

## Original Article

# Upward running is more beneficial than level surface or downslope running in reverting tibia bone degeneration in ovariectomized rats

Keigo Tamakoshi<sup>1,2</sup>, Yasue Nishii<sup>3</sup>, Akira Minematsu<sup>3</sup><sup>1</sup>Department of Physical Therapy, Niigata University of Health and Welfare, Japan; <sup>2</sup>Institute for Human Movement and Medical Sciences, Niigata University of Health and Welfare, Japan; <sup>3</sup>Department of Physical therapy, Faculty of Health Science, Kio University, Japan**Abstract**

**Objective:** We investigated the effects of upslope, level surface, and downslope running on indices of tibia and femur bone recovery in ovariectomized (OVX) rats. **Methods:** Rats were randomly divided into five groups: one sham-operated (SHAM) group and four OVX groups. One OVX group was a non-running control (OVX-Cont) and the others performed upslope running (OVX-Up), level surface running (OVX-Level), or downslope running (OVX-Down) on a treadmill after ovariectomy. The metaphysis region of the proximal tibia, distal femur, and proximal femur were scanned by micro-computed tomography and various geometric and microarchitectural parameters as well as bone mineral density measured using bone analysis software. **Results:** Tibial bone geometric parameters, BV/TV and trabecular thickness, were significantly improved in OVX-Up and OVX-Level groups compared to that in OVX-Cont and OVX-Down groups, and improved to a greater degree in OVX-Up group than in OVX-Level group. **Conclusions:** Therefore, running slope substantially influences the beneficial effects of treadmill running on OVX-induced bone degeneration, with upward running being more effective than level surface running or downslope running, likely due to the greater bone loads associated with upslope running. The benefits of upslope treadmill running were particularly observed in the proximal tibia.

**Keywords:** Osteoporosis, Slope Running, Bone Microarchitecture, Tibia, Femur

**Introduction**

Post-menopausal estrogen deficiency accelerates the rate of osteoclastic resorption relative to osteoblastic formation, leading to net bone loss and osteoporosis<sup>1</sup>. It is well known that exercise is effective for preventing osteoporosis, with numerous reports showing positive effects of exercise on bone mass in postmenopausal or elderly women<sup>2,3</sup>.

The characteristics of bone loss in ovariectomized (OVX) animals resemble those of postmenopausal women. Animals

studies have examined the osteogenic responses to several forms of training, including treadmill running<sup>4</sup>, jumping<sup>5</sup>, swimming<sup>6</sup>, tower climbing<sup>7</sup>, weight-bearing exercise<sup>8</sup>, and vibration therapy<sup>9</sup>. These training regimens have been shown to inhibit bone loss with variable efficacy in OVX animals<sup>10-12</sup>. The benefits of these regimens are thought to depend on the magnitude of mechanical stress placed on actively moving bones<sup>13</sup>. For instance, the effects of treadmill running vary according to the intensity and duration<sup>14,15</sup>, and weight-bearing and high-impact exercises are considered particularly beneficial<sup>5,16</sup>.

Muscle length changes, muscle loads, and ground reaction force in the hindlimb of quadrupedal animals differ according to the walking surface angle (level, downslope, or upslope)<sup>17</sup>. The mechanical stress on bone likely also differs with surface incline. Thus, we speculated that inhibition of bone loss after ovariectomy may vary according to the slope used during treadmill running. Overall bone strength depends on cortical bone thickness/volume and 3D trabecular microarchitecture, so we quantified the effects of treadmill running at different

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Abbreviations	
ANOVA	Analysis of variance
BMC	Bone mineral content
BMD	Bone mineral density
BV	Trabecular bone volume
Conn.D	Connectivity density
Micro-CT	Micro-computed tomography
Ct.Ar	Cortical bone sectional area
Ct.Th	Cortical thickness
CV	Cortical bone volume
Ec.Pm	Endocortical perimeters
MV	Medullary volume
OVX	Ovariectomized
Ps.Pm	Periosteal perimeter
ROI	Regions of interest
SMI	Structural model index
Tb.N	Trabecular number
Tb.Sp	Trabecular separation
Tb.Th	Trabecular thickness
TV	Total bone volume

slopes on multiple indices of trabecular and cortical bone structure and microarchitecture in OVX rats.

## Materials and methods

### Animal care

Seven-week-old female Wistar rats (n = 32) were housed in standard cages under controlled room temperature (23±2°C) and humidity (55±5%) with a 12-h light-dark cycle and food and water *ad libitum*. The body weight of each rat was measured weekly. The experiment was approved by the Animal Ethics Committee of Kio University, Japan. After 1 week of acclimation to the diet and new environment, rats were randomly assigned to five groups, one sham-operated (SHAM; n=6) group with no training and four groups subjected to bilateral OVX under intraperitoneal anesthesia with sodium pentobarbital (40 mg/kg). One OVX group was a non-running control (OVX-Cont; n=8) and the other three groups performed the same running regimen (below) differing only by the angle of the treadmill: upslope running (OVX-Up, n=6), level surface running (OVX-Level; n=6), and downslope running (OVX-Down, n=6).

### Treadmill running

The rats in the OVX running groups exercised on a motor-driven treadmill (Model 1055R, Bioresearch, Aichi, Japan) at 20 m/min for 30 min, 5 days/week, for 8 weeks starting at 1 week after the operation, using a slightly modified exercise protocol of previous research<sup>18,19</sup>. The

angle of the treadmill was as follows: OVX-UP, +10%; OVX-Level, 0%; OVX-Down, -10%.

### Tissue preparation and micro-computed tomography scanning

After completion of the intervention, rats were sacrificed under sodium pentobarbital anesthesia. The soleus muscle was removed, cleaned of excess fat and connective tissues, and weighed (wet weight). The bilateral tibias and femurs were excised from each rat and cleaned of soft tissue. The length of each tibia was measured using a digital caliper. Dry weight was also determined. The ratio of soleus wet weight to body weight was then calculated. Cortical and trabecular bone microstructure was analyzed using a cone-beam X-ray micro-computed tomography (micro-CT) system (CBSTAR, MCT-100CB; Hitachi Medical Corporation, Japan) as described previously<sup>20</sup>. Bone specimens were scanned continuously in increments of 19 µm for 512 slices at a tube voltage of 62 kV, tube current of 90 mA, and voxel size of 19 µm.

### Trabecular bone assessment

After micro-CT scanning, the raw data were transferred to a workstation and structural and microarchitectural indices calculated using 3D image analysis software (TRI/3D-BON; Ratoc System Engineering Co. Ltd, Tokyo, Japan). TRI/3D-BON builds 3D models from serial tomographic datasets for visualization and morphometric analysis<sup>4</sup>. The 3D images were segmented into voxels identified as bone and marrow. Three regions of interest (ROIs) were created: the proximal tibial metaphysis, the distal femoral metaphysis, and the femoral neck. The ROI for trabecular bone microarchitecture included 100 contiguous slices of metaphyses, with the first slice scanned at 1 mm to the physal-metaphyseal demarcation<sup>21</sup>. Briefly, the gray-scale images were segmented using a median filter to remove noise, and the mineralized bone phase was extracted using a fixed threshold. Subsequently, the isolated small particles in marrow space and the isolated small gaps in bone were removed using a cluster-labeling algorithm. Cortical and trabecular bone were analyzed separately for structural indices. The following parameters were analyzed: fractional trabecular bone volume (BV) relative to total bone volume (TV) or BV/TV ([%]), trabecular thickness (Tb.Th [mm]), trabecular number (Tb.N [1/mm]), trabecular separation (Tb.Sp [mm]), connectivity density (Conn.D [1/mm<sup>3</sup>]), and structural model index (SMI). The SMI for trabecular structure expresses the ratio of plate-like to rod-like structures, with SMI of 0 indicating a perfect plate-like structure and SMI of 3 a perfect rod-like structure<sup>22</sup>, whereas Conn.D is a topological parameter that estimates the number of trabecular connections per cubic millimeter<sup>13</sup>.

### Cortical bone assessment

Tibial and femoral cortical geometry was assessed using 100 contiguous slices located at the diaphysis of mid-

	SHAM	OVX-Cont	OVX-Up	OVX-Level	OVX-Down
Initial body weight (g)	191.8 ± 13.2	192.9 ± 7.8	202.1 ± 4.3	201.5 ± 7.6	195.1 ± 10.1
Final body weight (g)	254.5 ± 27.6	312.2 ± 16.0*	316.9 ± 20.4*	334.4 ± 31.5*	317.7 ± 25.0*
Body weight gain (%)	132.5 ± 5.5	161.8 ± 4.9*	162.4 ± 7.0*	165.3 ± 13.8*	157.7 ± 11.1*
Tibial length (mm)	37.2 ± 1.2	38.6 ± 0.7*	39.0 ± 0.6*	38.2 ± 0.9*	38.5 ± 0.7*
Dry tibial weight (mg)	435.0 ± 37.9	462.8 ± 15.8*	521.6 ± 27.1*	482.9 ± 24.6*	494.9 ± 24.7*
Dry tibial weight / wt (%)	0.171 ± 0.005	0.148 ± 0.005*	0.165 ± 0.012†	0.146 ± 0.017*‡	0.156 ± 0.006*
Femoral length (mm)	33.6 ± 1.2	35.3 ± 1.7*	36.1 ± 0.5*	34.8 ± 1.3*	34.8 ± 0.6
Dry femoral weight (mg)	543.1 ± 54.1	571.9 ± 25.8	606.4 ± 51.1*	560.4 ± 28.4	581.8 ± 32.3
Dry femoral weight / wt (%)	0.215 ± 0.03	0.184 ± 0.01*	0.193 ± 0.02	0.169 ± 0.017*‡	0.186 ± 0.016*
Wet soleus muscle mass (%)	0.35 ± 0.00	0.37 ± 0.02	0.36 ± 0.01	0.39 ± 0.01	0.4 ± 0.02

Values are means ± SD. \*P < 0.05 vs. SHAM group, †P < 0.05 vs. OVX group, ‡P < 0.05 vs. OVX-Up group

**Table 1.** Physical parameter of the experimental rats.

	SHAM	OVX-Cont	OVX-Up	OVX-Level	OVX-Down
BV/TV (%)	19.19 ± 2.86	12.66 ± 2.08*	24.07 ± 4.45†	18.23 ± 7.87†‡	10.29 ± 1.94*‡§
Tb.Th (µm)	47.82 ± 4.88	39.59 ± 2.88*	56.80 ± 5.71*†	45.91 ± 7.97†‡	37.31 ± 2.58*‡§
Tb.N (1/mm)	4.01 ± 0.39	3.19 ± 0.42*	4.22 ± 0.51†	3.82 ± 1.10	2.74 ± 0.39*‡§
Tb.Sp (µm)	203.77 ± 24.09	279.58 ± 46.37*	183.47 ± 31.56†	238.48 ± 95.12	334.74 ± 59.28*‡§
SMI	0.95 ± 0.29	2.71 ± 0.11*	2.42 ± 0.23*†	2.50 ± 0.32*	2.81 ± 0.13*‡§
Conn.D (1/mm <sup>3</sup> )	74.16 ± 10.00	51.94 ± 12.97*	69.49 ± 14.79	62.99 ± 29.09	35.98 ± 10.02*‡§
vBMD (g/cm <sup>3</sup> )	106.57 ± 23.35	55.41 ± 11.94*	128.69 ± 26.68†	94.25 ± 42.08†‡	52.75 ± 10.92*‡§
BMC (g)	1.27 ± 0.27	0.66 ± 0.15*	1.75 ± 0.49*†	1.14 ± 0.47†‡	0.67 ± 0.15*‡§

BMC: Bone mineral content, vBMD: Volume bone mineral density, BV: Trabecular bone volume, Conn.D: Connectivity density, SMI: Structural model index,

Tb.N: Trabecular number, Tb.Sp: Trabecular separation, Tb.Th: Trabecular thickness, TV: Total bone volume

Values are means ± SD. \*P < 0.05 vs. SHAM group, †P < 0.05 vs. OVX group, ‡P < 0.05 vs. OVX-Up group, §P < 0.05 vs. OVX-Level group

**Table 2.** Trabecular bone morphometric parameter and vBMD in the tibia.

tibia and mid-femur. We measured cortical bone volume (CV [mm<sup>3</sup>]), the average cortical bone sectional area (Ct.Ar [mm<sup>2</sup>]), medullary volume (MV [mm<sup>3</sup>]), average cortical thickness (Ct.Th [mm<sup>2</sup>]), and periosteal perimeter (Ps.Pm [mm]) and endocortical perimeters (Ec.Pm [mm]) as reported previously 23 using TRI/3D-BON-C.

#### Bone mineral density assessment

Bone mineral density (BMD) was measured in trabecular bone of the proximal tibial metaphysis, distal femoral metaphysis, and femoral neck using the micro-CT system. To calibrate CT units to equivalent bone mineral concentration, all bone samples were scanned together with a calibration phantom. Bone mineral content (BMC) and volume BMD were calculated using TRI/3D-BON-BMD.

#### Statistical analysis

All data are expressed as mean ± SD. All statistical analyses were performed using SPSS software version 16.0 for Windows (SPSS, Chicago, IL). Group means were compared by one-way analysis of variance (one-way ANOVA) with Tukey-HSD tests for pair-wise comparisons. Values of P < 0.05 were considered statistically significant.

## Results

**Physical parameters of experimental rats (Table 1).** There were no differences in mean body weight among the 5 groups (SHAM, OVX-Cont, OVX-Up, OVX-Level, OVX-Down) before treatment. Ovariectomy significantly increased body weight, dry tibial weight, and tibial length with no influence of exercise. The ratio of soleus muscle weight to body weight also did not differ among groups.

**Trabecular bone morphometric parameters and vBMD in the tibia (Table 2).** At 9 weeks after surgery, all OVX groups displayed reduced BV/TV, Tb.Th, Tb.N, and Conn.D and increased Tb.Sp, and SMI compared to the SHAM group (P < 0.05 by Tukey-HSD test, used for all pair-wise comparisons), indicating development of osteopenia. These effects were partially reversed by treadmill running, especially with upslope, as BV/TV and Tb.Th were significantly higher in the OVX-Up and OVX-Level groups than in the OVX-Cont group, and higher in the OVX-Up group than the OVX-Level group (P < 0.05). Tb.N was also significantly higher and Conn.D numerically higher with near significance (P = 0.058) in the OVX-Up group compared to the OVX-Cont group, whereas Tb.Sp and SMI were significantly lower in the OVX-Up group compared to the OVX-Cont group (P < 0.05). In contrast to

	SHAM	OVX-Cont	OVX-Up	OVX-Level	OVX-Down
CV (mm <sup>3</sup> )	4.50 ± 0.42	4.74 ± 0.22	5.09 ± 0.24*†	4.74 ± 0.28‡	4.81 ± 0.30
MV (mm <sup>3</sup> )	1.96 ± 0.32	1.98 ± 0.16	2.41 ± 0.40*†	2.18 ± 0.28	2.18 ± 0.13
Ct.Ar (mm <sup>2</sup> )	2.18 ± 0.21	2.30 ± 0.11	2.47 ± 0.12*†	2.31 ± 0.14	2.34 ± 0.15
Ct.Th (mm <sup>2</sup> )	434.42 ± 35.38	447.74 ± 17.35	446.04 ± 30.39	434.26 ± 18.83	439.58 ± 26.79
Ps.Pm (µm)	3779.23 ± 319.97	4018.47 ± 301.43	4532.27 ± 429.70*†	4316.93 ± 294.90*	4194.43 ± 147.29*
Ec.Pm (µm)	6688.80 ± 282.24	6906.54 ± 162.62	7334.29 ± 268.20*†	7039.63 ± 285.68*	7088.92 ± 146.27*

Ct.Ar: Cortical bone sectional area, Ct.Th: Cortical thickness, CV: Cortical bone volume, Ec.Pm: Endocortical perimeters, Ps.Pm: Periosteal perimeter  
Values are means ± SD. \*P < 0.05 vs. SHAM group, †P < 0.05 vs. OVX group, ‡P < 0.05 vs. OVX-Up group,

**Table 3.** Cortical bone morphometric parameter.

	SHAM	OVX-Cont	OVX-Up	OVX-Level	OVX-Down
BV/TV (%)	29.93 ± 5.35	11.66 ± 2.31*	17.52 ± 2.12*††	15.70 ± 3.92*†	13.43 ± 1.60*
Tb.Th (µm)	57.56 ± 4.46	47.42 ± 3.52*	53.76 ± 2.82††	51.69 ± 3.16*†	49.60 ± 2.95*
Tb.N (1/mm)	5.17 ± 0.57	2.44 ± 0.35*	3.25 ± 0.27*††	3.02 ± 0.20*†	2.70 ± 0.06*
Tb.Sp (µm)	138.28 ± 26.44	371.15 ± 70.28*	255.50 ± 26.56*††	304.46 ± 131.83*	322.58 ± 29.83*
SMI	1.50 ± 0.27	2.28 ± 0.15*	1.97 ± 0.11*††	2.02 ± 0.23*†	2.12 ± 0.15*
Conn.D (1/mm <sup>3</sup> )	95.59 ± 14.84	28.70 ± 6.76*	51.16 ± 7.52*†	43.29 ± 14.82*†	35.83 ± 6.16*‡
vBMD (g/cm <sup>3</sup> )	225.00 ± 48.34	85.27 ± 27.30*	97.87 ± 26.39*	113.55 ± 29.22*	92.13 ± 16.04*
BMC (g)	2.08 ± 0.49	0.71 ± 0.20*	1.20 ± 0.25*†	1.06 ± 0.32*	0.85 ± 0.19*

BMC: Bone mineral content, vBMD: Volume bone mineral density, BV: Trabecular bone volume, Conn.D: Connectivity density, SMI: Structural model index,  
Tb.N: Trabecular number, Tb.Sp: Trabecular separation, Tb.Th: Trabecular thickness, TV: Total bone volume  
Values are means ± SD. \*P < 0.05 vs. SHAM group, †P < 0.05 vs. OVX group, ‡P < 0.05 vs. OVX-Up group, §P < 0.05 vs. OVX-Level group

**Table 4.** Trabecular bone morphometric parameter and vBMD in the distal femur.

	SHAM	OVX-Cont	OVX-Up	OVX-Level	OVX-Down
BV/TV (%)	24.49 ± 2.09	19.51 ± 2.33*	24.15 ± 2.74†	24.88 ± 2.28†	22.12 ± 2.60
Tb.Th (µm)	56.60 ± 2.06	55.99 ± 1.97*	61.63 ± 2.36*†	31.03 ± 1.97†	59.41 ± 3.64†
Tb.N (1/mm)	4.32 ± 0.27	3.48 ± 0.30*	3.91 ± 0.34*†	4.07 ± 0.31†	3.71 ± 0.42*§
Tb.Sp (µm)	175.56 ± 15.12	233.63 ± 26.34*	195.67 ± 23.83†	185.88 ± 21.39†	210.98 ± 21.18*
SMI	1.91 ± 0.17	2.17 ± 0.15*	1.91 ± 0.19†	1.84 ± 0.09†	2.00 ± 0.14
Conn.D (1/mm <sup>3</sup> )	65.85 ± 9.57	39.74 ± 8.49*	49.21 ± 9.40*	54.95 ± 7.82*†	43.97 ± 5.99*§
vBMD (g/cm <sup>3</sup> )	244.95 ± 24.98	204.15 ± 36.70*	243.33 ± 31.60†	261.53 ± 24.24†	234.52 ± 32.02
BMC (g)	2.12 ± 0.22	2.17 ± 0.39*	2.65 ± 0.44*†	2.84 ± 0.33*†	2.65 ± 0.35*†

BMC: Bone mineral content, vBMD: Volume bone mineral density, BV: Trabecular bone volume, Conn.D: Connectivity density, SMI: Structural model index,  
Tb.N: Trabecular number, Tb.Sp: Trabecular separation, Tb.Th: Trabecular thickness, TV: Total bone volume  
Values are means ± SD. \*P < 0.05 vs. SHAM group, †P < 0.05 vs. OVX group, ‡P < 0.05 vs. OVX-Up group, §P < 0.05 vs. OVX-Level group

**Table 5.** Trabecular bone morphometric parameter and vBMD in the proximal femur.

	SHAM	OVX-Cont	OVX-Up	OVX-Level	OVX-Down
CV (mm <sup>3</sup> )	6.70 ± 0.67	6.99 ± 0.29	7.41 ± 0.53	6.87 ± 0.44	6.99 ± 0.35
MV (mm <sup>3</sup> )	3.63 ± 0.34	3.99 ± 0.43	4.36 ± 0.63*	4.26 ± 0.23*	4.07 ± 0.53
Ct.Ar (mm <sup>2</sup> )	3.26 ± 0.33	3.40 ± 0.14	3.61 ± 0.26*	3.34 ± 0.21	3.40 ± 0.17
Ct.Th (mm <sup>2</sup> )	494.99 ± 35.50	496.16 ± 26.70	511.10 ± 36.13	479.94 ± 21.97	493.85 ± 29.58
Ps.Pm (µm)	8325.15 ± 325.58	8646.94 ± 185.80*	8950.32 ± 271.90*†	8697.17 ± 293.17*	8738.30 ± 279.97*
Ec.Pm (µm)	5216.04 ± 416.35	5472.50 ± 350.04	5558.40 ± 395.87	5619.00 ± 355.77	5449.00 ± 347.15

Ct.Ar: Cortical bone sectional area, Ct.Th: Cortical thickness, CV: Cortical bone volume, Ec.Pm: Endocortical perimeters, Ps.Pm: Periosteal perimeter  
Values are means ± SD. \*P < 0.05 vs. SHAM group, †P < 0.05 vs. OVX group, ‡P < 0.05 vs. OVX-Up group,

**Table 6.** Cortical bone morphometric parameter.

upslope and level surface running, downslope running had no benefits on OVX-induced bone degeneration, as there was no significant difference in any bone morphometric parameter between OVX-Cont and OVX-Down groups ( $P>0.05$ ), while these parameters did differ significantly in the OVX-Down group compared to OVX-Up and OVX-Level groups ( $P<0.05$ ). Ovariectomy significantly decreased vBMD, an effect reversed in tibial trabecular bone by training ( $P<0.05$ ). Indeed, vBMD of the OVX-Up group increased to approximately the level of the SHAM group.

*Cortical bone morphometric parameters in the tibia (Table 3).* Ovariectomy had no influence on the mid-tibial cortical bone compared to SHAM, but exercise increased Ps.Pm and Ec.Pm ( $P<0.05$ ). All cortical bone morphometric parameters except Ct.Th were higher in the OVX-Up group than in the OVX-Cont group ( $P<0.05$ ).

*Trabecular bone morphometric parameters and vBMD in the distal femur (Table 4).* Ovariectomy reduced BV/TV, Tb.Th, Tb.N, Conn.D, and vBMD, and increased Tb.Sp and in distal femur compared to the SHAM group ( $P<0.05$ ). The BV/TV, Tb.Th, Tb.N, and Conn.D were all significantly higher in OVX-Up and OVX-Level groups than in the OVX-Cont group ( $P<0.05$ ). The Tb.Sp and were significantly lower in the OVX-Up than in the OVX-Cont group ( $P<0.05$ ). BV/TV, Tb.Th, Tb.N, and vBMD were significantly higher in the OVX-Up group than in the OVX-Cont group ( $P<0.01$ ) and in the OVX-Level group than in the OVX-Cont group ( $P<0.05$ ). BMC was significantly higher in the OVX-Up group compared to the OVX-Cont group ( $P<0.05$ ).

*Trabecular bone morphometric parameters and BMD in the proximal femur (Table 5).* The BV/TV, Tb.Th, Tb.N, and Conn.D were reduced and Tb.Sp increased in proximal femur by ovariectomy ( $P<0.05$ ). The BV/TV, Tb.N, and vBMD were significantly higher in OVX-Up and OVX-Level groups than in the OVX-Cont group ( $P<0.05$ ), and Tb.Sp and SMI were significantly lower in OVX-Up and OVX-Level groups than in the OVX-Cont group ( $P<0.05$ ).

*Cortical bone morphometric parameter in the femur (Table 6).* No morphometric parameter of the femur except for Ps.Pm was affected by OVX ( $P>0.05$ ). Ct.Ar significantly increased in the OVX-Up group compared to that in the SHAM group ( $P<0.05$ ). Ps.Pm was significantly higher in the OVX-Up group than in the OVX-Cont group ( $P<0.05$ ).

## Discussion

We report that the mineral density of tibial trabecular bone was maintained by treadmill running following ovariectomy in rats, consistent with many studies showing that exercise regimens can prevent or reverse bone loss from estrogen deficiency in model animals<sup>24,25</sup>. Our major finding; however, is that treadmill running with an upward slope was significantly more effective for inhibiting the loss of trabecular bone volume in the proximal tibia and distal femur after ovariectomy than level surface running. While

level running inhibited the decrease in trabecular thickness induced by OVX, upward slope running also enhanced tibial cortical bone volume and inhibited both the decreases in trabecular number and bone connectivity and the increase in trabecular spacing. In contrast, downward slope running had little effect on bone loss in either tibia or femur. Adding an upward incline to treadmill regimens may provide additional benefits to those at risk for osteoporosis.

Numerous forms of training, including treadmill running, have been reported to improve bone strength in OVX rats. However, the benefits differ according to training intensity, duration, period, frequency, and other exercise parameters<sup>2,14,26</sup>. Peng et al. reported that slow running (10 m/minute) was more effective than faster running (18 m/minute) for reversing bone degeneration in OVX rats. Iwamoto et al. studied three different running protocols, 12 m/minute for 1 h/day, 18 m/minute for 1 h/day, and 12 m/minute for 2 h/day, and found that only the first protocol was effective in OVX rats<sup>27</sup>. Therefore, the beneficial effects of treadmill running require the optimal level of exercise, such as provided by use of an incline. Although exercise condition of previous studies using inclined treadmill were referred, different effects according to training intensity, duration, period, and frequency need to be investigated in future studies.

The principal factor driving increased bone strength with exercise is mechanical stress<sup>28</sup>. For instance, jumping training places strong mechanical stress on bones. Notomi et al. reported that jumping training increased bone compressive load, mass, BV/TV, Tb.Th, and Tb.Sp<sup>29</sup>. In another study, jump training was more effective than treadmill running for improving compressive load, BV/TV, and Tb.Th, and the effects were not limited by age<sup>30</sup>. A daily 10-jump program for 8 weeks also increased tibial bone mass and strength, as well as cortical area, periosteal perimeter, and moment of inertia at the tibia mid-shaft, but not endosteal perimeter at tibia mid-shaft in OVX rats<sup>16,31</sup>. Thus, training with larger mechanical stress on bones, such as jumping training, is more effective for enhancing parameters related to bone strength. In the present study, greater inhibition of tibia and femur bone loss with upward running angle may also be due to higher mechanical stress. Robert et al. recorded the ground reaction force on the hindlimb while cats walked on a walkway under three conditions: downslope (26.6°), level (0°), and upslope (26.6°). Peak ground reaction forces, representing the peak load on the hindlimb, were highest for the upslope condition, while downslope walking resulted in significantly lower normal force than level walking<sup>17</sup>.

Upslope running inhibited the OVX-induced decrease in trabecular number, thickness, and bone connectivity, as well as the increase in trabecular spacing and the shift to rod-shaped trabecular geometry. It was reported that jumping training reversed the deterioration of trabecular architecture in the distal femur after tail suspension<sup>5</sup>. In the proximal tibia and distal femoral trabecular bone, upslope running may have an effect similar to higher impact training such as jumping in OVX rats<sup>16</sup>. Upslope running increased

cortical bone volume, average cortical area, and endosteal and periosteal perimeters in the tibia by promoting endosteal expansion and periosteal resorption. High-impact training has also been reported to increase cortical area<sup>6,31</sup>. Thus, we demonstrate that upslope running in OVX rats has benefits on tibial trabecular and cortical bone comparable to higher impact regimens. In contrast, downslope running had minimal effects on morphometric and microarchitectural parameters in the tibia and femur. It was reported to increase trabecular BMD but not cortical BMD compared to the control group, and failed to alter trabecular bone morphological parameters in the femoral epiphysis. Our data is consistent with this previous study. Downslope running induces more eccentric contraction of muscle than level or upslope running<sup>32</sup>. Hubal et al. reported that although eccentric training improved bone stiffness in OVX mice, bone geometry was not improved<sup>33</sup>. Thus, it is unclear if eccentric exercise can facilitate bone recovery following OVX.

Treadmill running inhibited the decrease in trabecular BMD induced by OVX, consistent with many previous studies<sup>18</sup>. Upslope running best maintained trabecular BMD of the three slopes tested. Running exercise was found to increase trabecular BMD more than swimming but less than jumping exercise in rats<sup>34,35</sup>, again suggesting that the magnitude of the mechanical load determines the degree of BMD maintenance following OVX.

The influence of slope differed between the tibia and femur, which is consistent with several previous studies. Exercise had a greater effect on tibia ash weight than on femur ash weight in OVX rats<sup>36</sup>. Voluntary running significantly increased BMD of the tibia and proximal femur but not of the distal femur<sup>37</sup>. Iwamoto et al. suggested that the response of cancellous bone to treadmill exercise may be greater in distal than in proximal sites of the tibia due to differential biomechanical loading<sup>15</sup>. Each running slope angle is associated with a unique load distribution and so may uniquely influence the bone at different sites.

There are several methodological limitations of this study. First, we studied young adult rats, and the increase in body weight during this developmental period may mask the influence of OVX. Additionally, age-related changes in femur cortical bone geometry to compensate for age-related degeneration could also mask changes due to the intervention<sup>38</sup>.

In this study, the tibial and femoral length in OVX rats increased. Similar phenomenon has been reported in OVX rats in previous research, i.e., an increased rate of bone length and longitudinal bone growth occur temporally during the early stages of estrogen deficiency<sup>39,40</sup>. Clinical picture in male patients with the ER-mutation or with aromatase deficiency indicated that estrogen is also essential for proper fusion of the growth plate<sup>41-43</sup>. We believe that estrogen deficiency inhibited the fusion of the growth plate to use young adult rat. In future studies, age of the rats should also be considered. This study focused solely on bone geometry, so further research is needed to investigate bone stiffness and bone cell activities. Finally, we detected a few statistical

trends that would have likely reached significance with a greater number of animals per group.

In conclusion, we demonstrate that of upslope, downslope, and level treadmill running angle, upslope running was the most effective for inhibition of bone loss and for maintaining bone microarchitecture and mineral density, particularly in the proximal tibia, in OVX rats. The present research suggests that upslope training may enhance the benefits of treadmill exercise for prevention of bone loss due to an estrogen deficit.

#### Ethical approval

*The experimental procedures were performed according to the guidelines for the care and use of laboratory animals approved by the Animal Ethics Committee of Kio University, Japan. This article does not contain any studies with human participants performed by any of the authors.*

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