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Swim-trained rats have greater bone mass, density, strength, and dynamics

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Received 17 November 2000; accepted in final form 1 June 2001

Hart, K. J., J. M. Shaw, E. Vajda, M. Hegsted, and S. C. Miller. Swim-trained rats have greater bone mass, density, strength, and dynamics. *J Appl Physiol* 91: 1663–1668, 2001.—Weight-bearing exercise is traditionally recommended for improving bone health in postmenopausal women. Effects of swim exercise were studied as an alternative to weight-bearing exercise in ovariectomized rats. Rats in a swim group (Sw, $n = 8$) swam for 12 wk, 5 days/wk for 60 min per session. A control group (Con, $n = 9$) engaged in no structured exercise. Femurs were analyzed for bone mineral density and for bone mineral content by dual energy X-ray absorptiometry, biomechanical properties by three-point bending (Instron), and bone structure and formation by histomorphometry. Food intake did not differ among groups. Final body weights were significantly lower in Sw compared with Con ($P < 0.05$). Swimmers had significantly greater femoral shaft bone mineral density and content ($P < 0.05$) compared with Con. Femurs of the Sw group had greater mechanical properties ($P < 0.05$) compared with Con. Histomorphometric data were significantly better in the Sw group compared with Con after the 12-wk intervention ($P < 0.05$). In conclusion, data from this study demonstrate some beneficial effects of swim exercise on bone structure, turnover, and strength.

swimming; bone; rats; aging; ovariectomy

OSTEOPOROSIS IS A DEBILITATING disease, characterized by loss of bone mass, which leads to an increased risk of bone fracture. Cortical bone is a major component of bone mass, and high bone mineral density (BMD, g/cm^2) in cortical bone ensures mechanical competence of the bone (9, 11, 22). Rapid declines in BMD occur with estrogen deficiency at menopause, placing women at a greater risk for the development of osteoporosis compared with men (6, 14). A decrease in physical activity is also associated with the development of postmenopausal osteoporosis.

Forces generated through mechanical loads during exercise promote osteogenesis (17). Therefore, exercise intervention is considered a strategy for reducing risk for osteoporosis. Various types of exercise, particularly those that load the skeleton in an atypical manner, are

associated with maintenance or increased skeletal mass. Cross-sectional studies have shown that physically active individuals, who experience high skeletal forces, have greater BMD compared with inactive individuals (4, 25). Forces are highest during weight-bearing modes of exercise; therefore weight-bearing exercise is conventionally prescribed for bone health.

Little is known about the role that a non-weight-bearing exercise, such as swimming, has on bone status. Most studies on swimming and bone mass have been conducted in younger, elite athletes, are cross-sectional in design, and usually demonstrate few skeletal benefits. In three studies, collegiate male and female swimmers had BMD values that were not significantly different from nonathletic controls (8, 13, 29). Courteix (5) made a similar observation with prepubertal girls. However, little information exists about the effects of swimming on bone mass in noncompetitive aging or postmenopausal individuals. Cross-sectional comparisons may reflect a selection bias, which does not represent the aging population. Orwoll (19) reported that male swimmers, ranging in age from 40–85 years of age, had greater BMD than did the nonexercising controls in this cross-sectional study. Further reports in humans are limited to noninvasive methods of determining the influences of swimming on skeletal status.

Some studies have demonstrated a beneficial effect of swim exercise in young, rapidly growing animals; however, limited swim exercise studies have been conducted in older animals or in animals with estrogen deficiency, which resembles the postmenopausal state. In young animals, swim exercise was correlated with increased periosteal apposition, longitudinal bone growth, increased bone mineral content, and bone strength (23, 27, 28). Simkin et al. (23) reported changes in bone geometry in swim-trained rats, which reflected a more favorable distribution of bone tissue and, hence, stronger tissue. Swim exercise was also reported to improve bone density in aging mice (10) and in normal and glucocorticosteroid-treated rats (27). Bourrin et al. (3), using histomorphometry, re-

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ported that swim exercise in young rats decreased the resorption and osteoid surface, suggesting a decrease in bone turnover.

Swim exercise is a popular exercise for many older individuals because it offers an alternative to weight-bearing exercise, particularly for those for whom weight-bearing exercise is difficult. Few data exist in either human or experimental studies to predict the benefits from swim exercise in maintaining skeletal health during and after menopause. To address this issue, we used aging, retired breeder rats that were ovariectomized to investigate the effects of swim exercise on cortical bone properties. The ovariectomized rat is an established model of estrogen-deficiency bone loss, replicating many aspects of postmenopausal osteoporosis (12, 15). The data from this study demonstrate some beneficial effects of swim exercise on bone structure, turnover, and strength.

METHODS

Retired breeder, female Sprague-Dawley rats (Indianapolis, IN) with 9 previous reproductive cycles were used (age 12–14 mo). Rats were fed a standard diet (AIN-93M from DYETS; Ref. 21), provided water ad libitum, and maintained on a 12:12-h light-dark cycle. After an acclimatization period of 2 wk, rats were ovariectomized, weight matched, and randomized to swim (Sw) or control (Con) groups. There were nine Con and eight Sw rats in the study. The success of the ovariectomy was determined at necropsy by the absence of ovarian tissue and atrophy of the uterine horns. Rats were weighed and fed every other day. The study was conducted according to the timeline presented in Table 1.

Swim exercise occurred in large containers filled with lukewarm water (30–34°C). Rats could not touch the bottom

or hang on the sides and were supervised the entire swim time. Swimming progressed as detailed Table 1. No additional weight was placed on the rats, and no forced exercise was imposed on the Con group.

Twelve days before necropsy, an intraperitoneal injection of oxytetracycline-hydrochloride (25 mg/kg body wt; Durvet, Blue Springs, MI) was given to Sw and Con groups. Two days before necropsy, rats were given calcein (Sigma Chemical, St. Louis, MO) by an intraperitoneal injection at a dose of 10 mg/kg body wt. The tetracycline and calcein are fluorochromes used for the histomorphometric measurement of bone formation. The right femurs were collected at necropsy and cleaned of adherent tissue. The bones were wrapped in saline-soaked gauze, placed in a plastic vial, and frozen at –20°F.

Three measurement techniques were used to assess cortical bone properties at the femoral middiaphyseal shaft; dual energy X-ray absorptiometry, mechanical testing using three point bending, and bone structure, formation, and turnover using histomorphometric methods. To assess BMD, femurs were scanned frozen by using a portable dual energy X-ray absorptiometer (Norland, Medical Systems, Fort Atkinson, WI). Small animal software was used to scan the middiaphyseal femoral shaft. The coefficient of variability on repeated scans and standards was <0.5%.

For biomechanical testing, the femurs were thawed at room temperature, the total length and diameter were measured by using vernier calipers (Mitutoyo, Japan), and the middiaphysis was marked with a soft lead pencil for later orientation. The femurs were kept moist with saline and placed in a servohydraulic material-testing machine (Instron, Canton, MA). The femurs were loaded to failure in three-point bending at the middiaphysis with a crosshead speed of 10 mm/min. The testing conditions follow published standards for laboratory testing of animal bones (1) with the exception that the dimensions of the rat femurs precluded placing the support length >10 times the diameter of the bone. However, support length was always >20 mm to ensure that 85–90% of the bone flexure was due to bending (30). Load data were obtained from a 50-lb (23-kg) compression load cell interfaced with an analog-to-digital converter and a personal computer. The coefficient of variation of the load cell was <0.1%. Deformation data were obtained from crosshead displacement. Maximum stress and Young's modulus (E) were calculated using standard engineering beam theory (30). Calculation of Young's modulus requires a measurement of cross-sectional moment of inertia (I), which was determined as described below. Flexural rigidity is calculated for each femur as $E \times I$ (30). Work to fracture was calculated as the area underneath the load-deformation curve, normalized to the total tissue volume tested (i.e., cross-sectional cortical area \times support length). Some femurs may have small cracks (discontinuities in the load-deformation curve) before complete failure; however, work to fracture was calculated from the point of complete bone failure for all specimens.

After mechanical testing, the broken halves of each femur were glued together with cyanoacrylate glue (Super Glue) and embedded in methyl methacrylate, as previously described (2). Transverse sections were cut through the middiaphysis with a low-speed saw (Isomet, Buehler, Lake Bluff, IL) by using the pencil mark as a guide. Sections are mounted on plastic slides and manually ground to ~30–40 μ m in thickness on a rotary grinding wheel (Ecomet 5, Buehler).

Video images of the sections were collected from each slide by use of a charge-coupled device camera (Panasonic, Model WV-1850, Japan) attached to a light microscope. The fracture spaces that were created by the mechanical testing and

Table 1. Study time line

Week	Stage
1	Rats arrived, placed on casein protein diet; rats were weight matched and randomly assigned to swim or control group. Exercise groups began swimming 5 min per day. Nonexercise groups remained in cages.
2	Day 1: swim 5 min Day 2: swim 10 min Day 3: swim 10 min Day 4: swim 15 min Day 5: swim 15 min End of week: ovariectomy surgery
3	Recover from surgery, no swim exercise
4	Day 1: swim 15 min Day 2: swim 20 min Day 3: swim 25 min Day 4: swim 30 min Day 5: swim 40 min
5	Day 1: swim 45 min Day 2: swim 50 min Day 3: swim 55 min Day 4: swim 60 min Day 5: swim 60 min
6–13	• Remainder of study swam Monday–Friday 60 min/session • 12 days before necropsy, oxytetracycline-hydrochloride injection
14	• 2 days before necropsy, calcein injection • Necropsy

Table 2. *Body weight and food intake*

Group	Control (n = 9)	Swim (n = 8)	P Value
Final body weight, g	354.4 ± 22.3	311.0 ± 25.3*	0.003
Weight change, g	+27.5	-24.0	
Food intake, g/day	13.6 ± 0.99	11.75 ± 1.06	

Values are means ± SD. * $P < 0.05$.

gluing process were edited from the final images. These images were analyzed on an Apple Power Macintosh computer (Apple, Cupertino, CA) running a public-domain software program (NIH Image 1.55, available at <http://rsb.info.nih.gov/NIH-Image>). From the images, the maximum and minimum cross-sectional moments of inertia were calculated for each femur by use of custom software routines based on published algorithms (16). The ratio of maximum to minimum cross-sectional moment of inertia (the anisometry ratio) is calculated for each femur.

A digitizing tablet, a microcomputer (Apple SE, Cupertino, CA), a fluorescence microscope (Nikon, Tokyo, Japan), and histomorphometry software (KSS Scientific Consultants, Magna, UT) were used to collect cortical bone static and dynamic histomorphometric indexes. The static measurements included cortical and marrow cross-sectional area and average and minimum cortical thickness (2). The measurements used to calculate the dynamic indexes included total surface perimeter, perimeter of single- and double-labeled surface, and the interlabel width on both the periosteal and endocortical surfaces. From these, the percentage of single-labeled, double-labeled, and mineralizing surfaces, mineral appositional rate, and surface-referent bone formation rates were calculated. Additionally, the eroded perimeter was measured on the endocortical surface. Eroded surface was defined as scalloped surfaces (Howship's lacunae) that did not have fluorochrome markers or other evidence of new bone formation when viewed by ultraviolet or polarized light. The histomorphometric data were calculated and expressed by use of standardized methods (20). At least two nonadjacent sections were quantified from each animal.

Statistical analysis. Differences between groups were tested for significance by a one-tailed Student's *t*-test. Values are expressed as means ± SD and were considered significant if $P < 0.05$.

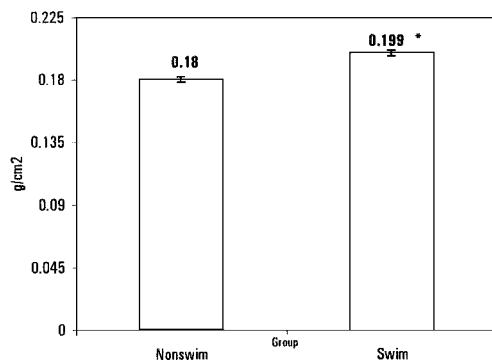


Fig. 1. Bone mineral density (BMD) measured by dual X-ray absorptiometry of the middiaphyseal shaft of the femur from nonswim controls (Con) and swim-exercised (Sw) retired breeder, ovariectomized rats. *Significantly different from nonswim group, $P < 0.05$.

Table 3. *Mechanical testing*

Group	Control	Swim	P Value
Maximum load, N	149.23 ± 20.0	155.65 ± 11.5	0.519
Flexural rigidity, N/mm	392.27 ± 20.5	510.08 ± 19.1	0.001†
Young's modulus, GPa	7.10 ± 1.0	10.43 ± 1.5	0.000†
Maximum stress, MPa	108.99 ± 17.9	127.42 ± 17.1	0.063
Work to fracture, mJ/mm ³	8.22 ± 1.7	5.73 ± 1.7	0.013*

Values are means ± SD. *Significantly different from control, $P < 0.05$; †significantly different from control, $P < 0.001$.

RESULTS

The Con groups gained weight during the study whereas the Sw group lost body weight resulting in a significant difference in their final body weights (Table 2). There was no significant difference in the average daily food intake between the groups (Table 2).

The BMD of the middiaphyseal femoral cortical bone was significantly greater in the Sw group compared with the Con group (Fig. 1). Bone mineral content was also significantly greater in the Sw group (data not shown).

Results from the biomechanical testing are shown in Table 3. The maximal load was slightly greater in the Sw group, but the differences were not statistically significant. Maximum stress in Sw rats showed a trend toward statistical significance ($P = 0.06$). Flexural rigidity was significantly greater in the Sw group compared with the Con, indicating that the Sw bones deformed less for a given load. The differences in stiffness were also observed at the tissue level, as evidenced by a significantly greater Young's modulus in the Sw group compared the Con group. In contrast, work to fracture was significantly less in the Sw group compared with the Con group.

The static morphometric data are displayed in Table 4. The average cortical area was significantly greater in the Sw group compared with the Con group. Additionally, the minimum cortical width was significantly less in the Con group than in the Sw group. There were no significant differences in the total periosteal perim-

Table 4. *Static histomorphometry*

Group	Control (n = 9)	Swim (n = 8)	P Value
Periosteal perimeter, mm	12.05 ± 0.68	12.36 ± 0.35	0.132
Cortical area, mm ²	5.91 ± 0.49	6.46 ± 0.20	0.028*
Marrow area, mm ²	4.12 ± 0.87	3.96 ± 0.48	0.327
Average cortical thickness, μm	569 ± 46	588 ± 54	0.239
Minimum cortical thickness, μm	467 ± 37	511 ± 42	0.026*

Values are means ± SD. *Significantly different from control, $P < 0.05$.

Table 5. *Dynamic histomorphometry*

Group	Control (n = 9)	Swim (n = 8)	P Value
Single-labeled surface, %			
Periosteal	19.05 ± 9.4	25.60 ± 13.3	0.011*
Endocortical	11.4 ± 3.7	18.1 ± 6.5	0.012*
Double-labeled surface, %			
Periosteal	35.72 ± 14.4	41.58 ± 34.1	0.302
Endocortical	5.6 ± 4.5	12.2 ± 4.2	0.004*
Mineralizing surface, %			
Periosteal	45.18 ± 14.7	49.43 ± 18.2	0.245
Endocortical	11.3 ± 5.6	21.2 ± 6.4	0.002*
Mineral appositional rate, %			
Periosteal	0.64 ± 0.2	0.66 ± 0.2	0.590
Endocortical	0.94 ± 0.3	0.92 ± 0.1	0.445
Bone formation rate, $\mu\text{m}^2 \cdot \mu\text{m}^{-1} \cdot \text{d}^{-1}$, %			
Periosteal	0.297 ± 0.158	0.344 ± 0.183	0.268
Endocortical	0.056 ± 0.049	0.112 ± 0.041	0.0137*
Eroded perimeter, %	45.1 ± 9.4	29.4 ± 13.4	0.0082*

Values are means ± SD. *Significantly different from control, $P < 0.05$.

eter, average marrow area, or average cortical thickness between groups.

The dynamic histomorphometric data are displayed in Table 5. On the periosteal envelope, the percentage of single-labeled surface was significantly greater in the Sw group compared with the Con group. On the endocortical envelope, the percentages of single-labeled, double-labeled, and mineralizing surfaces and surface-referent bone formation rate were significantly greater in the Sw group compared with Con. The percent eroded perimeter on the endocortical surface was less in the Sw group than in the Con group.

DISCUSSION

Our findings suggest that swimming has a positive effect on bone mineral properties in ovariectomized rats, indicated by greater BMD, mechanical properties, and histomorphometric indexes in Sw compared with the Con group. Histomorphometric changes were most pronounced on the endocortical surface of the femurs of our Sw rats. This indicates increased bone activity and supports the BMD and bone stiffness changes. Significant improvement in the Sw group is a point of interest given that all measurements were taken at the same anatomical location (midthiaphyseal shaft of the femur) that was void of direct muscle attachments. The significant difference between groups suggests that mechanical benefits occur after a regimen of non-weight-bearing exercise at skeletal sites other than where muscles originate or insert.

This aggressive non-weight-bearing program, 60 min/day for 5 days/wk, provided the necessary stimulus for a bone response. Bone in these rats accommodated in response to an increased functional demand, as explained by Wolff's Law (9). This is in contrast to the cross-sectional findings that demonstrate lower BMD in swimming athletes compared with weight-bearing athletes (29). This discrepancy may be a result

of selection bias or significant differences in swim regimens. Taafe et al. (29) studied collegiate-level swimmers, and the cross-sectional study design does not rule out the possibility that elite swimmers have a propensity for low bone mass. Duration of swimming may also be an important factor. The swimmers in the Taafe et al. study swam an average of 22.3 h/wk, which was approximately four times greater in duration than the present study. Too much swim exercise can have deleterious effects on bone (3). Bourrin et al. (3) utilized an intense regimen that involved swimming rats for 6 h/day, which more closely resembles the regimen of collegiate swimmers at the peak of their training season. They found decrements in bone status with this swimming protocol, which could be a function of prolonged weightlessness in the water and/or prolonged exposure to stress hormones such as corticosterone, which was not measured. Exercise of long duration is also known to result in elevated glucocorticoids for metabolic purposes (e.g., mobilization of free fatty acids), which have negative effects on bone.

Other studies have found improved bone properties in the femur that also imply the importance of swim duration. Results from the current BMD data are supported by similar studies, in which increased bone density in the ovariectomized rat was found (27, 28). Swim protocols of these studies were similar on hours per day for 5 days/wk over a span of 10 wk. Snyder et al. (26) found swimming to affect the humerus more than the femur, using a longer swim protocol of 2 h/day. However, Nyska et al. (18) used a similar protocol to our study and demonstrated that 1 h of swimming per day yielded greater histomorphometric changes in the femur of young adult rats compared with the humerus. A more recent study by Hoshi et al. (10) found significantly greater bone density in aged male and female mice on a 6-wk swim program, indicating an age-related effect. Hoshi et al. found few changes in mechanical properties between groups. Only the female mice exhibited improvements in elasticity. However, a lack of mechanical changes in this group could have been a function of the short (6 wk) swim intervention.

Sw rats the present study had significantly greater bone stiffness as determined by three-point bending. Maximum stress was also slightly (nonsignificantly) greater in Sw rats. Work to fracture was decreased by swim training, but this is to be expected. Previous studies have demonstrated that increased stiffness in bone is often inversely related to energy absorption (7). These findings are notable especially in light of the weight differences between the animals at the end of the present study. Ovariectomy is known to induce weight gain in the rat, which is considered protective of the skeletal system by providing an increased mechanical stimulus in the face of estrogen deficiency (31). Interestingly, the Sw rats in the study had significantly less weight gain after ovariectomy than did Con rats, with no difference in food intake, yet still had a greater femoral BMD and bone stiffness with swimming training. Hoshi et al. (10) demonstrated an in-

crease in BMD in their swim-trained mice with a tendency for weight loss as well; however, they did not show considerable improvements in mechanical properties of cortical bone, and the females in the study were estrogen replete. To our knowledge, this is the first study to demonstrate that BMD and mechanical properties of bone are greater with a non-weight-bearing exercise, given the double insult of ovariectomy and reduced body weight.

The greater cortical area and smaller minimum cortical width observed in the Sw compared with the Con group are consistent with the greater BMD and the better biomechanical properties observed as a result of swim exercise. On the periosteal surface, the fraction of the single-labeled perimeter was greater in the Sw group, suggestive of some increased bone formation activity. However, the other indexes of bone formation were not significantly different on this surface between the Sw and Con groups. Because the bone markers were given near the end of the study, it is possible that greater periosteal activity may have been observed earlier. There were, however, some significant differences observed on the endocortical surface that help explain the changes in BMD and biomechanical properties in the swim-exercised animals. The fraction of single-labeled surface, double-labeled surface, and mineralizing surface and surface-reference bone formation rates were all greater in the Sw animals. Additionally, the fraction of the eroded perimeter was less than in the Con group. The smaller eroded surface may have been due to a decrease in bone resorption and/or a more rapid filling of previously resorbed surfaces due to the increases in bone formation. These differences on the endocortical surface in response to swim exercise are similar to responses to mechanical stimuli reported in other studies. Bagi et al. (2) observed increased bone formation and decreased resorption on the endocortical surfaces of rat femurs that had been mildly overloaded compared with the contralateral underloaded limb. Bagi et al. also observed a greater cortical bone area, smaller minimum cortical thickness, and better biomechanical properties on the overloaded vs. the underloaded bone.

In summary, the present study demonstrates that cortical bone formation, density, structure, and mechanics were greater in the Sw group compared with Con as a result of swim exercise in the adult, estrogen-deficient female rat. This occurred even though Sw rats had a lower body weight compared with the nonswimming groups but consumed similar amounts of food. These results lend additional support that moderate swim exercise may be beneficial in preserving and perhaps improving bone mass and strength in an adult, aging human.

We express thanks to Beth Bowman, Sally Warner, and Trudy Hill for technical assistance.

This work was supported in part by Grant AR-44806 from the National Institute of Arthritis and Musculoskeletal and Skin Diseases.

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