JOURNAL OF BONE AND MINERAL RESEARCH
Volume 16, Number 1, 2001

Effects of Tower Climbing Exercise on Bone Mass, Strength, and Turnover in Growing Rats

TAKUYA NOTOMI,[1] NOBUKAZU OKIMOTO,[2] YUICHI OKAZAKI,[2] YURI TANAKA,[1] TOSHITAKA NAKAMURA,[2] and MASASHIGE SUZUKI[1]

ABSTRACT

To determine the effects of tower climbing exercise on mass, strength, and local turnover of bone, 50 Sprague–Dawley rats, 10 weeks of age, were assigned to five groups: a baseline control and two groups of sedentary and exercise rats. Rats voluntarily climbed the 200-cm tower to drink water from the bottle set at the top of it. In 4 weeks, the trabecular bone formation rate (BFR/bone surface [BS]), bone volume (BV/TV), and trabecular thickness (Tb.Th) of both the lumbar vertebra and tibia and the bone mineral density (BMD) of the tibia increased, while the osteoclast surface (Oc.S) decreased. The parameter values in the midfemur, such as the total cross-sectional area, the moment of inertia, the periosteal mineralizing surface (MS/BS), mineral apposition rate (MAR), BFR/BS, and bending load increased, while the endosteal MAR decreased. In 8 weeks, the increases in the bone mineral content (BMC), BMD of the femur and tibia, and the bending load values of the femur were significant, but the climbing exercise did not increase BMC, BMD, or the compression load of the lumbar vertebra. Although the periosteal MS/BS, MAR, and BFR/BS increased, the endosteal MS/BS, MAR, and BFR/BS decreased. These results show that climbing exercise has a beneficial effect on the femoral cortex and tibia trabecular, rather than the vertebral trabecular. In the midfemur, effects on bone formation are site specific, supporting accelerated cortical drift by mechanical stimulation. (J Bone Miner Res 2001;16:166–174)

Key words: climbing, voluntarily exercise, bone formation, osteoclast, cortical drift

INTRODUCTION

A WIDELY accepted rationale for the positive effects of exercise on bone is that mechanical strain plays an important role in the maintenance of bone mass and strength,[1,2] and a variety of exercise interventions have been successful in increasing bone mineral density (BMD).[3,4] Cross-sectional data in a human study suggested that resistance-trained athletes have greater bone mass than endurance-trained athletes or sedentary control subjects.[5–7] However, the mechanisms by which exercise leads to changes in bone metabolism and morphology are not fully understood.

In rodent studies, aerobic exercises, including running and swimming on a nonvoluntary basis, were applied to study the effects of exercise on bone morphology and metabolism[8–11] but rarely was resistance training applied.[12–14] These studies examined the effects of resistance exercise induced by electric stimulation on the floor of a metal cage and found an increase in the femoral cortical bone strength[12] and trabecular bone mass of the proximal tibia.[13] The effects of resistance exercise on local bone turnover and mechanical properties of bone have yet to be studied in detail.[12,13] The rat training models do not seem to exclude the effects from electrical stimulus and/or a nonvoluntary training regimen.[8–14]

[1]Laboratory and Biochemistry of Exercise and Nutrition, Institute of Health and Sport Sciences, University of Tsukuba, Tsukuba, Japan.
[2]Department of Orthopedic Surgery, University of Occupational and Environmental Health, Kitakyushu, Japan.

We modified the resistance exercise models, such as the rat climbing reported by Yarasheski et al.[15] and Duncan et al.,[16] who estimated skeletal muscle. In our model, rats voluntarily climbed the 200-cm tower to drink water from a bottle set at the top of it. The purpose of this study was to find out the effects of voluntary resistance exercise on the mass, strength, and local turnover of bone in growing rats.

MATERIALS AND METHODS

Animals

Male Sprague–Dawley rats were purchased from Japan CLEA, Inc. (Tokyo, Japan) and were acclimatized for 1 week under standard laboratory conditions (22 ± 2°C, 60% humidity). The light/dark cycle was 12 h with lights on from 6:00 a.m. to 6:00 p.m. All rats were housed in metal cages. Drinking water was available at all times. The amount of food taken was equalized among the groups. All rats were fed commercial rat chow (Japan CLEA; calcium, 1200 mg/100 g; phosphorus, 1080 mg/100 g). The body weight of the rats was measured weekly. All rats remained healthy. The protocol was approved by The University of Tsukuba's Institutional Animal Care and Use Committee.

Fifty rats, 10 weeks of age, were randomized by body weight to five groups. Group C was the baseline control, killed at the start. Groups 4S and 4E were the sedentary and the exercise groups, respectively, killed after 4 weeks of the experiment. Groups 8S and 8E were killed after 8 weeks. Mean body weight at 10 weeks of age in groups C, 4S, 4E, 8S, and 8E was 249 ± 7 g, 250 ± 3 g, 252 ± 3 g, 256 ± 4 g, and 255 ± 3 g, respectively. No significant differences were found among these values (Tukey's honestly significant difference after one-way analysis of variance [ANOVA]). In the preliminary experiment, 20 male Sprague–Dawley rats, 10 weeks of age, were assigned randomly to the sedentary and exercise groups by body weight. At 4 weeks, body weight in the sedentary and exercise groups was 340 ± 3 g and 337 ± 4 g, respectively, and food intake during the 4 weeks was 972 ± 24 g and 964 ± 31 g, respectively. No significant differences in body weight and food intake values between the sedentary and exercise groups were found (Student's *t*-test after *f* test). To avoid any difference in food intake, the sedentary and exercise groups were given the same amount of food, using the same methods of feeding as in our previous reports.[17,18] We measured the minimum amount of food consumed by the groups, and the amount of food was then reduced to the minimum amount on the next day. If the food was consumed completely, the amount of food was increased by 4–6 g/day. Therefore, at 4 weeks and 8 weeks, the amount of food consumed by the sedentary and exercise groups was equal.

At the end of the experiments, rats were killed by exsanguination under ether anesthesia. Soon after the death, the hindlimb muscles (gastrocnemius, plantaris, soleus, tibia, and extensor digitorum longus) and abdominal fats (epididymal fat, perigastric fat, mesenteric fat, and abdominal subcutaneous fat) were isolated. The combined weight of the hindlimb muscles and abdominal fats was measured. The third, fourth, and fifth lumbar vertebra (L3, L4, and L5) and bilateral tibias and femora were harvested. The L3, L4, right femur (RF), and bilateral tibias were fixed immediately with 4% paraformaldehyde in a 0.1 M phosphate buffer containing 2% sucrose and stored at 4°C. L5 and the left femur (LF) were stored at −80°C until the mechanical tests. Bone labeling of rats with a subcutaneous injection of calcein (8 mg/kg body weight) was performed 7 days and 3 days before death.

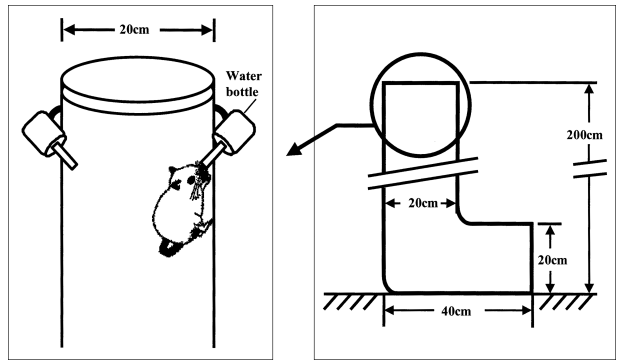

FIG. 1. Diagram illustrating the resistance exercise apparatus.

Resistance exercise

The groups of exercise rats were housed in metal cages with a wire meshed tower with two water bottles set at the top (Fig. 1). At the beginning, the bottles were set at a height of 20 cm. The set point of the drink bottles was elevated gradually to 200 cm over a week. The rats were monitored for 24 h/day every 2 weeks during the experimental period, using a charge-coupled device (CCD) camera (CCD-TRV95; Sony, Tokyo, Japan). The daily distances and times of climbing activity were obtained from the monitoring records.

Bone mineral measurement

BMD (mg/cm^2) and bone mineral content (BMC; mg) were measured on the LF and right tibia (RT) using dual-energy X-ray absorptiometry (DXA; DCS-3000; Aloka, Tokyo, Japan). The L5 body was prepared by removing the posterior segment and bone mineral measurements were performed. The mineralization profiles of the specimens were stored with the monitoring images, and the BMD and BMC values for the lumbar body, the femur mid-diaphyseal region (12 mm in length), and the tibia proximal region (12 mm in length) were obtained.

Mechanical testing

Femur: A three-point bending test was performed as previously described[19,20] using a load tester (Tensilon UTA-1T; Orientec, Tokyo, Japan). The LF specimen was placed on a holding device with supports located at a

distance of 12 mm, with the lesser trochanter proximal to and in contact with the proximal transverse bar. The midpoint served as the anterior (upper) loading point. A bending force was applied by the crosshead at a speed of 10 mm/minute until fracture occurred. The maximum load (N) and structural stiffness (N/mm) were obtained directly from the load-deformation curves that were recorded continually in the computerized monitor linked to the load tester.

Lumbar vertebral body: The L5 body specimen was fixed with a clamp at the bases of the transverse processes in the holder of a diamond band saw (Exakt, Norderstedt, Germany). By removing the cranial and caudal ends of the specimens, the planoparallel ends at a height of 3.5 mm were obtained.[20] The cylinder samples were placed centrally on the smooth surface of a steel disk attached to the load tester. A craniocaudal compression force was applied to the specimen via a steel disk at a nominal deformation rate of 2 mm/minute. The maximum load (N) and structural stiffness (N/mm) were obtained, as were those of the femur specimens.

Bone histomorphometry

Lumbar vertebral body and proximal tibia: The L4 and RT specimens were embedded in methylmethacrylate (MMA) after Villanueva's bone staining. From the middle portion of the specimen, 10-μm-thick undecalcified sagittal sections were cut on a microtome (Reichert Jung Supercut 2050; Reichert Jung, Heidelberg, Germany). The L3 and left tibia (LT) specimens were embedded in a mixture of MMA, hydroxyglycol methacrylate, and 2-hydroxyethylacrylate polymerized at 4°C. Six-μm-thick specimens of the L3 and LT were obtained as described for those of L4 and RT. The L3 and LT sections were then stained for tartrate-resistant acid phosphatase (TRAP).[21] Histomorphometry of L3, L4, and bilateral tibia specimens was performed with a semiautomatic image-analyzing system linked to a light microscope (Cosmozone 1S; Nikon, Tokyo, Japan). For each section, the area of the secondary spongiosa was measured, but the region within 1.0 mm of the growth plate/metaphyseal junction and one cortical shell width of the endocortical surface was not measured, to exclude the primary spongiosa.

For the structure parameters, the trabecular bone volume (BV; μm^2), trabecular tissue volume (TV; μm^2), and trabecular bone surface (BS; μm) were measured. The trabecular thickness (Tb.Th; μm), trabecular number (Tb.N; 1/mm), and trabecular bone separation (Tb.Sp; μm) were calculated by a parallel plate model assuming constant geometry.[22,23] For the bone formation parameters of L4 and RT, the single-labeled surface (sLS; μm), double-labeled surface (dLS; μm), trabecular bone surface (B.S: μm), and the mean distance between double labels (Ir.L.th; μm) were measured. The mineral apposition rate (MAR; μm/day) was calculated as the Ir.L.th multiplied by $\pi/4$. The mineralizing surface per BS (MS/BS; %) was obtained by adding the values of the dLS/BS and half of the sLS/BS value. The surface referent bone formation rate (BFR/BS; μm%/day), was calculated by multiplying the MS/BS value by the MAR.[22–24] For the bone resorption parameters of L3 and LT, the osteoclast surface (Oc.S; μm) and trabecular BS (μm) were measured.[22–24] TRAP-positive cells that formed resorption lacunae at the surface of the trabeculae and contained one or more nuclei were identified as osteoclasts.[25]

Femoral midshaft: An undecalcified section was obtained from the site of the middiaphysis of the RF. The specimen was embedded in MMA without staining to yield a 40-μm-thick crosscut ground section. Measurements were made on a cathode-ray tube monitor with a CCD camera (CCD High Gain Camera-1600A; Flovel Co., Tokyo, Japan) setting on the microscope using a semiautomatic image-analyzing program (Mac Scope; Mitani Corp., Fukui, Japan) on a computer.[20] The total cross-sectional area (mm^2), cortical bone area (mm^2), and bone marrow area (mm^2) were obtained. The endocortical surface was approximated as a regular, continuous line. The moment of inertia (mm^4) of the cortical bone area for the medial-lateral axis was calculated directly by the image-analyzing computer.[20] Kinetic parameters such as dLS/BS, sLS/BS, MS/BS, MAR, and BFR/BS were measured in the periosteal and endocortical envelopes. The eroded surface (ES/BS; %) also was measured in the endocortical perimeter.

Statistical analysis

All values are expressed as mean and SEM. One-way ANOVA with repeated measurement was used to examine the significance of changes in the climbing distances and time. Data were assessed by two-way ANOVA for the time and treatment. When the treatment was found to have a significant overall effect, the difference between the exercise and sedentary groups was assessed by Student's *t*-test for each time period.[14,26] The analysis of covariance (ANCOVA) was done to examine the significance of changes in bone mass and strength, using the hindlimb muscles or the abdominal adipose tissue values as covariate. Statistical significance was set at less than 0.05. All the analyses were performed with a commercially available statistical package (SPSS Ver 9.0; SPSS, Tokyo, Japan).

RESULTS

Climbing distances and time periods

The daily climbing distances in the exercise group after 2, 4, 6, and 8 weeks were 141 $\pm$ 1.6 m/day, 148 $\pm$ 2.6 m/day, 151 $\pm$ 3.9 m/day, and 137 $\pm$ 2.4 m/day, respectively. The respective time periods for the activity were 26.2 $\pm$ 1.2 minutes/day, 25.3 $\pm$ 0.2 minutes/day, 26.5 $\pm$ 0.9 minutes/day, and 24.5 $\pm$ 0.8 minutes/day. No significant differences were found among these values.

Body weight, hindlimb muscles, abdominal fats, and bone mineral measurements

Body weights did not significantly differ between the exercise and sedentary groups throughout the experimental period (Table 1). The hindlimb muscles value in the exercise group was significantly higher than that in the sedentary

TABLE 1. EXERCISE TIME, BODY CONDITION, AND BONE MASS OF THE MIDFEMUR, THE LUMBAR VERTEBRA, AND THE PROXIMAL TIBIA OF SEDENTARY AND EXERCISED RATS

						Midfemur		*Lumbar vertebra*		*Proximal tibia*	
Group	*Age of rats (weeks)*	*Exercise time (minutes/day)*	*Body weight (g)*	*Hindlimb muscles (mg)*	*Abdominal fats (mg)*	*BMC (mg)*	*BMD (mg/cm^2)*	*BMC (mg)*	*BMD (mg/cm^2)*	*BMC (mg)*	*BMD (mg/cm^2)*
C. Control	10		249 ± 7	4.92 ± 0.08	9.8 ± 0.9	79.9 ± 1.4	119.5 ± 1.6	21.7 ± 0.4	68.1 ± 1.8	70.5 ± 1.0	96.8 ± 1.0
4S. Sed (4 weeks)	14		327 ± 9	6.14 ± 0.09	21.0 ± 1.4	104.6 ± 1.6	156.6 ± 2.8	32.5 ± 1.4	90.6 ± 1.3	101.6 ± 2.4	138.7 ± 2.7
4E. Ex (4 weeks)		25.0 ± 0.4	332 ± 5	6.54 ± 0.09*	14.1 ± 1.2**	115.0 ± 7.8	161.1 ± 3.0	35.6 ± 1.4	94.7 ± 2.7	105.3 ± 1.4	148.6 ± 2.3*
8S. Sed (8 weeks)	18		380 ± 14	6.91 ± 0.13	32.0 ± 2.0	107.1 ± 1.3	162.7 ± 3.7	38.0 ± 2.1	97.2 ± 1.6	115.4 ± 1.2	160.6 ± 1.3
8E. Ex (8 weeks)		24.5 ± 0.8	364 ± 14	7.04 ± 0.10	21.3 ± 1.6**	117.9 ± 2.5**	177.0 ± 1.4**	40.0 ± 1.4	99.7 ± 1.8	124.3 ± 1.7**	171.8 ± 1.8**
Two-way ANOVA			NS	<0.05	<0.01	<0.05	<0.01	NS	NS	<0.01	<0.01

Exercise time in each of the groups; body weight at time of death.
Values are mean ± SEM.
*$p < 0.05$; **$p < 0.01$; significantly different from the corresponding sedentary group (Student's *t*-test after two-way ANOVA).

group after 4 weeks but not after 8 weeks. The abdominal fats value in the exercise group was lower than those in the sedentary group after both 4 weeks and 8 weeks. Except for the BMD value of the proximal tibia after 4 weeks, no difference was found between the two groups for these parameters. However, after 8 weeks the BMC and BMD values of the midfemur and the proximal tibia in the exercise group were significantly higher than the values in the sedentary group. After 4 weeks or 8 weeks, the parameters of BMC and BMD in the lumbar vertebra did not significantly differ between the exercise and sedentary groups. The test of regression in ANCOVA showed no significance in all the BMC or BMD values, using the hindlimb muscles or abdominal fats values as covariate.

Mechanical properties of the midfemur and lumbar vertebra, and the geometry of the femoral cortex

After 4 weeks, the values of the maximum load of the midfemur, total cross-sectional area, and moment of inertia in the exercise group were significantly higher than those in the sedentary group (Table 2). After 8 weeks, the maximum load and structural stiffness of the midfemur and total cross-sectional area, cortical bone area, and moment of inertia values in the exercise group were higher than those in the sedentary group. The maximum load or structural stiffness values of the lumbar vertebra did not significantly differ throughout the experimental period. The test of regression in ANCOVA showed no significance in all the maximum load or structural stiffness values, using the hindlimb muscles or abdominal fats values as covariate.

Kinetic parameters of the periosteal and endosteal surfaces

Periosteal surface: After both 4 weeks and 8 weeks, the values of MS/BS, MAR, and BFR/BS in the exercise group were significantly higher than those in the sedentary group (Table 3).

Endosteal surface: After 4 weeks, the MAR values in the exercise group were significantly lower in the sedentary group. However, after 8 weeks the MS/BS, MAR, and BFR/BS values in the exercise group were significantly lower than the values in the sedentary group. The ES/BS values did not significantly differ throughout the experimental period.

Structural and kinetic parameters of the lumbar vertebra

Structural indices: After both 4 weeks and 8 weeks, the parameters of BV/TV and Tb.Th in the exercise group were significantly higher than those in the sedentary group (Table 4). The Tb.N or Tb.Sp values did not significantly differ in either period.

Bone formation and resorption: After 4 weeks, the value of BFR/BS in the exercise group was significantly higher and the Oc.S/BS value was significantly lower than those in the sedentary groups. However, after 8 weeks the values of MS/BS, MAR, and BFR/BS in the exercise groups were

TABLE 2. MECHANICAL PARAMETERS OF THE MIDFEMUR AND LUMBAR VERTEBRA AND MORPHOLOGY OF THE MIDFEMUR OF SEDENTARY AND EXERCISED RATS

		Midfemur		*Lumbar vertebra*		*Cross-sectional morphology*			
Group	*Age of rats (weeks)*	*Maximum load (N)*	*Structural stiffness (N/mm)*	*Maximum load (N)*	*Structural stiffness (N/mm)*	*Total cross-sectional area (mm^2)*	*Cortical bone area (mm^2)*	*Bone marrow area (mm^2)*	*Moment of Inertia (mm^4)*
C. Control	10	172 ± 6	388 ± 13	174 ± 8	2607 ± 153	10.1 ± 0.1	4.7 ± 0.1	5.4 ± 0.2	4.6 ± 0.1
4S. Sed (4 weeks)	14	236 ± 4	590 ± 19	311 ± 20	4187 ± 287	11.3 ± 0.2	5.8 ± 0.1	5.5 ± 0.2	5.8 ± 0.2
4E. Ex (4 weeks)		277 ± 9**	630 ± 27	323 ± 23	4624 ± 247	12.0 ± 0.2*	6.1 ± 0.1	5.7 ± 0.1	6.9 ± 0.2**
8S. Sed (8 weeks)	18	255 ± 7	748 ± 20	386 ± 14	5488 ± 147	11.1 ± 0.2	6.0 ± 0.1	5.2 ± 0.1	5.9 ± 0.2
8E. Ex (8 weeks)		327 ± 12**	817 ± 22*	405 ± 11	5686 ± 161	12.2 ± 0.2**	6.6 ± 0.1**	5.6 ± 0.2	7.1 ± 0.2**
Two-way ANOVA		<0.01	<0.05	NS	NS	<0.01	<0.01	NS	<0.01

Values are mean ± SEM.
*$p < 0.05$; **$p < 0.01$; significantly different from the corresponding sedentary group (Student's *t*-test after two-way ANOVA).

TABLE 3. KINETIC PARAMETERS OF THE MIDFEMORAL PERIOSTEAL AND ENDOCORTICAL SURFACE OF THE SEDENTARY AND EXERCISED RATS

		Periosteal			*Endocortical*			
Group	*Age of rats (weeks)*	*MS/BS (%)*	*MAR (μm/day)*	*BFR/BS (μm-%/day)*	*MS/BS (%)*	*MAR (μm/day)*	*BFR/BS (μm-%/day)*	*ES/BS (%)*
C. Control	10	60.0 ± 0.8	2.80 ± 0.02	167.7 ± 1.3	87.3 ± 1.5	3.11 ± 0.09	273.1 ± 13.3	13.2 ± 0.3
4S. Sed (4 weeks)	14	47.9 ± 0.8	1.89 ± 0.02	90.3 ± 1.5	77.2 ± 4.8	2.63 ± 0.12	206.8 ± 14.4	10.7 ± 0.6
4E. Ex (4 weeks)		59.3 ± 2.9**	2.17 ± 0.04**	129.2 ± 8.5**	78.1 ± 3.4	2.27 ± 0.06*	178.0 ± 9.6	10.8 ± 0.9
8S. Sed (8 weeks)	18	47.7 ± 3.9	1.68 ± 0.06	81.2 ± 9.8	67.7 ± 2.3	2.29 ± 0.02	154.8 ± 6.2	10.1 ± 0.3
8E. Ex (8 weeks)		59.3 ± 3.6*	2.08 ± 0.02**	123.4 ± 7.9**	50.7 ± 2.1**	1.81 ± 0.05**	91.0 ± 5.6**	11.2 ± 0.7
Two-way ANOVA		<0.01	<0.01	<0.01	<0.05	<0.01	<0.01	NS

Values are mean ± SEM.
*$p < 0.05$; **$p < 0.01$; significantly different from the corresponding sedentary group (Student's *t*-test after two-way ANOVA).

TABLE 4. STRUCTURAL AND KINETIC PARAMETERS OF THE LUMBAR VERTEBRA OF SEDENTARY AND EXERCISED RATS

Group	Age of rats (weeks)	BV/TV (%)	Tb.Th (μm)	Tb.N (1/mm)	Tb.Sp (μm)	MS/BS (%)	MAR (μm/day)	BFR/BS (μm-%/day)	Oc.S/BS (%)
C. Control	10	22.2 ± 0.4	60.7 ± 0.4	3.65 ± 0.06	214.4 ± 4.8	34.1 ± 1.1	1.88 ± 0.04	64.1 ± 2.2	5.23 ± 0.15
4S. Sed (4 weeks)	14	24.8 ± 0.6	68.9 ± 1.4	3.61 ± 0.11	209.7 ± 7.4	24.0 ± 1.4	1.19 ± 0.04	28.7 ± 2.2	3.42 ± 0.10
4E. Ex (4 weeks)		27.8 ± 0.8**	73.6 ± 1.0*	3.79 ± 0.14	193.5 ± 9.5	27.4 ± 1.3	1.31 ± 0.06	36.3 ± 2.7*	3.00 ± 0.09**
8S. Sed (8 weeks)	18	29.8 ± 0.7	74.8 ± 0.5	3.98 ± 0.08	176.9 ± 5.4	18.8 ± 1.5	1.14 ± 0.03	21.3 ± 1.5	2.48 ± 0.13
8E. Ex (8 weeks)		33.5 ± 0.4**	83.4 ± 1.0**	4.03 ± 0.07	165.5 ± 3.6	22.7 ± 0.9*	1.31 ± 0.05*	29.7 ± 1.5**	2.07 ± 0.09*
Two-way ANOVA		<0.01	<0.01	NS	NS	<0.05	<0.05	<0.05	<0.01

Values are mean ± SEM.
*$p < 0.05$; **$p < 0.01$; significantly different from the corresponding sedentary group (Student's *t*-test after two-way ANOVA).

significantly larger, and the Oc.S/BS values were significantly lower, than those in the sedentary groups.

Structural and kinetic parameters of the tibia

Structural indices: After both 4 weeks and 8 weeks, the parameters of BV/TV and Tb.Th in the exercise group were significantly higher than those in the sedentary group (Table 5). The Tb.N or Tb.Sp values did not significantly differ in either period.

Bone formation and resorption: Except for the MS/BS value after 4 weeks, the values of MS/BS, MAR, and BFR/BS in the exercise groups were significantly higher and the Oc.S/BS values were significantly lower, compared with those values in the sedentary groups after both 4 weeks and 8 weeks.

DISCUSSION

This study showed that voluntary climbing exercise for 24–27 minutes/day accelerates age-dependent increases in mass and strength of the femur and trabecular bone mass of the lumbar vertebra and tibia in growing rats. After 4 weeks, the parameters of trabecular and periosteal bone formation increased and those of trabecular osteoclasts decreased. Although the increases in the BMC and BMD values of the femur and tibia and the bending load values of the femur were significant, exercise did not significantly increase the BMC, BMD, or compressive load of the lumbar vertebra. Climbing exercise had a beneficial effect on femoral cortex and tibia trabecular, rather than the vertebral trabecular. In the femur, the parameters of cortical bone structure and bending load values both significantly increased after 4 weeks. The endocortical BSs were less sensitive to exercise.

It has been suggested that treadmill-running exercise increases trabecular bone formation and decreases resorption in the tibia.[27,28] Our present findings on the local turnovers of the lumbar vertebra and tibia are consistent with these findings. Because the parameters of osteoclasts were reduced, resistance exercise seems to increase bone mass by both increasing bone formation and reducing bone resorption in the trabecular bone. However, in the femoral cortex, the ES at the endocortical surface did not change after exercise. Thus, the effects of climbing exercise on the cortical envelopes were mainly on periosteal bone formation. Because the cortical bone structure and the bending load increased together after 4 weeks, the parameters of cortical bone structure seem to be sensitive enough to detect the morphological changes induced by climbing exercise in the early stages.

The climbing exercise increased the total cross-sectional area and cortical area of the midfemur. The data are consistent with previous reports on the effect of electrically stimulated jumping exercise on the femur in growing and mature rats.[12–14] In mature rats, there was a trend toward a greater value in both the total cross-sectional area and the cortical area in the exercise group, with a significant increase in osteocalcin messenger RNA (mRNA) content in the bone matrix.[13] Thus, resistance exercises such as jump-

TABLE 5. STRUCTURAL AND KINETIC PARAMETERS OF THE TIBIA OF SEDENTARY AND EXERCISED RATS

Group	Age of rats (weeks)	BV/TV (%)	Tb.Th (μm)	Tb.N (1/mm)	Tb.Sp (μm)	MS/BS (%)	MAR (μm/day)	BFR/BS (μm-%/day)	Oc.S/BS (%)
C. Control	10	7.9 ± 0.3	37.1 ± 0.7	2.14 ± 0.09	446.5 ± 19.2	37.5 ± 0.8	1.93 ± 0.03	72.3 ± 1.8	8.73 ± 0.14
4S. Sed (4 weeks)	14	10.9 ± 0.5	48.0 ± 1.0	2.27 ± 0.08	397.5 ± 15.8	26.3 ± 1.0	1.39 ± 0.03	36.5 ± 1.5	5.84 ± 0.23
4E. Ex (4 weeks)		12.5 ± 0.3*	54.4 ± 0.8**	2.31 ± 0.06	381.4 ± 11.7	28.3 ± 1.1	1.80 ± 0.03**	50.8 ± 2.0**	4.67 ± 0.14**
8S. Sed (8 weeks)	18	14.4 ± 0.4	49.8 ± 0.3	2.88 ± 0.07	299.4 ± 9.7	21.2 ± 1.0	1.33 ± 0.05	27.9 ± 1.1	3.54 ± 0.20
8E. Ex (8 weeks)		17.0 ± 0.4**	60.8 ± 0.9**	2.81 ± 0.09	298.4 ± 11.1	25.8 ± 1.1*	1.46 ± 0.04*	37.6 ± 1.5**	2.94 ± 0.15**
Two-way ANOVA		<0.01	<0.01	NS	NS	<0.05	<0.01	<0.01	<0.01

Values are mean ± SEM.
*$p < 0.05$; **$p < 0.01$; significantly different from the corresponding sedentary group (Student's *t*-test after two-way ANOVA).

ing and climbing seem to be potent for stimulating periosteal bone formation. In our experiment, although neither the femoral BMC nor the BMD significantly increased, the parameters of total cross-sectional area, moment of inertia, and bending load increased after 4 weeks. These observations are in agreement with previous observations that the biomechanical properties of the femur were improved despite a lack of increase in BMC.[14,29,30]

The BMC, BMD, and compressive load values of the lumbar vertebra did not seem to be caused by the trabecular bone mass and structure, because the parameters of BV/TV, Tb.Th significantly increased after both 4 weeks and 8 weeks. The relationship between mechanical stress and structure is not a one to one simple correspondence.[31,32] The lumbar vertebrae are likely to receive less mechanical stress than the tibia during climbing exercise, because the BMD values of the proximal tibia were significantly increased after both 4 weeks and 8 weeks. Changes in the thickness of the cortical shell, which we did not measure, could be another possibility.[20]

Bone formation was augmented in the early stages of exercise in both the cortical and the trabecular bones, as was suggested in previous studies.[9,14,33] The resistance exercise significantly increased the parameters of periosteal bone formation in the midfemur. However, endocortical bone formation parameters decreased. Thus, the effects of climbing exercise on bone formation were site specific in the femoral cortex, supporting accelerated cortical drift by mechanical stimulation. It has been shown that treadmill-running exercise significantly increased the endocortical BFR of the tibial shaft.[9] Our findings are inconsistent with a previous report. One putative explanation is that different mechanical thresholds exist on cortical envelopes, as proposed by Frost.[1,2]

Concerning exercise and bone, most animal studies have used treadmill exercise on a nonvoluntary basis.[9–11,27,28,34] Some studies found a beneficial effect on femoral and tibial bone, but not vertebrae,[9,10] or a beneficial effect on those bones[34] with a training regimen including daily running of approximately 1.4–1.5 km for 12 weeks,[9] 1.0–1.1 km for 16 weeks,[10] and 2.0–2.1 km for 24 weeks.[34] Our voluntary exercise included the daily climbing of approximately 0.12–0.16 km for 4 weeks and 8 weeks and had beneficial effects on bone in growing rats. These data suggest that the effect of exercise on bone mass and strength does not depend on the distance or duration of the exercise, but may depend on the magnitude of the workload on bone while exercising. It also has been suggested that increased bone formation after exercise is not caused by simply the intensity and/or duration of the exercise, but rather to the change in the exercise level that is necessary to stimulate bone formation.[35] Therefore, the greater mechanical load induced by resistance exercise affected bone in this study, more than that of treadmill running.

Body weight did not differ between the sedentary and exercise groups. However, the lean body mass in the exercise group would be increased, because the exercise increased muscle mass and decreased abdominal adipose tissue. In this experiment, the test of regression in ANCOVA showed no differences in the bone mass and strength, using

muscle or fat mass as covariate. These results suggested that the increased bone mass and strength mainly were caused by the loading by the exercise, but not the secondary effects of an increase in muscle mass or lean body mass (LBM).

In human study, resistance training is more effective than aerobic training for increasing the bone mass.[5–7] Resistance training increases bone formation and decreases resorption in young males as assessed from serum osteocalcin and urinary deoxypyridinoline, as we previously reported.[6] The local turnover of the lumbar and femur in rat are consistent with these findings. Although the studies use different species, this exercise model has the potential to facilitate understanding of the role that resistance exercise might have in preventing bone loss and potentially to increase bone gain.

In conclusion, voluntary climbing exercise accelerated the radial cortical growth of the midfemur by stimulating the periosteal bone formation. The cortical bone formation induced by the climbing exercise seemed to strengthen the structure of the midfemur. The increase in trabecular bone mass of the lumbar vertebrae and tibia was caused by both increased bone formation and reduced bone resorption. The effect of the exercise was site specific on the cortical envelope in the midfemur, supporting accelerated cortical drift by mechanical stimulation.

ACKNOWLEDGMENTS

The authors thank Mr. Yushi Kato, KOKUYO Corp., Osaka, Japan, for help with making the resistance exercise apparatus and Dr. Tatsuhiro Matsuo, University of Kagawa, and Mr. Yasuhiro Mizushima, University of Tsukuba, for helpful discussion.

REFERENCES

1. Frost HM 1997 On our age-related bone loss: Insights from a new paradigm. J Bone Miner Res **12:**1539–1546.
2. Schissl H, Frost HM, Jee WSS 1998 Estrogen and bone-muscle strength and mass relationships. Bone **22:**1–6.
3. Davee AM, Rosen CJ, Adler RA 1990 Exercise patterns and trabecular bone density in college women. J Bone Miner Res **5:**245–250.
4. Menkes A, Mazel S, Redmond RA, Koffler K, Libanati CR, Gundberg CM, Zizic TM, Hagberg JM, Partley RE, Hurley BF 1993 Strength training increases regional bone mineral density and bone remodeling in middle-aged and older men. J Appl Physiol **74:**2478–2484.
5. Dalsky GP, Stocke KS, Ehsani AA, Slatopolsky E, Lee WC, Birge SJ 1988 Weight bearing training and bone mineral content in postmenopausal women. Ann Intern Med **108:**824–828.
6. Fujimura R, Ashizawa N, Watanabe M, Mukai N, Amagai H, Fukubayashi T, Hayashi K, Tokuyama K, Suzuki M 1997 Effect of resistance exercise training on bone formation and resorption in young male subjects assessed by biomarkers of bone metabolism. J Bone Miner Res **12:**656–662.
7. Heinrich CH, Going SB, Pamenter RW, Perry CD, Boyden TW, Lohman TG 1990 Bone mineral content of cyclically menstruating female resistance and endurance trained athletes. Med Sci Sports Exerc **22:**558–563.
8. Bourrin S, Ghaemmaghami F, Vico L, Chappard D, Gharib C, Alexandre C 1992 Effect of a five-week swimming program on rat bone: A histomorphometric study. Calcif Tissue Int **51:**137–142.
9. Iwamoto J, Yeh JK, Aloia JF 1999 Differential effect of treadmill exercise on three cancellous bone sites in the young growing rat. Bone **24:**163–169.
10. Yeh JK, Aloia JF, Chen M 1994 Growth hormone administration potentiates the effect of treadmill exercise on long bone formation but not vertebrae in middle-aged rats. Calcif Tissue Int **54:**38–43.
11. Bourrin S, Genty C, Palle S, Gharib C, Alexandre C 1994 Adverse effects of strenuous exercise: A densitometric and histomorphometric study in the rat. J Appl Physiol **76:**1999–2005.
12. Umemura Y, Toshihiro I, Takeshi Y, Masashi K, Shoji M 1997 Five jumps per day increase bone mass and breaking force in rats. J Bone Miner Res **12:**1480–1485.
13. Westerlind KC, Fluckey JD, Gordon ST, Kraemer WJ, Farrell PA, Turner RT 1998 Effect of resistance exercise training on cortical and cancellous bone in mature rats. J Appl Physiol **84:**459–464.
14. Notomi T, Lee SJ, Okimoto N, Okazaki Y, Takamoto T, Nakamura T, Suzuki M 2000 Effects of resistance exercise training on mass, strength, and turnover of bone in growing rats. Eur J Appl Physiol **82:**268–274.
15. Yarasheski KE, Lemon WR, Gilloteaux 1990 Effect of heavy-resistance exercise training on muscle fiber composition in young rats. J Appl Physiol **69:**434–437.
16. Duncan ND, Williams DA, Lynch GS 1998 Adaptation in rat skeletal muscle following long-term resistance exercise training. Eur J Appl Physiol **77:**372–378.
17. Matsuo T, Suzuki M 1997 Brain β-adrenergic receptor binding in rats with obesity induced by a beef tallow diet. Metabolism **46:**18–22.
18. Matsuo T, Sumida H, Suzuki M 1995 Effects of chemical sympathectomy on lipoprotein lipase activities in peripheral tissues of rats fed high fat diets consisting of different fats. J Nutr Sci Vitaminol **41:**377–386.
19. Tanaka Y, Nakamura T, Nishida S, Suzuki K, Takeda S, Sato K, Nishi Y 1996 Effects of a synthetic vitamin D analog, ED-71, on bone dynamics and strength in cancellous and cortical bone in prednisolone-treated rats. J Bone Miner Res **11:**325–336.
20. Ohnishi H, Nakamura T, Narusawa K, Murakami H, Abe M, Barbier A, Suzuki K 1997 Bisphosphonate tiludronate increases bone strength by improving mass and structure in established osteopenia after ovariectomy in rats. Bone **21:**335–343.
21. Tsurukami H, Nakamura T, Suzuki K, Sato K, Higuchi Y, Nishii Y 1994 A novel synthetic vitamin D analogue, 2β-(3-hydroxypropoxy) 1α,25-dihydroxyvitamin D_3 (ED-71), increases bone mass by stimulating the bone formation in normal and ovariectomized rats. Calcif Tissue Int **54:**142–149.
22. Parfitt AM, Drezner MK, Glorieux FH, Kanis JA, Malluche H, Muenier PJ, Ott SM, Recker RR 1987 Bone histomorphometry: Standardization of nomenclature, symbols and units. Report of the ASBMR Histomorphometry Nomenclature Committee. J Bone Miner Res **2:**595–610.
23. Parfitt AM, Mathews CH, Villanueva AR, Kleerkoper M, Frame B, Rao DS 1983 Relationship between surface, volume, and thickness of iliac trabecular bone in aging and in osteoporosis: Implications for the micro anatomic and cellular mechanisms for bone loss. J Clin Invest **72:**1396–1409.
24. Frost HM 1983 Bone histomorphometry: Analysis of trabecular bone dynamics. In: Recker RR (ed.) Bone Histomorphometry: Techniques and Interpretations. CRC Press Boca Raton, FL, USA, pp. 109–131.

25. Murakami H, Nakamura T, Tsurukami H, Abe M, Barbier A, Suzuki K 1994 Effect of tiludronate on bone mass, structure, and turn over at the epiphyseal, primary, and secondary spongiosa in the proximal tibia of growing rats after sciatic neurectomy. J Bone Miner Res **9:**1355–1364.
26. Samuels A, Perry MJ, Tobias JH 1999 High-dose estrogen induces de novo medullary bone formation in female mice. J Bone Miner Res **14:**178–186.
27. Bourrin S, Palle S, Pupier R, Vico L, Alexandre C 1995 Effects of physical exercise training on bone adaptation in three zone of rat tibia. J Bone Miner Res **10:**1745–1752.
28. Yeh JK, Aloia JF, Chen MM, Tierney JM, Sprintz S 1993 Influence of exercise on cancellous bone of the aged female rat. J Bone Miner Res **8:**1117–1125.
29. Barrengolts EI, Curry DJ, Bapna MS, Kukreja SC 1993 Effects of endurance exercise on bone mass and mechanical properties in intact and ovariectomized rats. J Bone Miner Res **8:**937–942.
30. Raab DM, Smith EL, Crenshaw TD, Thomas DP 1990 Bone mechanical properties after exercise training in young and old rats. J Appl Physiol **68:**130–134.
31. Landis WJ, Librizzi JJ, Dunn MG, Silver FH 1995 A study of the relationship between mineral content and mechanical properties of turkey gastrocnemius tendon. J Bone Miner Res **10:**859–867.
32. Mosekilde L, Danielsen CC, Sogaard CH, Mcosker JE, Wronski TJ 1995 The anabolic effects of parathyroid hormone on cortical bone mass, dimensions and strength-assessed in a sexually mature, ovariectomized rat model. Bone **16:**223–230.
33. Kristoffersson A, Hultdin J, Holmlund I, Thorsen K, Lorentzon R 1995 Effects of short-term maximal work-on plasma calcium, parathyroid hormone, osteocalcin and biochemical markers of collagen metabolism. Int J Sports Med **16:**145–149.
34. Mosekilede L, Danielsen CC, Sogaard CH, Thorling R 1994 The effect of long-term exercise on vertebral and femoral bone mass, dimension and strength-assessed in a rat model. Bone **15:**293–301.
35. Raab DM, Smith EL, Crenshaw TD, Thomas DP 1994 Bone response to alternate-day mechanical loading of the rat tibia. J Bone Miner Res **9:**203–211.

Address reprint requests to:
Masashige Suzuki
Institute of Health and Sports Sciences
University of Tsukuba
Tsukuba-shi
Ibaragi-ken 305-8574, Japan

Received in original form February 17, 2000; in revised form July 10, 2000; accepted August 22, 2000.