

Original Article

Long-term wheel-running can prevent deterioration of bone properties in diabetes mellitus model rats

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Abstract

Objectives: The purpose of this study was to examine the effects of long-term wheel-running on tibia bone properties in T2DM Otsuka Long-Evans Tokushima Fatty (OLETF) rats. **Methods:** Ten five-week-old male OLETF rats were used as experimental animals and 5 Long-Evans Tokushima Otsuka (LETO) rats as controls. Half of OLETF rats performed daily voluntary wheel-running for 17 months (OLETF-EXE), while neither the remainder of OLETF nor LETO rats had exercise. At the end of experiment, in addition to serum biochemical and bone formation/resorption marker analyses, bone mass, trabecular bone microarchitecture and cortical bone geometry were analyzed in left tibia, and bone mechanical strength of right tibia was measured. **Results:** Tibia bone mass, trabecular bone microarchitecture, cortical bone geometry and bone mechanical strength deteriorated in diabetic OLETF rats. However, such deterioration was obviously attenuated in OLETF-EXE rats, which maintained normal levels of blood glucose, HbA1c and blood urea nitrogen. **Conclusions:** Daily wheel-running could prevent the deterioration of bone properties in OLETF rats. This would be induced mainly by suppressing the development of T2DM. Regular physical exercise may be a potent strategy for preventing not only the development of diabetes but also the deterioration of bone properties in patients with chronic T2DM.

Keywords: Type 2 Diabetes Mellitus, Voluntary Wheel-running, Trabecular Bone Microarchitecture, Cortical Bone Geometry, Bone Mechanical Strength

Introduction

The prevalence of diabetes has been increasing worldwide in recent decades, and 9% of adults over 18 years old had diabetes in 2014¹. In addition, the number of 20-79-year-old adults with diabetes appears to increase by 69% in developing countries and by 20% in developed countries for 20 years between 2010 and 2030². Thus, it should be noted that the burden of diabetes will be bigger and bigger in future. Diabetes showing manifestation of chronic hyperglycemia is

a metabolic disorder induced by multiple etiology³, which affects various organ systems including the visual, nervous, circulatory and urinary systems, etc.

Osteoporosis is one of the diabetic complications. Both type 1 and type 2 diabetes (T1DM and T2DM) are well-known to cause bone fragility and to increase the risk of fracture^{4,5}. The patients with T2DM showed a 1.4- to 1.7-fold increase in the fracture risk as compared with healthy people without diabetes^{4,5}. In addition, both the diabetes with different pathophysiology showed the different process of bone fragility^{6,7}. The fracture risk rose not only in the T1DM patients with the low levels of bone mineral density (BMD)^{6,7}, but also in the T2DM patients with the normal to high levels of BMD⁸⁻¹⁰, suggesting that the bone fragility observed in T2DM results from the deterioration of bone quality rather than of BMD.

More than 90% of the patients with diabetes are T2DM³. The onset of T2DM could be prevented by physical activity, healthy diet and regular life style, because it resulted from overweight, obesity, less physical activity, harmful diet and unhealthy life style^{1,4-7}. Therefore, both the prevalence and in-

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cidence of T2DM were reported to be associated with physical activity¹¹. Moreover, physical inactivity was considered as etiology in 27% of the patients with diabetes⁸. In addition, regular physical activity could reduce the risk of developing T2DM by 20-60% in an intensity-response manner¹². For instance, the moderate-intensity exercise for at least 150 min per week reduced both the risk of noncommunicable diseases including diabetes and mortality by every cause¹. Moreover, the intensive exercise stimulated bone metabolism, and thereby the bone mass was augmented by 5-15% as compared with that of sedentary adults¹¹. Thus, regular exercise may improve not only diabetic symptoms but also bone mass in T2DM.

Otsuka Long-Evans Tokushima Fatty (OLETF) rat is a polygenic model of T2DM, which is characterized by late onset of hyperglycemia, a chronic course of disease, mild obesity, inheritance by males, hyperplastic foci of pancreatic islets and renal complication¹³. Also, it was reported that the OLETF rats can be used as a useful model for investigating bone metabolism in T2DM osteopenia¹⁴. In the OLETF rats, more recently, the voluntary wheel-running for 36 weeks was found to prevent the excessive weight gain, obesity, insulin resistance and development of T2DM, and also to enhance biomechanical properties of the femur at 40 weeks of age¹⁵. However, the effects of long-term regular exercise on microstructure, geometry and mechanical properties of the tibia have not been reported in the terminal phase of T2DM as yet. The purpose of this study was to examine the effects of long-term daily voluntary exercise on bone properties of the tibia, including trabecular bone microarchitecture, cortical bone geometry and bone mechanical strength, in the aged OLETF rats. In the present study, therefore, the voluntary wheel-running exercise started at 1 month of age and ended at 18 months of age.

Methods

Animals and experimental design

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).

Ten five-week-old male OLETF rats were used as experimental animals and 5 five-week-old male Long-Evans Tokushima Otsuka (LETO) rats as control animals (Japan SLC Inc., Hamamatsu, Japan). Both the OLETF and LETO rats were established from the same colony of Long-Evans rats, which had been purchased from Charles River Canada (St. Constant, Quebec, Canada) in 1982¹³. The OLETF rats have been often used as an animal model of spontaneous T2DM, while the LETO rats with no manifestation of T2DM have been generally used as control animals of the experimental OLETF rats¹³. They were divided into 3 groups as follows; LETO, OLETF and OLETF+EXE groups (n=5 each). The OLETF+EXE group was housed in cages equipped with wheels for 17

months, and the LETO and OLETF groups in standard cages, in an animal facility where the room temperature and lighting were controlled (temperature, 23±2°C; lighting, 12:12-h light-dark cycle). All the rats were fed a standard rodent chow (CE-2; Clea Japan Inc., Tokyo, Japan) and water ad libitum throughout the experiment. At the end of 17 month-experimental periods, the collections of blood, epididymal fat and tibia were performed. After 5-6 hours of fasting, the rats were anesthetized with inhaled 2% (volume/volume) isoflurane on a mechanical ventilator at 12:00 p.m. - 03:00 p.m. The chest was opened through a median sternotomy, and more than 10 ml of blood was obtained from the left ventricle using a 20-gauge needle. Blood samples were analyzed for blood glucose concentration and HbA1c. Serum samples, obtained from blood centrifugation at 3500 rpm for 10 minutes, were stored at -80°C until biochemical analysis and ELISA. The length of bilateral tibias was measured with digital caliper. Right and left tibias were stored in saline and 70% ethanol until analyzed, respectively.

Biochemical analyses and ELISA

Serum samples were analyzed for calcium, inorganic phosphate, total protein and blood urea nitrogen (BUN), and also with a commercially available enzyme-linked immunosorbent assay (ELISA) kit for insulin (Mercodia, Uppsala, Sweden), bone specific alkaline phosphatase (BAP; Biomedical Technology Inc., Stoughton, MA, USA) and tartrate-resistant acid phosphatase-5b (TRACP-5b; Immunodiagnostic Systems Ltd., Boldon, UK).

Analyses of bone mass, trabecular bone microarchitecture and cortical bone geometry

Analyses of bone mass and trabecular bone microarchitecture were performed as previously reported by us^{16,17}. Using an x-ray micro-computed tomography (Micro-CT; Hitachi Medical Corporation, Tokyo, Japan), the proximal left tibia was scanned at 65 kV, 90 µA, with a voxel size of 21.3 µm in the high-definition mode for trabecular bone microarchitecture analysis. The region of interest (ROI) for trabecular bone microarchitecture was a 2 mm-length portion of tibia metaphysis, and the first slice was scanned 1 mm-distal from the physal-metaphyseal demarcation. In addition, the center of tibia was simultaneously scanned with the Micro-CT at 65 kV, 90 µA, with a voxel size of 16.1 µm in the high-definition mode for cortical bone geometry analysis. The ROI for cortical bone geometry was a 2 mm-portion of tibia diaphysis, and the first slice was scanned 1 mm-proximal to tibia-fibro connection. Scanned data were transmitted to a personal computer, and trabecular bone microarchitecture and cortical bone geometry of the ROI were analyzed using the bone analysis software, TRI/3D-BON (Ratoc System Engineering Co. Ltd., Tokyo, Japan). Tissue volume (TV), bone volume (BV), bone volume fraction (BV/TV), trabecular thickness (Tb.Th), trabecular number (Tb.N), trabecular separation (Tb.Sp) and connectivity density (Conn.D) were assessed as trabecular bone microarchitec-

Table 1. Running distance, body weight, diabetes indices and serum biochemical data.

	LETO	OETF	OETF-EXE
Total running distance (km)	-	-	1667±375
Body weight (g)	575.7±18.3	496.1±87.6	529.2±42.7
Epididymal fat wet weight (g)	6.0±0.7	2.6±1.9*	2.9±1.0*
Blood glucose (mg/dL)	70.8±11.5	229.3±104.5*	68.6±25.6 [†]
HbA1c (%)	5.0±0.1	8.3±1.3*	4.8±0.2 [†]
Blood urea nitrogen (mg/dL)	29.9±1.2	92.2±53.7*	26.9±1.8 [†]
Insulin (ng/mL)	0.44±0.12	0.23±1.2	0.97±0.36 [†]
Calcium (mg/dL)	9.6±0.2	9.0±0.6	8.8±0.1*
Inorganic phosphate (mg/dL)	4.5±0.4	5.9±1.6	5.2±1.0
Total protein (g/dL)	5.7±0.2	5.3±0.5	5.2±0.3*

Values are expressed as mean±SD. *Significantly different from the LETO group ($p<0.05$), [†]Significantly different from the OETF group ($p<0.05$).

ture parameters in the tibia metaphysis. Cortical bone volume (CV), all bone volume (AV; CV+MV), medullary volume (MV), cortical volume fraction (CV/AV), cortical bone thickness (Ct.Th), cortical bone sectional area (Ct.Ar), periosteal perimeter (Ps.Pm) and endocortical perimeter (Ec.Pm) were assessed as cortical bone geometry parameters in the tibia diaphysis. 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). TMD was calculated by collating luminance of micro CT with BMD phantom. vBMD, obtained from an equation of BMC/TV, means BMC per unit volume of TV.

Measurement of bone mechanical strength

The maximum load of right tibia was measured by a 3-point bending strength test using a Universal Testing Machine (Autograph AGS; Shimadzu Corp., Kyoto, Japan). Tibia was supported by 2 fulcrums with 5 mm-diameter, and the distance between both the fulcrums was one third of bone length. Next, tibia was pressed downward on the center of bone at a speed of 0.5 mm/min. The load-displacement curves, thus obtained, were analyzed for the slope and area under the curve to fracture using a software of Autograph AGS (Trapezium Lite X).

Dry bone and ash weight measurements

After the tibias were used for the measurements of trabecular bone microarchitecture / cortical bone geometry parameters and bone mechanical strength, they were dehydrated in 100% ethanol for 48 hours and then heated at 100°C for 24 hours in a drying machine (Advantec, Tokyo, Japan) to obtain the data of dry bone weight. Finally, the tibias were burned to ash at 600°C for 24 hours with an electric furnace (Nitto Kagaku Co. Ltd., Nagoya, Japan), and the ash thus obtained was weighed.

Statistical analysis

All values were expressed as mean ± standard deviation. Overall difference among the LETO, OETF and OETF+EXE groups was determined by a Kruskal Wallis test, and difference between individual groups was examined using a Steel-Dwass post-hoc test. In addition, Pearson's correlation coefficients were determined to examine the relationship between bone property parameters and body weight or bone mechanical strength. All statistical analyses were performed using the Excel Statistics software (Excel 2012 version 1.08 for Windows; Social Survey Research Information Co., Ltd., Tokyo, Japan). A *P* value less than 0.05 was considered statistically significant. Most of the figures are expressed by a box-and-whisker plot.

Results

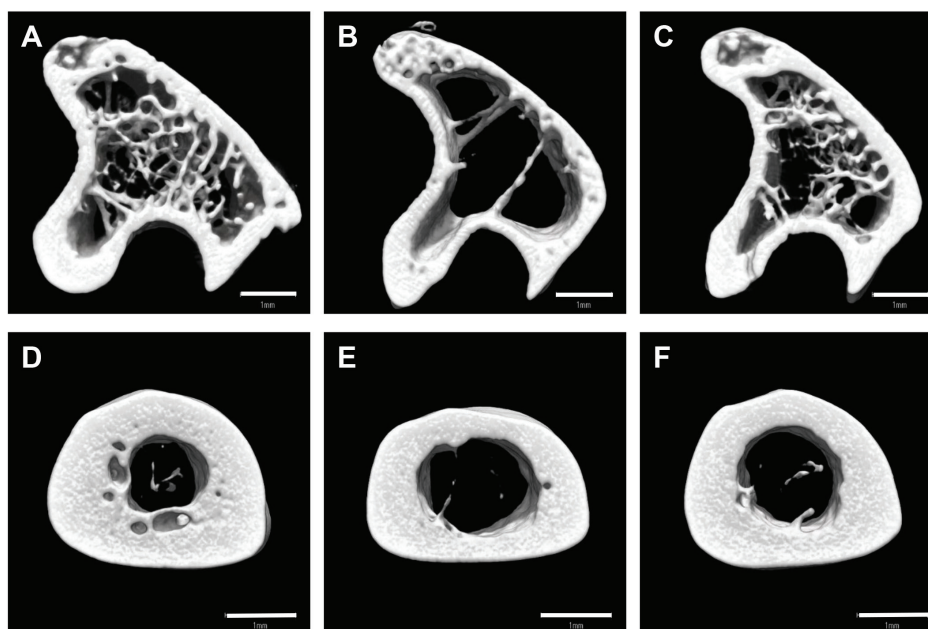
Running distance, body weight, diabetes indices and serum biochemical data

In the OETF-EXE group, the average total wheel-running distance during the experimental period of 17 months was 1667±375 km (Table 1). However, the total running distance varied widely among the individual rats, i.e., ranging between 1171 km and 2219 km. At 9 weeks of age, the body weight was significantly heavier in the OETF group than in the OETF-EXE and LETO groups. The body weight of the OETF group reached a maximum (643.6±69.3 g) at 37 weeks of age, and thereafter was gradually lost. At the end of experiment, the diabetic OETF group showed a considerable variation in final body weight. There was no statistically significant difference in body weight among the 3 groups (Table 1). On the other hand, epididymal fat wet weight was much lighter in the OETF and OETF-EXE groups than in the LETO group, suggesting that the diabetic OETF rats utilized body fat for generating energy because of the restricted utilization of blood glucose while

Table 2. Tibia bone length, dry bone weight and bone ash weight.

	LETO	OETF	OETF-EXE
Bone length (mm)	45.8±0.6	44.9±1.0	45.7±0.5
Dry bone weight (mg)	863.6±28.5	747.8±68.5*	781.7±32.4*
Bone ash weight (mg)	548.0±15.6	463.4±46.3*	501.2±18.4*
Bone ash weight/dry bone weight	0.635±0.008	0.619±0.014*	0.641±0.01†

Values are expressed as mean±SD. *Significantly different from the LETO group ($p<0.05$), †Significantly different from the OETF group ($p<0.05$). N=5 in each group.

**Figure 1.** Representative micro-CT images of tibia trabecular/cortical bone transverse sections. Trabecular bone of proximal tibia metaphysis in the LETO (A), OETF (B) and OETF-EXE (C) rat. Cortical bone of tibia diaphysis in the LETO (D), OETF (E) and OETF-EXE (F) rat. Bar=1 mm.

the exercised OETF rats metabolized body fat, in addition to blood glucose, to get more energy (Table 1). Diabetes indices (blood glucose and HbA1c levels) and BUN levels were much lower in the OETF-EXE group, as well as in the LETO group, than in the OETF group (Table 1). On the other hand, serum insulin concentration was significantly higher in the OETF-EXE group than in the OETF group (Table 1). In addition, the OETF-EXE group showed a decreased serum levels of calcium and of total protein as compared with the LETO group (Table 1).

Tibia bone length, dry bone weight and bone ash weight

Bone length of the OETF groups tended to be shorter, albeit not significantly, as compared with other 2 groups

(Table 2). On the other hand, both dry bone weight and bone ash weight were significantly lighter in the OETF-EXE and OETF groups than in the LETO groups (Table 2). However, the ratio of bone ash weight to dry bone weight was significantly higher in the OETF-EXE group, as well as in the LETO group, than in the OETF group (Table 2).

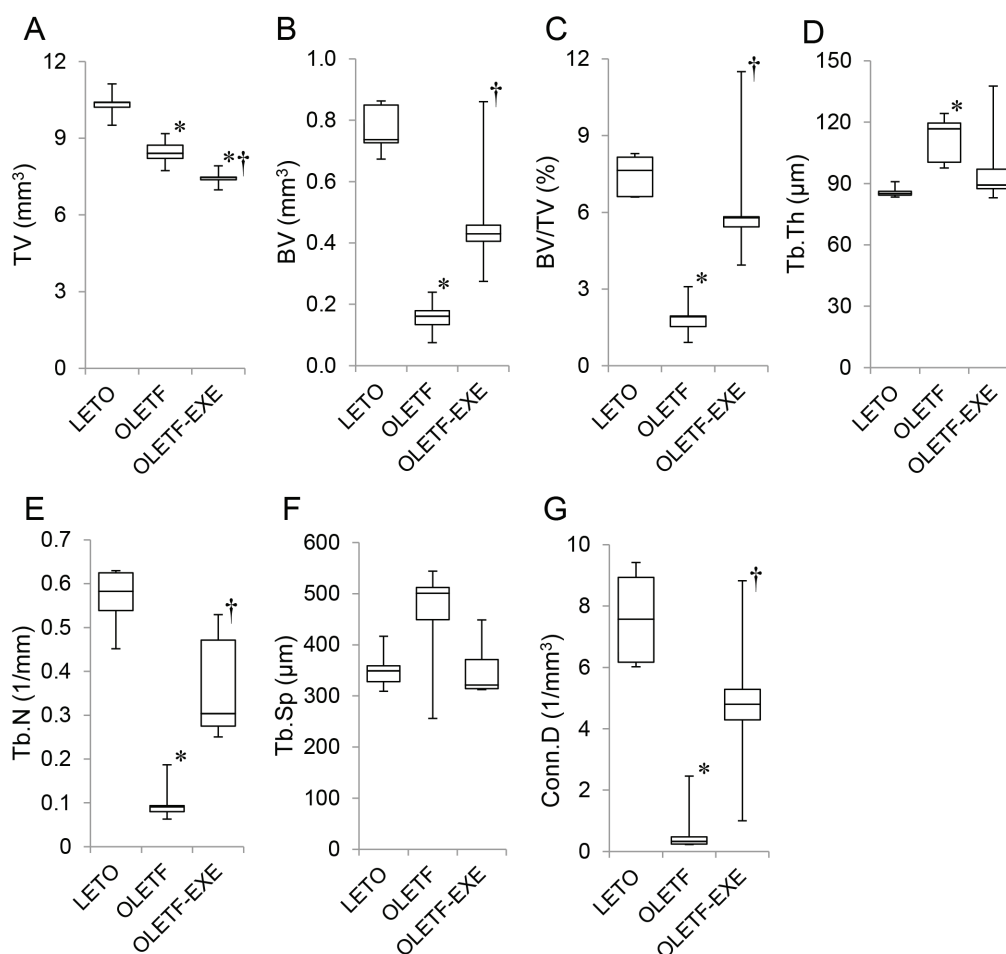
Micro-CT images

Figure 1 shows cortical and trabecular bone transverse sections of tibia ROI in the 3 groups. Trabecular bone connectivity at metaphysis of the proximal tibia appears to be relatively well maintained in the OETF-EXE group (Figure 1C), whereas trabecular bone of the OETF groups is disconnected (Figure 1B). On the other hand, the micro-CT images

Table 3. Bone mass parameters in tibia trabecular and cortical bones.

	LETO	OETF	OETF-EXE
Trabecular bone			
Tissue mineral density (mg/cm ³)	324.5±12.5	381.8±38.5*	370.4±37.2*
Bone mineral content (μg)	255.2±34.5	60.9±23.0*	187.3±10.9 [†]
Volume bone mineral density (mg/cm ³)	24.5±3.2	7.1±2.9*	24.8±14.3 [†]
Cortical bone			
Tissue mineral density (mg/cm ³)	956.8±22.8	1061.3±40.7*	1030.3±12.7*
Bone mineral content (mg)	9.0±0.3	7.9±0.4*	8.7±0.31 [†]

Values are expressed as mean±SD. *Significantly different from the LETO group (p<0.05), [†]Significantly different from the OETF group (p<0.05). N=5 in each group.

**Figure 2.** Trabecular bone microarchitecture parameters in tibia metaphysis. A, tissue volume (TV); B, bone volume (BV); C, bone volume fraction (BV/TV); D, trabecular thickness (Tb.Th); E, trabecular number (Tb.N); F, trabecular separation (Tb.Sp); G, connectivity density (Conn.D). *Significantly different from the LETO group (p<0.05), [†]Significantly different from the OETF group (p<0.05). N=5 in each group.

of cortical bone at diaphysis of tibia show that bone marrow area is slightly increased and bone is somewhat thinner in the OETF group (Figure 1E), as compared with in the OETF-

EXE group (Figure 1F). Thus, the preventive effects of wheel-running exercise on bone deterioration were evident in the T2DM rat model OETF.

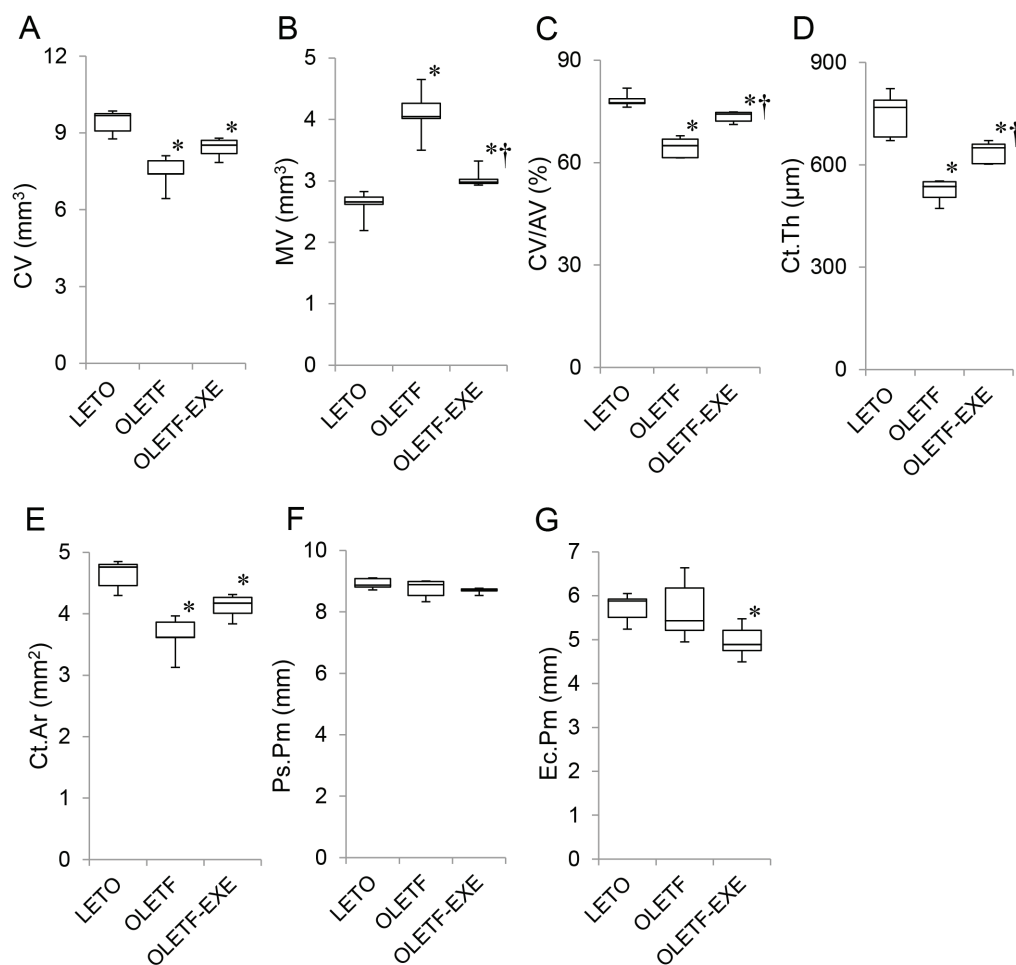


Figure 3. Cortical bone geometry parameters in tibia diaphysis. A, cortical bone volume (CV); B, medullary volume (MV); C, cortical volume fraction (CV/AV; AV, all bone volume); D, cortical bone thickness (Ct.Th); E, cortical bone sectional area (Ct.Ar); F, periosteal perimeter (Ps.Pm); G, endocortical perimeter (Ec.Pm). *Significantly different from the LETO group ($p < 0.05$). †Significantly different from the OLETF group ($p < 0.05$). N=5 in each group.

Bone mass parameters

In tibia trabecular bone, BMC and vBMD were much higher in the OLETF-EXE group, as well as in the LETO group, than in the OLETF group, and also TMD was significantly higher in the OLETF-EXE and OLETF groups than in the LETO group (Table 3). In tibia cortical bone, likewise, BMC was significantly, but slightly, higher in the OLETF-EXE and LETO groups than in the OLETF group, and also TMD was significantly higher in the OLETF-EXE and OLETF groups than in the LETO group (Table 3).

Trabecular bone microarchitecture and cortical bone geometry parameters

In trabecular bone microarchitecture parameters of the tibia metaphysis, BV, BV/TV, Tb.N and Conn.D were

considerably higher in the OLETF-EXE and LETO groups than in the OLETF group, though TV in the OLETF-EXE and OLETF groups were the lowest and medium, respectively (Figure 2). To the contrary, Tb.Th was significantly lower in the LETO group than in the OLETF group (Figure 2). There was no significant difference in Tb.Sp among the 3 groups. In cortical bone geometry parameters of the tibia diaphysis, on the other hand, CV, CV/AV, Ct.Th and Ct.Ar were significantly lower in the OLETF-EXE group, as well as in the OLETF group, than in the LETO group (Figure 3). However, CV/AV and Ct.Th in the OLETF-EXE group were significantly higher than those of the OLETF group. To the contrary, MV in the OLETF group was prominent as compared with two other groups. In addition, the OLETF-EXE group showed a decreased Ec.Pm, irrespective of similar Ps.Pm, compared with two other groups (Figure 3).

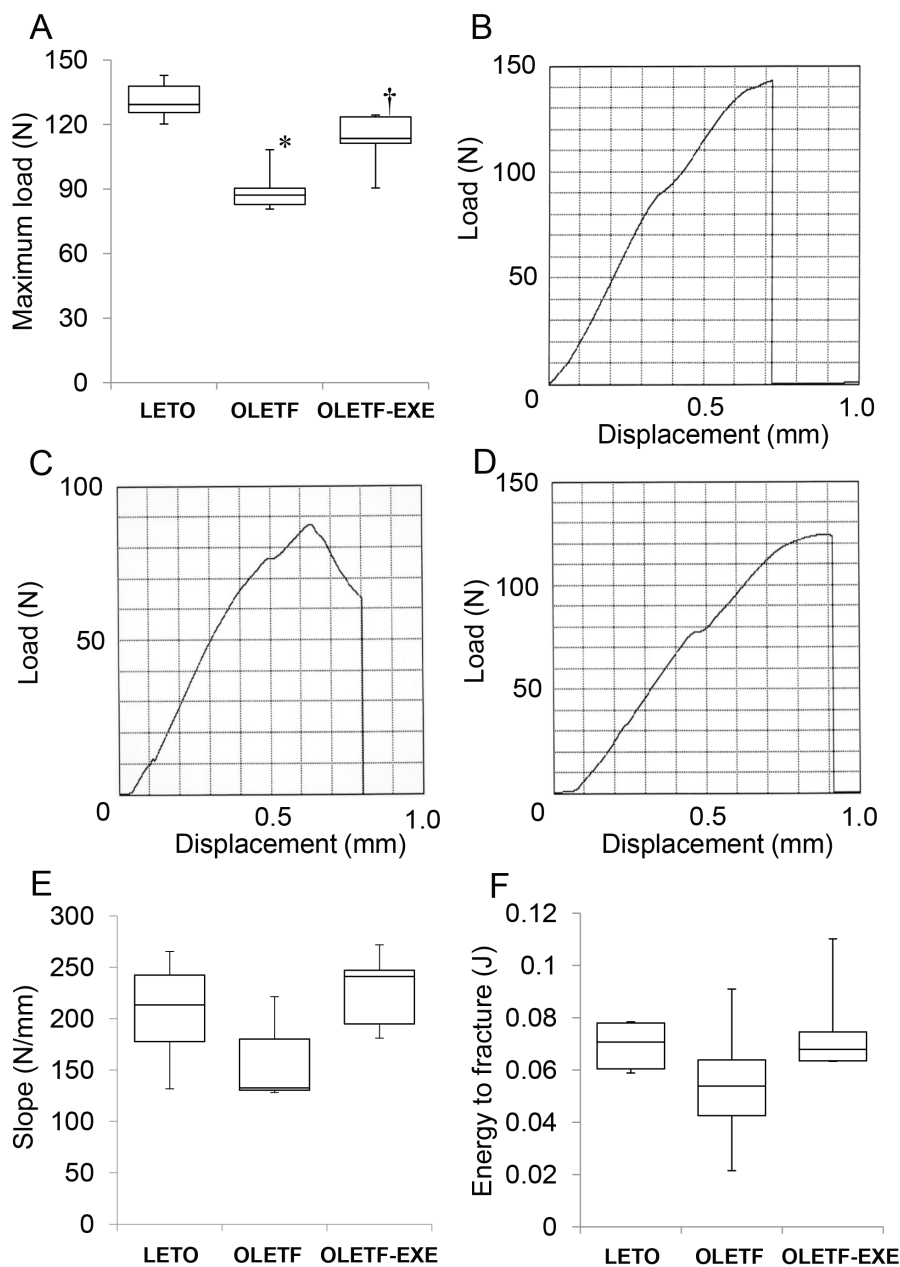


Figure 4. Bone mechanical strength of tibia (A), representative load-displacement curves in the LETO (B), OLETf (C) and OLETf-EXE (D) rats, slope of load-displacement curves (E) and energy to fracture obtained by area under the curve (F). *Significantly different from the LETO group ($p < 0.05$), †Significantly different from the OLETf group ($p < 0.05$). $N = 5$ in each group.

Bone mechanical strength of tibia

The OLETf group showed a significant decrease in the maximum load obtained by the bending strength test of tibia, as compared with the OLETf-EXE and LETO groups (Figure 4A). Figures 4B, 4C and 4D show representative load-displacement curves obtained from tibia of the LETO, OLETf and OLETf-EXE rats, respectively. In the load-dis-

placement curves, the OLETf group showed the lowest median value of the slope that implies stiffness and also of the energy to fracture, that is obtained by the area under the curve, as compared with two other groups, although there was no significant difference in these slope and energy to fracture among the 3 groups (Figures 4E and 4F).

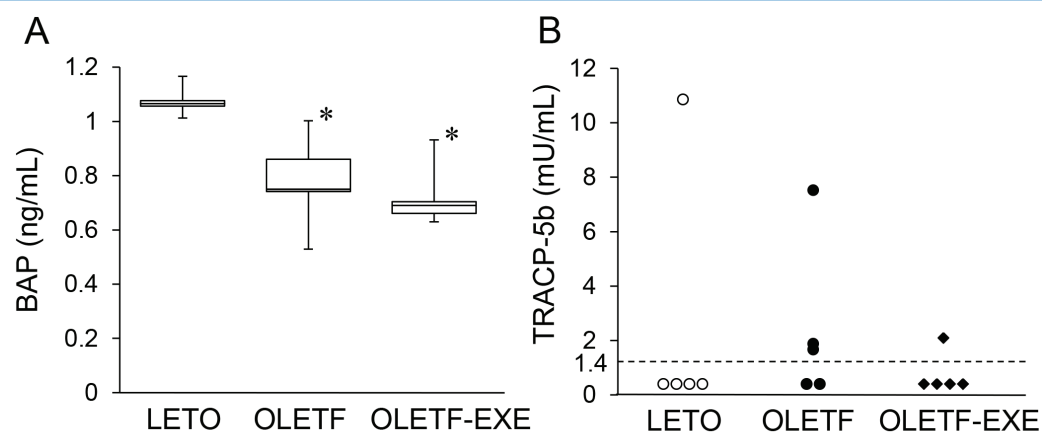


Figure 5. Serum levels of bone specific alkaline phosphatase (A) and tartrate-resistant acid phosphatase-5b (B). BAP, bone specific alkaline phosphatase; TRACP-5b, tartrate-resistant acid phosphatase-5b. Symbols represent data from individual animals. More than 1.4 mU/mL of serum TRACP-5b concentration could be detected with the present ELISA. *Significantly different from the LETO group ($p < 0.05$). $N = 5$ in each group.

Table 4. Correlation between bone parameters and body weight or bone mechanical strength.

	Body weight	Bone mechanical strength
- Bone mechanical strength	0.55	---
- Bone specific alkaline phosphatase	---	0.55
- Dry bone weight	0.75	0.86
- Bone ash weight	0.72	0.87
Bone mass parameters		
- Tissue mineral density (cortical)	-0.62	-0.83
- Bone mineral content (trabecular)	---	0.89
- Bone mineral content (cortical)	---	0.86
- Volume bone mineral density (trabecular)	---	0.85
TBMA parameters		
- Bone volume	---	0.88
- Bone volume fraction	---	0.86
- Trabecular number	---	0.79
- Connectivity density	---	0.83
CBG parameters		
- Cortical bone volume	0.54	0.94
- Medullary volume	-0.54	-0.89
- Cortical volume fraction	0.51	0.94
- Cortical bone thickness	---	0.96
- Cortical bone sectional area	0.54	0.94

Values are Pearson's correlation coefficient ($p < 0.05$).
TBMA, trabecular bone microarchitecture; CBG, cortical bone geometry.

Circulating levels of BAP and TRACP-5b

Serum levels of the bone formation marker BAP were significantly lower not only in the OLETF group but also in the OLETF-EXE group than in the LETO group (Figure 5). On the other hand, the present ELISA was unable to detect the bone resorption marker TRACP-5b in 4 serum samples from the OLETF-EXE group as well as from the LETO group, but was able to detect it in 3 serum samples from the OLETF group (Figure 5).

Correlation between bone parameters and body weight or bone mechanical strength

Using the data obtained from the 3 groups, we examined correlations between bone parameters and body weight or bone mechanical strength (Table 4). There were somewhat good correlations between body weight and bone mechanical strength, dry bone weight, bone ash weight, TMD or cortical bone geometry parameters except Ct.Th. In contrast, there were fairly good correlations between bone mechanical strength and dry bone weight, bone ash weight, bone mass parameters, trabecular bone microarchitecture parameters or cortical bone geometry parameters. On the other hand, we could not find any correlations between bone mechanical strength and total wheel-running distance in the OLETF-EXE group.

Discussion

In this study, the long-term voluntary wheel-running could prevent not only the development of diabetes but also the deterioration of bone properties in the OLETF rats. The wheel-running-induced prevention of chronic hyperglycemia seems to play a pivotal role in the prevention of bone deterioration. The intensity and duration of physical activity were associated with the risk of impairing glucose tolerance in diabetes¹⁸. On the other hand, the poor glycemic control, i.e., HbA1c >7.5%, was associated with a decrease in the trabecular bone score¹⁹, and also with the fracture risk in T2DM²⁰. Moreover, the long-term hyperglycemia weakened the bone material strength²¹. Hyperglycemia and oxidative stress were reported to induce the accumulation of advanced glycation end products (AGEs) and to result in the impairment in enzymatic cross-link formation and the excess in non-enzymatic cross-link formation^{22,23}. The AGEs thus produced led to a decline in the bone qualities including mineralization, microstructure and material properties, and thereby increased the risk of bone fragility^{22,23}. In addition, even in the diabetic rat bones with normal levels of BMD, the bone fragility was found to be caused increasingly by the impaired formation of enzymatic cross-links and excessive formation of non-enzymatic cross-links²⁴. Thus, in diabetes, hyperglycemia appears to be a key factor in the bone fragility. On the other hand, exercise was reported to prevent not only hyperglycemia but also inflammation in T2DM animal model²⁵. In the present study, blood glucose and HbA1c levels in the OLETF-EXE rats were

similar to those of the LETO rats. Based on these facts, in our OLETF-EXE rats, the long-term wheel-running exercise prevented chronic hyperglycemia, and would have caused no accumulation of AGEs. Therefore, the bone properties, assessed by bone mass, trabecular bone microarchitecture and cortical bone geometry parameters and bone mechanical strength, were well maintained in the OLETF-EXE rats, like in the LETO rats.

In the previous studies using the diabetic model rats, the changes in trabecular bone microarchitecture and cortical bone geometry parameters of the lower limb bone were reported to be inconsistent²⁶⁻³¹. For example, in trabecular bone microarchitecture parameters, BV/TV and Tb.Th were decreased or unchanged, and Tb.N was increased or unchanged. Likewise, in cortical bone geometry parameters, Ct.Th and Ct.Ar were decreased or unchanged, and Ps.Pm was increased or unchanged. Such inconsistency may derive from the different diabetic rat models and/or the different animal ages, though their ages were under 1 year. In the present study, although the long-term (about 11 months) diabetic condition caused the remarkable trabecular bone microarchitecture deterioration of the proximal tibia in the OLETF group, such trabecular bone microarchitecture deterioration could be prevented by the voluntary wheel-running in the OLETF-EXE group. Moreover, in the OLETF group, the decreased Ct.Th and increased MV were observed in the tibia shaft with the decreased CV, while in the OLETF-EXE group, such cortical bone geometry deterioration, too, could be prevented by the wheel-running exercise.

In diabetic model rats, the bone formation markers were more or less decreased in most cases, whereas the bone resorption markers were increased or unchanged^{26,28,31}. In addition, the osteoblast function was impaired, though the osteoclast function was not affected in a rat model of T2DM³¹. The previous long-term (69 weeks) study in the OLETF rats indicated an increase in circulating BAP and TRACP levels and a decrease in vitamin D levels¹⁴. Another study using 33-week-old OLETF rats showed a decrease in osteocalcin levels and no change in TRACP levels³². Our OLETF rats aged 75 weeks showed a decrease in serum BAP levels and also ELISA-detectable levels of TRACP-5b in 3 of 5 sera. Such inconsistency in bone formation/resorption markers still remains to be resolved in the OLETF rats. In the present study, our OLETF-EXE rats showed a decrease in serum BAP levels as seen in the OLETF rats. On the other hand, ELISA-undetectable levels of TRACP-5b were found in 4 of 5 sera from the OLETF-EXE rats, as well as from the LETO rats, and also in 2 of 5 sera from the OLETF rats. In view of these results, the voluntary wheel-running exercise might influence the bone resorption rather than the bone formation. However, this idea appears vague due to the small sample size and the overall lack of detection. To further examine this idea, the histomorphometry measurements for analyzing the osteoblast/osteoclast functions are required.

As mentioned above, it seems likely that the wheel-running exercise prevents the deterioration of bone properties via the suppression of hyperglycemia. In addition to such

suppression, there seems to be two possible mechanisms for wheel-running-induced prevention of bone deterioration in diabetic rats. First, the wheel-running exercise may prevent the impairment of mineral turnover in the bones of diabetic rats. In the tibia bones of our OLETF rats, a low mineral turnover was suggested by the much lower BMC and higher TMD values. However, the tibia BMC values of the OLETF-EXE rats were similar to those of the LETO rats. Second, in T2DM model rats, the insulin treatment did not restore the bone homeostasis but the bone defect regeneration³³. Therefore, in our OLETF-EXE rats, the increased serum insulin may play an important role in restoring the bone defect regeneration. There seem two possible mechanisms for higher levels of circulating insulin in the OLETF-EXE rats than in the diabetic OLETF rats. First, a myokine interleukin-6, released from contracting skeletal muscles, was reported to enhance insulin secretion via increased glucagon-like peptide-1 secretion³⁴. Second, percentage of collagen area in histological sections of spleen was smaller in the OLETF-EXE rats, as well as in LETO rats, than in the OLETF rats (data not shown). In relation to this, our OLETF-EXE rats showed the high insulin sensitivity induced by the enhanced GLUT-4 protein expression in skeletal muscles, whereas the OLETF rats showed the insulin resistance (data not shown).

In this study, the sample sizes were small and therefore most of data including the correlations were statistically underpowered. Studies using more animals are indispensable for further confirming the present results. In addition to the measurement of bone formation/resorption markers, moreover, biochemical analysis of bone metabolism-related substances, like hormones and vitamin D, may be required for fully understanding such preventive effects of exercise on bone deterioration in T2DM.

In conclusion, based on our results, we concluded that the daily voluntary wheel-running can prevent the deterioration of bone properties in the OLETF rats. This would be induced mainly by suppressing the development of T2DM. Therefore, regular physical exercise may be a potent strategy for preventing not only the development of diabetes but also the deterioration of bone properties in patients with chronic T2DM.

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