WBV groups compared with the CON group ($p<0.001$). Tibial bone length, body mass-corrected bone weight, and %ash were significantly lower in the DM and WBV groups compared with the CON group ($p<0.01$) (Table 1).

Changes of non-fasting BG levels are shown in Figure 1. Non-fasting BG levels did not exceed 170 mg/dL in the CON group throughout the experiment. Non-fasting BG levels in the DM and WBV groups did not exceed 200 mg/dL at

Table 1. Body mass, food intake, muscle weight, bone length, bone weight and ash content of tibias

	CON	DM	WBV
Final body mass (g)	340.4±9.0	381.6±24.7*	386.5±32.8*
Food Intake (g/day)	20.9±1.4	33.9±6.1*	30.5±5.7*†
Muscle weight-body mass ratio (mg/body mass)			
Soleus	0.42±0.02	0.31±0.02*	0.31±0.02*
Extensor digitorum longus	0.47±0.02	0.33±0.02*	0.34±0.01*
Bone length (mm)	39.8±0.5	38.1±0.5*	37.9±0.5*
Bone weight-body mass ratio (mg/body mass)	2.18±0.10	1.71±0.08*	1.66±0.11*
DBW-bone weight ratio (mg/bone weight)	0.65±0.01	0.66±0.01	0.66±0.01
% ash	61.2±0.2	60.0±2.2*	60.0±0.8*

*Data are expressed as mean ± standard deviation. *: Significantly different from the CON group (p<0.05). † Significantly different from the DM group (p<0.05). CON, age-matched control; DM, diabetic rat model without whole-body vibration; WBV, diabetic rat model with whole-body vibration. DBW, dry bone weight; %ash, ash content corrected for DBW.*

Table 2. Bone mass parameters of trabecular and cortical bone of tibias.

	CON	DM	WBV
Trabecular bone			
Tissue mineral density (mg/cm^3)	624.2±10.3	591.0±8.7*	596.6±12.9*
Bone mineral content (mg)	3.21±0.49	2.35±0.36*	2.80±1.13
Volume bone mineral density (mg/cm^3)	124.8±16.3	102.2±14.6*	118.0±38.1
Cortical bone			
Tissue mineral density (mg/cm^3)	1371.5±7.3	1372.2±18.0	1360.0±6.4*
Bone mineral content (mg)	2.80±0.10	2.44±0.13*	2.41±0.09*

*Data are expressed as mean ± standard deviation. *: Significantly different from the CON group (p<0.05). CON, age-matched control; DM, diabetic rat model without whole-body vibration; WBV, diabetic rat model with whole-body vibration.*

Table 3. Trabecular bone microstructure and cortical bone geometry in tibias.

	CON	DM	WBV
Trabecular bone microstructure			
Trabecular bone volume fraction (%)	20.3±2.4	17.5±2.5*	20.0±6.0
Trabecular bone thickness (μm)	105.9±4.7	95.8±4.0*	98.1±6.1*
Trabecular bone number (mm^{-1})	1.56±0.19	1.47±0.23	1.60±0.35
Trabecular bone separation (μm)	193.1±17.5	192.0±21.3	183.9±19.4
Connectivity density (mm^{-3})	37.3±4.7	36.2±6.2	40.3±12.2
Trabecular bone pattern factor (mm^{-1})	9.9±0.7	11.2±0.7*	10.8±2.2
Cortical bone geometry			
Cortical bone volume (mm^3)	2.04±0.07	1.78±0.10*	1.76±0.07*
Medullary volume (mm^3)	0.78±0.09	0.77±0.05	0.78±0.05
Cortical bone volume fraction (%)	72.4±1.9	69.9±1.3*	69.5±1.5*
Cortical bone thickness (μm)	585.4±66.5	558.9±37.9	564.9±18.5
Cortical bone sectional area (mm^2)	4.08/±0.13	3.59±0.19*	3.57±0.13*
Periosteal perimeter (mm)	8.94±0.23	8.36±0.21*	8.43±0.16*
Endocortical perimeter (mm)	5.26±0.32	5.14±0.16	5.13±0.17

*Data are expressed as mean ± standard deviation. *: Significantly different from the CON group (p<0.05). CON, age-matched control; DM, diabetic rat model without whole-body vibration; WBV, diabetic rat model with whole-body vibration.*

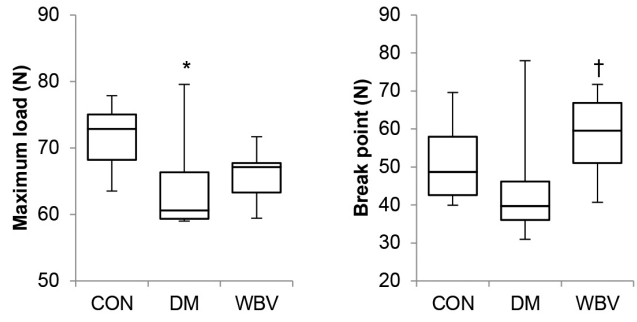

Figure 2. Maximum load and break point of tibia. **: Significantly different from the CON group (p<0.05). †: Significantly different from the DM group (p<0.05). CON, age-matched control; DM, diabetic rat model without whole-body vibration; WBV, diabetic rat model with whole-body vibration.*

Table 4. Biochemical analysis.

	CON	DM	WBV
Calcium (mg/dL)	9.8±0.2	10.4±0.4*	10.7±0.5*
Inorganic phosphorus (mg/dL)	6.0±0.4	6.1±0.5	6.8±0.7*†
Albumin (g/dL)	4.1±0.1	4.0±0.2	4.3±0.3†
Blood urea nitrogen (mg/dL)	22.5±2.5	30.4±2.6*	26.2±1.9*†
Creatinine (mg/dL)	0.30±0.02	0.24±0.03*	0.21±0.02*†
Total protein (g/dL)	5.7±0.1	6.1±0.2*	6.4±0.7*
Total cholesterol (mg/dL)	74.1±7.1	131.7±15.2*	121.9±29.7*
Triglyceride (mg/dL)	38.1±13.6	295.5±53.0*	395.1±266.4*
Non-esterified fatty acid (μEq/L)	418.0±67.7	630.2±127.6*	626.0±108.1*
Low density lipoprotein cholesterol (mg/dL)	8.3±0.9	9.2±1.5	9.3±3.6
High density lipoprotein cholestero (mg/dL)	31.3±1.9	77.1±8.3*	64.7±13.6*†
Glucose (mg/dL)	185.7±27.6	595.5±45.7*	471.9±117.0*†
Glycoalbumin (%)	1.6±0.2	4.7±0.8*	4.6±0.9*
Total ketone body (μmol/L)	406.2±39.4	428.0±98.4	492.4±155.2
Glycated hemoglobin A1c (%)	5.0±0.1	8.8±0.5*	7.9±1.0*

*Data are expressed as mean ± standard deviation. *: Significantly different from the CON group (p<0.05). †: Significantly different from the DM group (p<0.05). CON, age-matched control; DM, diabetic rat model without whole-body vibration; WBV, diabetic rat model with whole-body vibration.*

the beginning of the experiment. All rats in the DM group exceeded 200, 300, 400, and 500 mg/dL of non-fasting BG levels at the 3rd, 4th, 5th, and 8th week of the experiment, respectively, and all rats except for one in the WBV group exceeded 300 and 400 mg/dL at the 5th and 6th week of the experiment, respectively.

Bone mass and TBMS/CBG parameters

Trabecular TMD of the tibia was significantly lower in the DM and WBV groups compared with the CON group (p<0.01) (Table 2). Trabecular BMC and vBMD of the tibia were significantly lower in the DM group compared with the CON group (p<0.01) (Table 2). Cortical TMD of the tibia was significantly lower in the WBV group compared with the CON group (p<0.05), and cortical BMC of the tibia was significantly lower in the DM and WBV groups compared with the CON group (p<0.01) (Table 2).

BV/TV in the DM group and Tb.Th in the DM and WBV groups were significantly lower compared with those in the CON group (p<0.05), and TBPf was significantly higher in the DM group compared with the CON group (p<0.01) (Table 3).

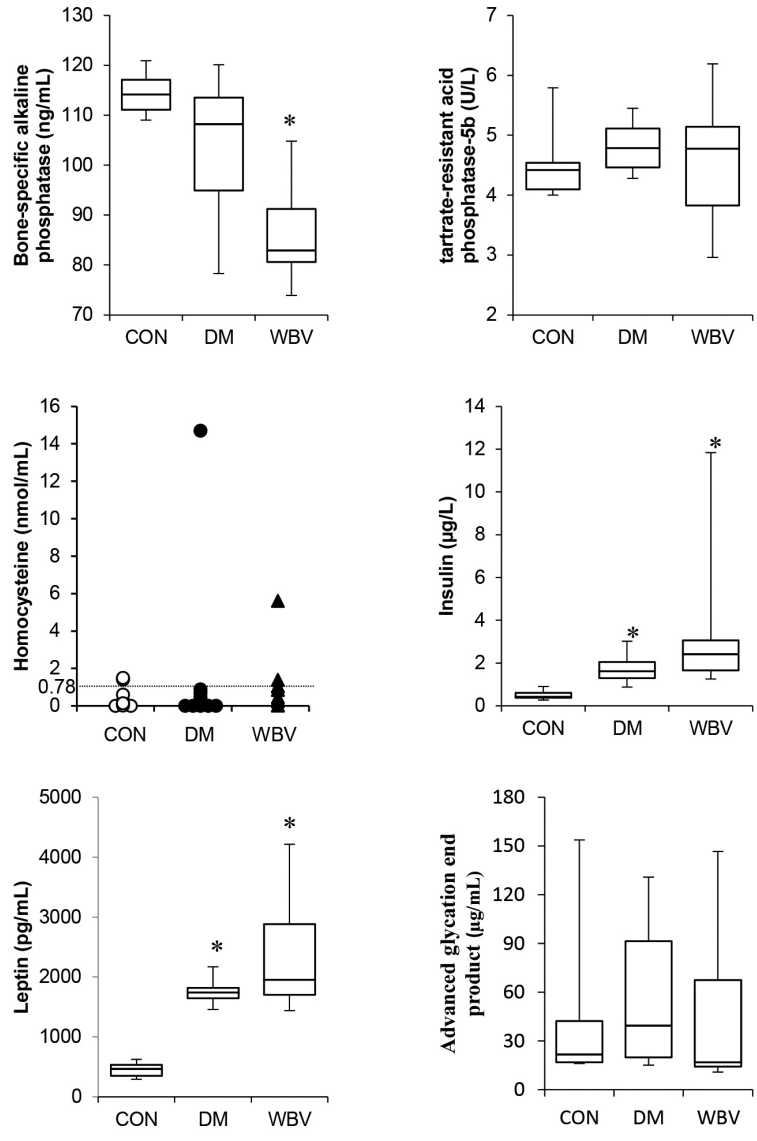

Figure 3. Serum levels of bone metabolic markers. **: Significantly different from the CON group (p<0.05). CON, age-matched control; DM, diabetic rat model without whole-body vibration; WBV, diabetic rat model with whole-body vibration.*

Ct.V, Ct.V/AV, Ct.Ar, and Ps.Pm were significantly lower in the DM and WBV groups compared with the CON group (p<0.05) (Table 3).

Bone mechanical strength

Maximum load was significantly lower in the DM group compared with the CON group (p<0.05), and the break point was significantly higher in the WBV group compared with the DM group (p<0.05) (Figure 2).

Biochemical analyses

Serum levels of Ca, BUN, TP, TC, TG, NEFA, HDL-C, glucose, GA, insulin, and leptin and HbA1c concentration, were significantly higher in the DM and WBV groups compared with the CON group (p<0.05), and serum levels of BUN, HDL-C, and glucose were significantly lower in the WBV group compared with the DM group (p<0.05) (Table 4). Serum levels of creatinine were significantly lower in the DM and WBV groups compared with the CON group (p<0.01), and significantly lower in the WBV group compared with the DM group (p<0.05) (Table 4). Serum levels of IP were significantly higher in the WBV group compared with the CON and DM groups (p<0.05), and serum levels of albumin were significantly higher in the WBV group compared with the DM group (p<0.05) (Table 4). Serum levels of BALP were significantly lower in the WBV group compared with the CON group (p<0.001) (Figure 3).

Table 5. Correlation coefficients between maximum load and cortical bone geometry parameters and biochemical indices.

Cortical bone geometry	
Cortical bone mineral content	0.676
Cortical bone volume	0.684
Cortical bone volume fraction	0.441
Cortical bone sectional area	0.694
Periosteal perimeter	0.640
Biochemical index	
Blood urea nitrogen	-0.415
Total cholesterol	-0.482
Non-esterified fatty acid	-0.394
High density lipoprotein cholesterol	-0.571
Glucose	-0.558
Glycoalbumin	-0.602
Glycated hemoglobin A1c	-0.601
Values are Spearman's rank correlation coefficients (p<0.05).	

Relationships between maximum load and cortical bone properties and biochemical index

Table 5 summarizes significant rank correlation coefficients (p<0.05). Maximum load was positively correlated with cortical BMC, Ct.V, Ct.V/AV, Ct.Ar, and Ps.Pm, and negatively correlated with BUN, TC, NEFA, HDL-C, glucose, GA, and HbA1c concentration.

Discussion

In the present study, bone properties (quantity, quality, and mechanical strength) were deteriorated in pre-DM, ZFDM, *fa/fa* rats. WBV for 8 weeks attenuated the decrease in the maximum load of the tibia, but not the deterioration of TBMS and CBG. The DM group showed a reduction of trabecular and cortical BMC and bone mechanical strength, as well as deterioration of TBMA and CBG. However, the decrease in maximum load, a reflection of bone mechanical strength, was delayed in the WBV group. Treadmill running (TR) reportedly has no effect on bone properties (BMD, bone strength, and TBMA) in OLETF rats and does not improve BG and serum sclerostin levels[20], although it improves tibial TBMA, CBG, biomechanical properties, BG, and HbA1c levels in hyperphagic OLETF rats[21]. In addition, long-term voluntary running from a young age was reported to prevent the deterioration of bone properties while improving BG and HbA1c levels[13]. Thus, preventing the worsening of DM conditions could contribute to the prevention of T2DM-induced bone fragility.

Although WBV differs from running, it also stimulates bone and results in cyclical muscle contractions[22]. WBV passively stimulates the musculoskeletal system without body movement. According to the previous study, WBV for 12 weeks improved bone microstructure and bone mechanical strength in db/db mice without improving BG and serum leptin levels; the decrease in levels of a bone formation marker (osteocalcin) and proteins involved in osteoblastogenesis (alkaline phosphatase, runt-related transcription factor 2, type I collagen, bone morphogenetic protein 2, Wnt3a, low-density lipoprotein receptor-related protein 6, and β-catenin) was attenuated and levels of bone resorption markers (TRACP-5b and C-terminal cross-linked telopeptides of type 1 collagen) were increased in this model[17]. This suggested that WBV may improve bone properties by increasing bone formation, decreasing bone resorption, upregulating skeletal gene expression of Wnt signaling components, downregulating skeletal gene levels of sclerostin, and the osteoprotegerin/RANKL/RANK system without improving hyperglycemia in db/db mice[17]. Passive muscle contraction by percutaneous electrical stimulation reportedly improves BMD and bone quality in OLETF rats[23]. In addition, WBV at a frequency of 35 Hz with 0.25 *g* acceleration for 8 weeks inhibited the deterioration of bone properties (BMD, TBMS, and mechanical strength) of the femur in streptozotocin-induced DM (STZ-DM) model rats exhibiting the characteristics of type 1 DM[24]. WBV also attenuated the inhibition of osteoblast functions by high BG condition and improved BG levels compared with non-treated DM rats, although not to the same levels as those in non-DM control rats[24]. Meanwhile, a different study found that WBV did not improve the deterioration of bone properties and glycemic control in db/db mice, although it increased serum osteocalcin levels and decreased insulin levels[16]. However, the impact of bone resorption and osteogenesis factors on bone properties were not assessed in that study[16].

In the present study, bone and muscle weights were smaller, and both trabecular and cortical BMC, TBMS, and CBG were deteriorated, in the DM group compared with the CON group. Deteriorations in these parameters were also observed in the WBV group, likely because WBV could not prevent the onset of T2DM, as reflected in the increase in levels of BUN, TC, TG, NEFA, glucose, GA, HbA1c, insulin, and leptin under T2DM conditions. The lower levels of serum creatinine in the WBV and DM groups were considered to have contributed to muscle mass atrophy. According to the previous study, muscle fiber atrophy in EDL was observed in db/db mice[16], and T2DM conditions impaired bone remodeling and resulted in bone fragility due to osteoblast and osteocyte dysfunctions, accumulation of AGEs cross-links, and abnormal bone microstructure[25]. However, in the present study, WBV attenuated the decrease in maximum load, despite that it did not inhibit the deterioration of BMC and CBG.

As described above, the effects of T2DM were apparent in the WBV group, as in the DM group. Maximum load was positively correlated with cortical BMC and CBG (Ct.V, Ct.V/AV, Ct.Ar, and Ps.Pm) and negatively correlated with DM-related biochemical indices (BUN, TC, NEFA, glucose, GA, and HbA1c). This suggests that maximum load may depend, at least in part, on cortical bone and T2DM conditions. In

addition, serum BUN and glucose levels were significantly lower and HbA1c concentration tended to be lower (p=0.05) in the WBV group compared with the DM group. While serum AGE levels were lower in the WBV group compared with the DM group, the difference was not significant. The distribution of serum homocysteine levels was similar among the three groups, except for one data point in the WBV and DM groups. These findings suggest the possibility that renal impairment and accumulation of AGEs cross-links in the WBV group were not as advanced as those observed in the DM group. In addition, Liu, et al. reported that WBV improved insulin resistance by activating the phosphoinositide 3-kinase/Akt pathway and improving insulin receptor substrate-1, Akt2, and glucose transporter type 4 (GLUT-4) in STZ-DM rats[26] and by attenuating oxidative stress to ameliorate liver injury in db/db mice[27]. Moreover, WBV improved wound and bone healing in DM model rodents by activating cytokines related to wound and fracture repair[28-30]. WBV improved blood microcirculation with increasing angiogenesis and related tissues by increased insulin-like growth factor 1 (IGF-1), vascular endothelial growth factor, and GLUT-4[28,29]. WBV enhanced muscle activities, which in turn led to improved glycemic control[29]. WBV also increased osteogenic and chondrogenic cell proliferation by increasing IGF-1 and decreasing RANKL in addition to improving bone callus quality and quantity in STZ-DM rats[30]. The effects of WBV, as demonstrated in the previous studies using DM model rodents[16,17,24,26,27,29], may have contribute to delay in the onset of T2DM, since WBV conditions in the present study were similar to those in the previous studies. Notably, there was no significant difference in maximum load in the WBV group compared with the CON and DM groups, although maximum load was significantly lower in the DM group compared with the CON group. This suggests that the decrease in maximum load was delayed rather than inhibited, and might be attributed to slow accumulation of AGEs cross-links and mild T2DM condition.

WBV reportedly improves glycemic control (BG or HbA1c levels) in DM rodents, but not to the extent observed in non-DM rodent[17,26,27,29,31]. This is consistent with our present findings and suggests that improvement of T2DM-induced bone deterioration by WBV is not solely dependent on glycemic control. In fact, increased osteocalcin levels, decreased TRACP-5b levels, and improved osteoblastogenesis were previously observed, which likely could be explained by improvements in T2DM-induced bone deterioration due to WBV[17], with these parameters improved to the extent they were similar to those in non-DM mice. These findings suggest that, to prevent T2DM-induced bone deterioration, improvements in T2DM conditions to the extent observed in non-DM conditions are important.

2DM rodent models generally show increased body mass due to hyperphagia[32], whereas STZ-DM rodent models show decreased body mass[33]. In the present study, body mass in the DM and WBV groups (ZFDM, *fa/fa* rats) was significantly increased compared with that in the CON group, which is consistent with previous reports on ZFDM, *fa/fa* rats[18,19]. In addition, FI was significantly higher in the DM and WBV groups compared with the CON group, and was significantly higher in the DM group compared with the WBV group (Table 1). Although FI was higher in the DM with TR group compared with the non-treated DM group in hyperphagic OLETF rats, body mass was lower in the DM with TR group compared with the non-treated DM group[14]. Moreover, although FI was lower in the WBV group compared with the non-treated DM group in STZ-DM rats, body mass was higher in the WBV group compared with the non-treated DM group[26]. In contrast, FI was increased in the WBV group with no difference in body mass compared with the other groups in non-DM rats, suggesting that the increase in FI might be related to the influence of WBV exercise[34]. It is possible that, in the present study, the lower FI in the WBV group was due to the influence of WBV and not because of pathological conditions, given the continuous increase in body mass throughout the course of experiment, similar to the DM group. Also, the relationship between body mass and FI is considered to change depending on the model of DM or type of exercise.

The strength of the present study is that effects of WBV on bone properties were examined in pre-T2DM model rats. Since few studies have examined how WBV affects bone properties in pre-T2DM or T2DM model rats, our results may help in developing WBV-based preventive measures for T2DM and DM-induced osteoporosis. In addition, to our knowledge, this study is the first to report the effects of WBV on bone properties in ZFDM, *fa/fa* rats. The choice of T2DM model rodents in bone research related to T2DM may increase in the future, depending on purposes. However, this study has limitations that should be noted. First, this study did not set the group of non-DM, ZFDM, *fa/+* rats with WBV stimulation. Nonetheless, the present study demonstrated for the first time the impact of WBV on bone properties, bone metabolic markers, and DM-related markers in ZFDM, *fa/fa* rats, providing important insights. Second, osteoblastogenesis and the upregulation/downregulation of skeletal gene expression were not assessed, although WBV improved glycemic control (glucose and HbA1c) but not bone metabolic markers such as BALP, TRACP-5b, and homocysteine levels.

Conclusions

The present study demonstrated a deterioration of bone properties, such as bone mass, TBMA, and CBG, in ZFDM rats, and that WBV did not prevent this deterioration but may have delayed the decrease in maximum load. WBV improved BUN, glucose, and HbA1c levels compared with the DM group, but the levels of these markers did not improve to the same levels observed in the CON group. Only a few studies have investigated the effects of WBV on bone properties in T2DM rodents, with conflicting results[16,17,24]. Given that bone properties can be both positively and negatively affected by WBV condition[35,36] and strain[37], further studies on effective WBV conditions for preventing

T2DM and improving bone properties, and the impact of these conditions on bone metabolic makers, osteogenesis-related gene expression, and regulatory system of bone homeostasis, are warranted.

Ethics approval

This study was approved by the Committee of Research Facilities of Laboratory Animal Science of Kio University (No. RO2-05) and was performed in accordance with the Guide for Animal Experimentation at Kio University.

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