Effect of swimming exercise on three-dimensional trabecular bone microarchitecture in ovariectomized rats

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Ju YI, Sone T, Ohnaru K, Tanaka K, Fukunaga M. Effect of swimming exercise on three-dimensional trabecular bone microarchitecture in ovariectomized rats. J Appl Physiol 119: 990–997, 2015. —Swimming is generally considered ineffective for increasing bone mass in humans, at least compared with weight-bearing sports. However, swimming exercise has sometimes been shown to have a strong positive effect on bone mass in small animals. This study investigated the effects of swimming on bone mass, strength, and microarchitecture in ovariectomized (OVX) rats. OVX or sham operations were performed on 18-wk-old female Fisher 344 rats. Rats were randomly divided into four groups: sham sedentary (Sham-CON), sham swimming exercised (Sham-SWI), OVX sedentary (OVX-CON), and OVX swimming exercised (OVX-SWI). Rats in exercise groups performed swimming in a water bath for 60 min/day, 5 days/wk, for 12 wk. Bone mineral density (BMD) in right femurs was analyzed using dual-energy X-ray absorptiometry. Three-dimensional trabecular architecture at the distal femoral metaphysis was analyzed using microcomputed tomography (μCT). Geometrical properties of diaphyseal cortical bone were evaluated in the midfemoral region using μCT. The biomechanical properties of femurs were analyzed using three-point bending. Femoral BMD was significantly decreased following ovariectomy. This change was suppressed by swimming. Trabecular bone thickness, number, and connectivity were decreased by ovariectomy, whereas structure model index (i.e., ratio of rod-like to plate-like trabeculae) increased. These changes were also suppressed by swimming exercise. Femurs displayed greater cortical width and maximum load in OVX-SWI. Moreover, the effects of swimming exercise on bone mass in the long bones of rats have shown that swimming exerts positive influences on bone mass (14, 20, 40, 44, 52–55). This disagreement over the effects of swimming exercise on bone mass between human and animal studies in the literature has created substantial confusion among researchers in this field. Because of the lack of consensus on this topic, the precise effects of swimming on bone remain inconclusive.

Concerning swimming exercise and its effects on bone, most animal studies have involved intact young, mature, or aging rodents, focusing on the ability of exercise to increase bone mass. However, the effects of swimming exercise on bone structure in osteoporotic animal models have yet to be fully elucidated. We have identified several studies that have evaluated the effects of swimming exercise on protecting against ovariectomy-induced bone loss (5, 16, 20, 41, 47). Moreover, there is scarce detail regarding the changes in trabecular bone microarchitecture in these studies.

Since discrepancies exist between human and animal studies regarding the effects of swimming on bone, as described above, the present study was intended to clarify the exact relationship between swimming exercise and trabecular bone microarchitecture in ovariectomized (OVX) rats. To achieve this objective, we used microcomputed tomography (μCT) to assess three-dimensional (3D) trabecular architectural changes in cancellous bone that occur in the distal femoral metaphysis after 12 wk of swimming in OVX-induced osteopenic rats.

MATERIALS AND METHODS

Animals and experimental design. For this study, 36 female Fisher 344 rats (mean weight, 45 g; 28 days old) were purchased from Clea Japan (Osaka, Japan) and were 18 wk old at the start of the study. Rats were housed individually in 20 × 33 × 33-cm cages at room temperature (22 ± 1°C) and 60 ± 5% humidity, with lights on a 12-h on/off cycle. Rats were fed standard MF laboratory animal chow (1.15% calcium, 0.88% phosphorus; Oriental Yeast, Chiba, Japan) and were provided ad libitum access to water. Bilateral ovariectomy was performed in 18 rats (OVX groups) using a dorsal approach under anesthesia (50 mg/kg ip pentobarbital sodium). The remaining 18 rats were subjected to a sham operation (Sham groups) in which ovaries were exteriorized but not removed. Postoperatively, all rats were provided 2 wk for recovery. Rats were divided randomly into the following four groups: sham sedentary (Sham-CON; n = 9), sham swimming exercised (Sham-SWI; n = 9), OVX sedentary (OVX-CON; n = 9), and OVX swimming exercised (OVX-SWI; n = 9). Body weight and food intake were measured daily before exercise or

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at the equivalent time on days without exercise. At the end of the experiment, rats were anesthetized with pentobarbital sodium (ip) and euthanized by exsanguination from the abdominal aorta. Soon after euthanasia, the uterus of each rat was collected and immediately weighed. Surgical success was confirmed in 16 of 18 rats in the O VX groups by the absence of ovarian tissue and uterine atrophy. Intact ovaries and uteri were found in the remaining two rats in the O VX -CON group, and therefore, these rats were excluded from the study. Both femurs were excised from each rat and cleaned of soft tissue. The length of the left femur was measured using digital calipers and then wrapped in saline-soaked gauze. The middiaphyseal region was then imaged using μ CT . Care was taken to ensure that samples did not dry out. After μ CT , the left femur was stored at −40°C until mechanical testing. To minimize the possible effects of freezing and thawing on biomechanical properties, the left femur was not subjected to repeated cycles of freezing and thawing. The right femur was stored at −40°C until further measurement. This study proceeded in strict accordance with the recommendations described in the National Institutes of Health’s Guide for the Care and Use of Laboratory Animals . The Committee for the Ethics of Animal Experiments at Kawasaki University of Medical Welfare approved the experimental protocol (permit no. 14-007).

Swim training protocol. Rats assigned to the exercise groups performed swimming for 60 min/day, 5 days/wk for 12 wk. Rats were placed in water for 10 min/day during the 3 days preceding the start of the training protocol to familiarize the animals with the exercise environment. Rats swam for 10 min/day for the first 2 days, and exercise duration was then gradually increased by 10 min/day until a continuous swimming time of 60 min/day was achieved. Additional weights such as a caudal dumbbell were not used to increase exercise intensity. Swim training was performed in a cylindrical plastic barrel (diameter, 50 cm; height, 70 cm) filled with tap water to a depth of 60 cm. Rats were trained in groups of three to four animals per barrel, and water temperature was maintained at 35–36°C. Throughout the swimming exercise, rats were carefully monitored to ensure that they did not touch the sidewall of the barrel. At the end of each training session, each rat was dried using a cotton towel and placed under a heating lamp until the fur was completely dry before being returned to the cage. Sedentary rats remained in cages during the exercise period.

Measurement of bone mineral density. Bone mineral density (BMD) of the right femur was measured by dual-energy X-ray absorptiometry using a QDR-2000 densitometer (Hologic, Waltham, MA). Briefly, the instrument was set on the small-animal ultra-high resolution scan mode with line spacing of 0.254 mm, point resolution of 0.127 mm, and a collimator diameter of 0.9 mm. Excised femurs were immersed in saline and scanned in a supine position on the table. The entire right femur was scanned, and femoral scan images were segmented using a 3 × 3 median filter to remove noise and a fixed threshold to extract the mineralized bone phase. Isolated small particles in marrow space were removed. Cortical and trabecular bone were subsequently separated by semiautomatically drawn contours, and the following 3D morphometric parameters were calculated by measuring distances in 3D directly in the trabecular network (21); trabecular bone volume fraction (BV/TV), trabecular thickness (Tb.Th), trabecular number (Tb.N), and trabecular separation (Tb.Sp). Connectivity density (B1/TV) (15), trabecular bone pattern factor (TBPF) (18), structural model index (SMI) (22), and degree of anisotropy (DA) (19) were calculated directly from segmented voxel representations.

Ash weight of the femur. After BMD measurement and μ CT , dry weight and ash weight were determined for the right femur of each animal. Femora were dried at 105°C for 1 day after fat extraction in solvent (2:1 chloroform-methanol by volume), and dry weights were recorded. Bones were then reduced to ash using a CM-150 muffle furnace (Shibata, Tokyo, Japan) at 600°C for 24 h. Degree of calcification was assessed by calculating the percentage ash weight, defined as ash weight divided by dry bone weight.

Geometry measurement. A middiaphyseal tomogram of the left femur was obtained by μ CT under identical scanning conditions as the distal metaphyseal region and used for the analysis of geometric properties of diaphyseal cortical bone. To calculate geometrical properties of the femoral diaphysis, cortical bone was separated using histogram-based thresholding. Cortical bone area (Ac) and bone marrow area (Abm) were measured using computerized planimetry, which was performed on a Macintosh computer using public domain NIH Image software (written by Wayne Rasband, NIH). Cortical width (Tc) was subsequently calculated, assuming a circular ring model, using the following formula:

\[ Tc = \sqrt{\frac{(Ac + Abm)}{\pi}} - \sqrt{\frac{Abm}{\pi}} \]

Maximum and minimum cross-sectional moment of inertia were calculated using the methods described by Cheng et al. (12).

Mechanical testing. After μ CT was performed, mechanical strength of the left femur was determined using a three-point bending

### Table 1. Physical parameters of experimental rats

<table>
<thead>
<tr>
<th>Group</th>
<th>ANOVA P Value</th>
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<tbody>
<tr>
<td></td>
<td>Sham-CON</td>
</tr>
<tr>
<td>Initial body weight, g</td>
<td>166.81 ± 8.75</td>
</tr>
<tr>
<td>Final body weight, g</td>
<td>175.99 ± 6.38</td>
</tr>
<tr>
<td>Uterine weight, mg</td>
<td>361.00 ± 83.92</td>
</tr>
<tr>
<td>Femoral length, mm</td>
<td>32.04 ± 0.59</td>
</tr>
</tbody>
</table>

All values represent means ± SD. Sham-CON, sham-operated sedentary group; Sham-SWI, sham-operated swimming group; O VX-CON, ovariecinated sedentary group; O VX-SWI, ovariecinated swimming group; NS, not significant. *P < 0.001 vs. Sham-CON; **P < 0.001 vs. Sham-SWI.
test (Model MZ-500D; Maruto Testing Machine, Tokyo, Japan), as described previously (43). Briefly, femurs were positioned so that one fulcrum was at the distal 1/6 point of the total length, and the other was at the proximal 5/6 point. Breaking force was applied with the cross-head moving at 6 mm/min until failure. The maximum load and stiffness of the bone were determined directly from the load deformation curve. Bending stress was then calculated using the methods outlined by Raab et al. (49) and Umemura et al. (59).

**Statistical analysis.** Statistical analyses were performed using the IBM SPSS Statistics 22.0 software package (IBM, Armonk, NY). Two-way (OVX × SWI) analysis of variance was used to examine individual main effects and interactions between treatments. When a statistically significant interaction was identified, the effects of swimming exercise or OVX were assessed using post hoc analysis. All data are reported as means ± SD. Values of P < 0.05 were considered statistically significant.

**RESULTS**

**Body weight, uterine weight, and femoral length.** Initial and final body weights, uterine weight, and femoral length in rats from each group are shown in Table 1. No significant differences in initial body weight and femoral length were found among groups. Final body weight was significantly higher in OVX rats than in sham rats. Uterine weight was significantly lower in OVX rats than in sham rats. Both final body weight and uterine weight were significantly lower in exercised rats than in sedentary rats. Significant interactions were identified between the effects of OVX and swimming exercise on uterine weight. In sham-treated rats, swimming exercise significantly decreased uterine weight, whereas swimming did not affect uterine weight in OVX rats.

**Bone mass measurements.** Dry weight, ash weight, and percent ash weight of the femur are shown in Table 2. Ash weight and percentage ash weight were significantly lower in OVX rats than in sham-treated rats. The decrease in ash weight for OVX rats was inhibited by swimming exercise, but the percentage ash weight was not. Changes in BMD with respect to anatomic location along the length of the femur are shown in Figs. 1 and 2. BMD values at R1 (femoral head, neck, and greater trochanter), R2 (intertrochanter), R6 (distal metaphysis), and R7 (distal epiphysis) were significantly lower for OVX rats than for sham-treated rats, indicating OVX-elicited decreases in BMD for these regions. Conversely, no significant differences were observed in BMD at R3, R4, and R5 (femoral diaphysis) between OVX and sham-treated rats. BMD was significantly higher in exercised rats than in the sedentary group for all femoral regions.

**Microstructural properties.** Trabecular BV/TV, Tb.Th, Tb.N, and Tp/TV were significantly lower in OVX rats than in sham-treated rats, whereas Tb.Sp, TBPf, and SMI were significantly higher in OVX rats than in sham-treated rats, indicating that OVX decreases trabecular bone volume fraction, number, and connectivity and increases the ratio of rod-like to plate-like trabeculae. Conversely, DA did not change with OVX. All changes were inhibited, and DA was also significantly altered, by swimming exercise (Table 3).

<table>
<thead>
<tr>
<th>Group</th>
<th>Femoral dry weight, mg</th>
<th>Femoral ash weight, mg</th>
<th>%Ash weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sham-CON</td>
<td>0.363 ± 0.015</td>
<td>0.218 ± 0.012</td>
<td>0.601 ± 0.004</td>
</tr>
<tr>
<td>Sham-SWI</td>
<td>0.378 ± 0.024</td>
<td>0.237 ± 0.016</td>
<td>0.626 ± 0.015</td>
</tr>
<tr>
<td>OVX-CON</td>
<td>0.353 ± 0.017</td>
<td>0.207 ± 0.010</td>
<td>0.588 ± 0.008</td>
</tr>
<tr>
<td>OVX-SWI</td>
<td>0.369 ± 0.014</td>
<td>0.214 ± 0.014</td>
<td>0.581 ± 0.049</td>
</tr>
</tbody>
</table>

**ANOVA P Value**

- SWI: p < 0.05
- OVX: p < 0.001
- SWI × OVX: p < 0.05

All values represent means ± SD.

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**Table 2. Dry weight, ash weight, and %ash of femurs**

<table>
<thead>
<tr>
<th>Group</th>
<th>Value</th>
<th>ANOVA P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Femoral dry weight, mg</td>
<td>0.363 ± 0.015</td>
<td>0.05 NS NS</td>
</tr>
<tr>
<td>Femoral ash weight, mg</td>
<td>0.218 ± 0.012</td>
<td>0.014 NS NS</td>
</tr>
<tr>
<td>%Ash weight</td>
<td>0.601 ± 0.004</td>
<td>0.049 NS NS</td>
</tr>
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</table>

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Geometry and mechanical properties. OVX had no effect on these parameters, except for cortical width, which was decreased by OVX. Conversely, cortical width, moment of inertia, and maximum load were significantly higher in exercised rats than in sedentary rats. Bending stress and stiffness also tended to be higher in exercised rats, although no significant differences were identified \((P = 0.273\) and \(P = 0.125\), respectively; Table 4).

**DISCUSSION**

Physical activity and exercise are well known to play crucial roles in skeletal development in both humans and animals. In contrast, skeletal unloading, whether due to spaceflight (50), prolonged bed rest (60), sciatic neurectomy (25), cast immobilization (31), or non-weight bearing during tail suspension (4), causes rapid and marked bone loss. Cross-sectional data in human studies have suggested that bone mass in swimmers was lower than or similar to that observed in weight-bearing sport protocols. They demonstrated that swimming showed a level of osteogenic effect similar to jumping and a slightly greater effect than vibration. Moreover, swimming exercise has been reported to produce greater bone adaptations than running in rapidly growing female rats (53). Our data were consistent with these results. In the present study, OVX rats exhibited marked deterioration of trabecular bone architecture, particularly from the central zone of the femur with the loss of trabeculae (Fig. 3), but this deterioration was attenuated by swimming exercise. Compared with OVX sedentary control rats, swimming-exercised OVX rats showed increased trabecular bone mass (102%), number of trabeculae (71%), and thickness (18%). In sham-treated rats, swimming exercise also induced a significant increase in trabecular bone mass (64%), number of trabeculae (42%), and thickness (16%) when compared with sedentary control rats. Interestingly, the increased mass of metaphyseal trabecular bone gained through swimming exercise in this study was similar to that in our previous report (30), which showed that jump exercise during the remobilization period induced a significant increase in BV/TV (64%), Tb.N (31%), and Tb.Th (63%) when compared with nonintervention recovery rats. Furthermore, rates of bone gain were greater than in our previous report (26); we found that the treadmill running exercise increased cancellous bone mass (33%) as a result of increasing the number (22%) and thickness (8%) of trabeculae. The percentage increase in trabecular bone mass of the femur was thus similar to that measured in jumping-exercised rats but was higher than that in running-exercised rats. However, direct comparisons are difficult because of the differences in animal species, sex, strain, age, and exercise protocols.

Gravitational or impact stressors acting on the body during swimming are counteracted by the buoyancy of water (44). Consequently, the osteogenic effect observed in swim-exercised rats is derived primarily from the mechanical loading generated by muscle contraction. This implicates that in small studies have examined the effects of noncompetitive-type moderate swimming exercise on bone mass in postmenopausal or elderly individuals, but conflicting data on this issue also exist (2, 9, 46, 57). However, results of studies using small laboratory animal models have been promising. Recently, Falcai et al. (14) compared the effects of swimming (60 min/day, 5 days/wk for 3 wk), jumping (20 jumps/day, 5 days/wk for 3 wk), and vibration therapies (20 min with a longitudinal amplitude of 1 mm and frequency of 50 Hz/day, 5 days/wk for 3 wk) on the prevention of bone loss using a hindlimb unloading rat model. They demonstrated that swimming showed a level of osteogenic effect similar to jumping and a slightly greater effect than vibration. Moreover, swimming exercise has been reported to produce greater bone adaptations than running in rapidly growing female rats (53). Our data were consistent with these results. In the present study, OVX rats exhibited marked deterioration of trabecular bone architecture, particularly from the central zone of the femur with the loss of trabeculae (Fig. 3), but this deterioration was attenuated by swimming exercise. Compared with OVX sedentary control rats, swimming-exercised OVX rats showed increased trabecular bone mass (102%), number of trabeculae (71%), and thickness (18%). In sham-treated rats, swimming exercise also induced a significant increase in trabecular bone mass (64%), number of trabeculae (42%), and thickness (16%) when compared with sedentary control rats. Interestingly, the increased mass of metaphyseal trabecular bone gained through swimming exercise in this study was similar to that in our previous report (30), which showed that jump exercise during the remobilization period induced a significant increase in BV/TV (64%), Tb.N (31%), and Tb.Th (63%) when compared with nonintervention recovery rats. Furthermore, rates of bone gain were greater than in our previous report (26); we found that the treadmill running exercise increased cancellous bone mass (33%) as a result of increasing the number (22%) and thickness (8%) of trabeculae. The percentage increase in trabecular bone mass of the femur was thus similar to that measured in jumping-exercised rats but was higher than that in running-exercised rats. However, direct comparisons are difficult because of the differences in animal species, sex, strain, age, and exercise protocols.

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laboratory animals, such as rats, muscle contraction in swimming may produce a level of mechanical stress that is comparable with jump exercise and greater than that in running exercise. This contrasts sharply with cross-sectional studies that demonstrate lower bone mass in swimmers compared with athletes engaged in weight-bearing sports. Based on human studies (13, 17, 38, 56), muscle forces on the skeleton during swimming do not appear to offset the concomitant reduced weight bearing that is crucial to bone mass maintenance. Since the gravitational force on the skeleton increases relative to body weight, its contribution to the osteogenic stimulus during exercise would be much lower than muscle contractile forces in rats than in humans. Supporting this notion, we recently reported that mechanical stress by muscle contraction in jump exercise seems to be more important than the ground reaction force as an osteogenic stimulus in rats (29). Alternatively, the sensing point of mechanical stimuli in the Wnt signaling pathway and associated regulatory factors such as sclerostin, which plays a crucial role in the regulation of bone mass and bone cell functions (7), may differ between small animals and humans. Direct extrapolation of results from rats to humans is thus inappropriate. Swimming exercise in humans would thus not be expected to provide an effective means of increasing bone mass unless some device is used to increase the mechanical loading on bone by muscle contraction. Nonetheless, the present results suggest that the swimming-induced direct muscle contraction force itself produces a positive effect on trabecular bone mass and the structure of limb bones.

Not all swim studies in small animals are associated with bone gains (5, 8, 10, 16, 23, 24, 47). The inconsistent results of the effects of swimming exercise on bone among different studies are probably due to differences in study protocols, such as the duration of swimming exercise, animal species, and age. Therefore, even in rats, extended periods of exercise in a hypogravity environment may result in deleterious effects on bone, as is the case in humans.

The loss of trabecular connectivity is known to be associated with a reduction in the physical strength of trabecular bone (51). In the present study, both TBPf and B1/TV showed significant decreases in trabecular connectivity after OVX, Table 4. Geometry and mechanical properties of femoral mid-diaphysis

<table>
<thead>
<tr>
<th>Group</th>
<th>Sham-CON</th>
<th>Sham-SWI</th>
<th>OVX-CON</th>
<th>OVX-SWI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tc, mm</td>
<td>0.43 ± 0.01</td>
<td>0.45 ± 0.01</td>
<td>0.42 ± 0.01</td>
<td>0.44 ± 0.01</td>
</tr>
<tr>
<td>Ac, mm²</td>
<td>2.33 ± 0.08</td>
<td>2.45 ± 0.11</td>
<td>2.28 ± 0.11</td>
<td>2.43 ± 0.05</td>
</tr>
<tr>
<td>Abm, mm²</td>
<td>1.31 ± 0.12</td>
<td>1.31 ± 0.09</td>
<td>1.35 ± 0.07</td>
<td>1.33 ± 0.10</td>
</tr>
<tr>
<td>Iₘₐₓ, mm⁴</td>
<td>1.57 ± 0.14</td>
<td>1.71 ± 0.14</td>
<td>1.55 ± 0.13</td>
<td>1.68 ± 0.11</td>
</tr>
<tr>
<td>Iₘᵦₚ, mm⁴</td>
<td>0.34 ± 0.03</td>
<td>0.37 ± 0.03</td>
<td>0.34 ± 0.03</td>
<td>0.37 ± 0.03</td>
</tr>
<tr>
<td>Bending stress, N/mm²</td>
<td>312.49 ± 16.00</td>
<td>322.42 ± 18.21</td>
<td>314.63 ± 22.15</td>
<td>318.49 ± 15.66</td>
</tr>
<tr>
<td>Maximum load, N</td>
<td>61.75 ± 3.38</td>
<td>67.15 ± 4.46</td>
<td>61.03 ± 3.93</td>
<td>65.92 ± 2.27</td>
</tr>
<tr>
<td>Stiffness, N/mm</td>
<td>151.67 ± 16.93</td>
<td>167.63 ± 16.93</td>
<td>153.95 ± 12.80</td>
<td>156.99 ± 21.12</td>
</tr>
</tbody>
</table>

All values represent means ± SD. Tc, cortical width; Ac, cortical bone area; Abm, bone marrow area; Iₘᵦₚ, maximum cross-sectional moment of inertia; Iₘᵦₚ, minimum cross-sectional moment of inertia.

Fig. 3. Typical 3-dimensional images of distal femoral metaphysis in Sham-CON (A), Sham-SWI (B), OVX-CON (C), and OVX-SWI rats (D), as visualized using micromputed tomography. Intact bone (top) and isolated cancellous bone (bottom) used for calculating trabecular bone parameters are shown.
supporting the results of other investigators (34, 39). Conversely, swimming exercise decreased TBPF and increased trabecular connectivity. Decreases in TBPF or increases in trabecular connectivity are usually coupled with increases in bone strength (33). Moreover, some studies have shown that restoration of trabecular connectivity is important for strength recovery (33, 35). Therefore, the changes in TBPF and B1/TV observed in the present study could quite conceivably have contributed to the beneficial effects of swimming exercise on cancellous bone strength. SMI offers an estimation of the plate-and rod-like characteristics of trabecular structures (22). Trabecular perforation results in shifting trabecular structure from plate-like to rod-like and represents a well-established characteristic of osteoporosis (36). In the present study, SMI was increased by OVX, indicating that trabecular structure changed from plate-like to rod-like with estrogen deficiency. Conversely, swimming exercise significantly decreased SMI compared with sham-treated sedentary rats, suggesting concomitant increases in mechanical strength. Trabecular alignment is another important parameter contributing to mechanical bone strength (45). In the present study, trabecular bone was oriented more anisotropically in exercised rats than in sedentary rats. The increase in trabecular anisotropy suggests that muscle contraction during swimming exercise induced bone formation in a manner similar to jump exercise (30).

The OVX rat represents an established model of estrogen deficiency-induced bone loss and has been validated as an excellent experimental model for the bone loss seen in human menopause. OVX is not limited to just young rats, as aged rats can also be used as an OVX rat model. Several animal studies have used OVX rat models at relatively old ages (36–64 wk old) to examine the effects of mechanical loading on the skeletal system (3, 62). Thus, 20- to 32-wk-old rats used in this study would be hormonally active and relevant for modeling estrogen deficiency by OVX. The effect of estrogen deficiency by OVX is clearly supported by the decrease in uterine weight. OVX rats did not exhibit any changes in BMD, bone geometry, or mechanical strength at the femoral diaphysis, probably because cortical bone is less susceptible than cancellous bone to ovariectomy-induced bone loss (58). On the other hand, swimming exercise increased cortical bone area and width at the midfemoral diaphysis without significant changes in marrow area, regardless of estrogen deficiency. These results were consistent with previous studies (11, 48), suggesting that exercise affects the periosteal surface of cortical bone rather than the endosteal surface. Swimming exercise also increased moment of inertia and maximum load in the midfemoral diaphysis but did not affect bending stress, indicating that the material properties of bone were unchanged in exercised rats.

The mineralization of bone tissue also determines bone strength, and the degree of mineralization reportedly decreases in OVX animals and postmenopausal women (6, 42). Contrastingly with the effect of OVX, no significant effect on the percentage ash weight was observed between the CON and SWI groups, suggesting that swimming exercise does not affect the degree of calcification in bone tissue. Compared with Sham and OVX sedentary control rats, uterine wet weight has decreased in rats subjected to swimming exercise (~32% in the Sham-SWI group, ~8% in the OVX-SWI group). This may be because increased levels of physical activity reduce ovarian steroidogenesis (1) or blood flow to the uterus of rats (37) and send extra blood to the muscles being used. In any case, our results suggest that the effects on bone of hormonal alteration in this study, if any, would be small compared with the effects on the uterus.

Some limitations to the current study must be considered when interpreting the results. We did not perform dynamic histomorphometric analyses or measurement of serum markers of bone turnover. We thus cannot confirm tissue-level or systemic mechanisms for the functional adaptation of trabecular bone to swimming exercise observed in the present study. Further detailed dynamic histomorphometric measures and serum bone marker assays will be helpful to validate the effects of swimming exercise on bone metabolism, and this parameter should be included in future studies.

In summary, swimming exercise attenuated the loss of trabecular bone of the distal femoral metaphysis in adult OVX rats, and ovariectomy-induced trabecular plate loss was fully inhibited by swimming exercise. Moreover, geometry parameters and mechanical properties in the femoral mid-diaphysis were significantly improved by swimming. These results, taken as a whole, provide additional support to the oft-mentioned notion that swimming exercise may be beneficial in improving bone mass, strength, and structure in small animals such as rats.

DISCLOSURES
No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS
Y.-I.J., T.S., and M.F. conception and design of research; Y.-I.J. and K.O. performed experiments; Y.-I.J., K.O., and K.T. analyzed data; Y.-I.J., T.S., and M.F. interpreted results of experiments; Y.-I.J. prepared figures; Y.-I.J. drafted manuscript; Y.-I.J. and T.S. edited and revised manuscript; Y.-I.J., T.S., K.O., K.T., and M.F. approved final version of manuscript.

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