Effects of swimming training and free mobilization on bone mineral densities of rats with the immobilization–induced osteopenia

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ABSTRACT

Objective: To investigate the possible effects of regular swimming exercise on bone mineral density (BMD) compared with free activity after cast immobilization of rats.

Methods: We carried out the study from April 2005 to June 2005 at the Department of Sports Medicine, Medical School of Suleyman Demirel University, Isparta, Turkey. The study included a total of 24 female Wistar rats. The rats were randomized to control (n = 6), swimming training (ST) n = 9, and free mobilization (FM) n = 9 groups. We measured Bone mineral densities of femur and vertebra of all rats with a total body scanner using software specifically designed for small animals, before study started and at weeks 3 and 7. Timepoints corresponded to basal, after cast removal (ACIM), and after 3 weeks of free mobilization (AFM) or swimming training (AST). We immobilized the right hindlimb of each ST and FM animal with a cast while the left hindlimbs were kept free. After 3 weeks, the casts were removed. Then we allowed the rats to move freely in their cage for one week, after which the animals in ST group started to swim for 5 days a week for 3 weeks for 30 minutes per day. The group FM rats moved freely in the cage.

Results: Bone mineral density of the femur and vertebra after cast immobilization was significantly decreased compared with both their basal and age-matched control group. After mobilization, significant increases occurred in both groups according to ACIM. Similar but milder changes were observed in free limbs femur BMD of rats. Interestingly, vertebra BMD of swimming group was also higher than its age-matched control group (p<0.05).

Conclusion: Our study showed that swimming exercise had a significant rehabilitative effect on BMD loss associated with immobilization. Further studies are needed to investigate the effects of swimming on other bone properties.

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Prolonged immobilization induces severe osteopenia. Experimental immobilization studies involving local disuse-induced bone loss models can lose up a baseline level of 60% of trabecular bone mass.[1] It is thought that the mechanical stress generated by exercise plays an important role in the osteogenic response.[2] Human and animal studies have demonstrated exercise to increase bone mineral density (BMD), as well as bone mass and strength in either intact[3-6] or osteopenic bones.[7-9] It is commonly accepted that weight-bearing activity provides these benefits. In cross-sectional human studies, increased BMD seen in participants of sports that involve weight bearing activities has been

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associated with increased mechanical stress placed on the bones.[10-16] However, little is known regarding the role that a non-weight-bearing exercise, such as swimming, has on bone status. Cross-sectional studies conducted in younger athletes on swimming and bone mass usually demonstrate no significant skeletal improvement.[17-20] Swimmers, who train in a non-weight-bearing environment, have been shown to gain less skeletal benefits than do athletes who participate in weight-bearing activities.[13,14,21-23] Weightlessness was reported causing even bone loss in scuba divers.[24] On the contrary, greater BMD in old men,[25] increased periosteal apposition, longitudinal bone growth, increased bone mineral content and bone strength[26-28] with decreased bone turnover[29] in growing young rats, greater bone mass, density, strength, and dynamics in ovariectomized older rats[30] were also reported in favor of benefits of swimming on bone mass. The BMD change response may vary due to chosen experimental procedure.[31-32] It seems logical to choose splintage of a single limb, rather than ovariectomy, to produce osteopenia for the primary purpose of elucidating the effects of non-impact exercise on the bones of that limb. This technique also provides a possibility of comparison between the free and immobilized limbs of splinted animals, which may be important for interpretation of results.[8-33]

Swimming exercise is a popular exercise. It is important to shed light on potential benefits of swimming as an easy to practice alternative to weight-bearing exercise or at least as a part of a positive lifestyle approach to osteoporosis prevention, particularly for people with debility or for those for whom weight-bearing exercise is difficult. We hypothesized that swimming exercise, even in levels, which are not well standardized, is more beneficial than free mobilization in restoring the BMD after immobilization. From this point of view, we aimed to determine differential effects of regular swimming training versus free mobilization on BMD in immobilized rats.

Methods. A total of 24 female Wistar rats were included in the study. At the beginning rats were 10 weeks old, with an average body weight of 198 g. Animals were obtained from the Animal Breeding Unit of Akdeniz University School of Medicine, Turkey. Animals were housed at room temperature of 20^{O}C and fed with rodent food and water ad libitum, and maintained on a 12:12-h light-dark cycle in Suleyman Demirel University, Isparta, Turkey. The rats were randomized to control (n = 6), swimming training (ST) n = 9 and free mobilization (FM) n = 9 groups. Bone mineral densities of femur and vertebra of all rats were measured before study start and at weeks 3 and 7. Timepoints correspond to basal, after cast removal (ACIM) and after 3 weeks of free mobilization (AFM) or swimming training (AST). The right hindlimb of each ST and FM animal was immobilized with cast from the toes to one cm above the knee. The knee was fixed in 100-degree flexion so that the calf muscles were relaxed. The fixation was checked daily for duration of 3 weeks. Left hindlimbs were kept free. After 3 weeks, the casts were removed. Then rats were allowed to move freely in their cage for one week, after which the animals in ST group started to swim 5 days a week for 3 weeks for 30 minutes a day in a 1.5 meter stainless tank, filled with water 50 cm in depth and 32 ± 4^{O}C in temperature. No additional weight was placed on the rats. The group of FM rats moved freely in the cage, and no forced exercise was imposed. During the training periods, all the animals were healthy and without infection.

Dual-energy x-ray absorptiometry (DEXA) scans were performed before the study start and at weeks 3 and 7. Timepoints corresponded to basal, ACIM and after 3 weeks AFM or AST. Animals were anesthetized with 0.1 mL/100 g ketamine HCl intraperitoneally (Ketamidor®; Richter Pharma, Austria) for BMD measurement. The rats were then allowed to wake up spontaneously after the procedure. Bone mineral density (grams per cm^2) was measured with a total body scanner using software specifically designed for small animals by DEXA (Norland XR-46 bone densitometer, Norland Corp, Fort Atkinson, USA). The Norland XR-46 was calibrated daily, 30 minutes after turning the apparatus on. Quality control was performed using calibration standard and QC phantom. Analyses of the different sub-areas were carried out on the image of the animal on the screen using a region of interest for the lumbar spine (trabecular bone) and the femoral diaphysis (cortical bone). To minimize the inter-observer variations all analyses were carried out by the same technician.Prior to this study, the protocol was reviewed and approved by the Suleyman Demirel University Ethic Committee. Differences between measurement timepoints (basal, ACIM, AST, AFM) were tested for significance by non parametric Wilcoxon test. Mann-Whitney U test were used for comparisons of all groups (ACIM, AST, AFM) with their age matched control groups. The Statistical Package for Social Sciences for Windows 9.0 computer program was used for statistical analyses. Values are expressed as median (minimum and maximum), and were considered significant if $p<0.05$.

Results. ***After cast immobilization:*** The BMD of the vertebra ACIM was significantly decreased when compared with both their basal group (18%, p=0.012) and age-matched control group (27%, p=0.001). Similarly, femur BMD value of casted limb was lower than that of basal group (14%, p=0.009), and control group (17%, p=0.002). Decrease in left (free) femur BMD was also significant, but smaller; according to basal (10%, p=0.011) and control group (13%, p<0.01).

After swimming training: In vertebra values, the measurements taken AST was significantly elevated than basal (15%, p=0.035), ACIM (40%, p=0.012), and control groups (6%, p=0.002). Also, AST casted femur BMD was significantly increased from basal (8%, p=0.035), and ACIM values (25%, p=0.001). No significant difference between casted femur BMD of ST and control groups was observed. Swimming training also caused an 18% increase (p=0.012) in left femur BMD compared with ACIM value. Last reached value in left femur was not significantly different from basal and control group.

After free mobilization: The vertebra AFM measurements was significantly higher than ACIM values (30%, p=0.012). An increase of 6% from basal value found in FM group, and was not statistically significant. However, compared with control group, the BMD of vertebra was lower, but not significant in this group (2%, p=0.32). Bone mineral density of right femur AFM was 6% lower than basal (p=0.012) and 11% lower than control group. A significant increase (9%, p=0.003) was found compared with ACIM. Other limbs femur BMD was not significantly different from basal and control values.

Bone mineral density values of basal measurement, ACIM, AST, AFM, and their age-matched controls are showed in **Table 1**.

The body weight after 3 week of immobilization period, a significant weight loss was observed in splinted animals (ST and FM groups) compared with their basal values and age-matched control groups. Measurements at the end of the study showed that decrease in body weight of FM group is less than decrease in body weight of ST group. Body weights of ST groups were significantly less than the weights of the control animals (17%, p=0.001), and FM animals (10%, p=0.012). The changes in body weight are showed in **Table 2.**

Discussion. Disuse bone, causing immobilization-induced bone resorption, is well known to necessitate greater than normal activity to restore BMD to normal levels,[34] however, effects of different forms of exercises is still not clear. It has been a traditional attitude to accept that only weight bearing exercises are responsible from beneficial effects.[35] However, several studies demonstrated that there were positive effects of non-weight-bearing exercise on some bone mechanical properties. Some of these studies involving rat models have investigated the effects of swimming versus running, upon bone growth in growing normal rats.[35,36] In another study, swimming training was found to be beneficial in improving bone structure of ovariectomized rats with osteopenia.[30] Comparing with free activity, positive effects of treadmill running on unilateral lower limb immobilization-induced bone changes have also been reported.[37]

Three weeks of unilateral cast immobilization showed to be an appropriate way to produce osteopenia in femur.[33] With this method, as concluded by Jarvinen et al,[37] careful consideration is required for side-to-side comparison between casted and free limbs, since immobilization of one limb had shown to be able to lead the contralateral limb to be either overloaded or underloaded.[38] However, by having a chance of repetitive evaluation of same rats without killing them, and by setting the age-matched control groups for comparisons at all measurement timepoints, reasonable intergroup comparisons could be made in our study. Contrary to a previous study,[37] in which immobilization of one limb did not change other limbs, we observed significant decrease in contralateral femur BMD as the evidence of underloading, which caused by a general diminution in physical activity.

DEXA analysis in this study showed that greater immobilization-induced BMD decrease took place in the vertebra trabecular bone in accordance with the literature.[1] Our findings suggest that swimming exercise has a positive effect on BMD of both extremities and vertebra in splinted animals, to an extent that even vertebra BMD values of immobilized rats were higher than age-matched controls. The latter results was especially surprising when considering the study of Iwamoto et al,[39] which reported that positive effects of increased mechanical loading was absent in the vertebra. Increase in femur BMD was higher than that found by Huang et al[35] in their rats, which swam with weight attached to their tail, and lower than Hart et al,[30] reported for ovariectomized rats with no additional weight. As an interesting result it was observed that, with swimming training, improvement of immobilized femur was more than that of free limbs of rats, suggesting that this form of exercise may be more beneficial to regain normal density in recovery from osteopenia, rather than improvement of normal bone to built a stronger structure. According to the literature, training protocol in this study was set as a minimum level to be effective.[29] It was reported that

Table 1 - Median (minimum and maximum) BMD values of vertebra and femur (g/cm²).

Measurement site	Basal	3rd week		7th week		
		ACIM	Control	AST	AFM	Control
Vertebra	0.160 (.14/.19)	0.131*,** (.13/.14)	0.180 (.16/.19)	0.184*,**,† (.16/.22)	0.170† (.16/.18)	0.173 (.17/.18)
Right femur (casted)	0.170 (.13/.19)	0.147*,** (.13/.17)	0.176 (.16/.18)	0.183*,† (.16/.19)	0.160† (.15/.19)	0.179 (.16/.19)
Left femur (free)	0.169 (.13/.19)	0.153*,** (.14/.18)	0.176 (.16/.18)	0.180† (.16/.20)	0.177 (.17/.18)	0.179 (.16/.19)

ACIM - After cast immobilization, AST - After swimming training, AFM - After free mobilization, BMD - bone mineral densities, *$p<0.05$ compared with basal BMD, **$p<0.05$ compared with their age-matched control, †$p<0.05$ compared with ACIM

Table 2 - Changes in body weight (as gram) during the experimental period.

Group	N	Basal	3rd week	4th week	7th week
ST	9	198	180	181	190
FM	9	198	180	183	210
Control	6	198	209	212	222

ST - swimming training, FM - free mobilization

immobilization-induced bone changes are recovered in proportion to the intensity of exercise.[37] A moderate load at frequencies of 4 and 5 days per week was suggested to increase BMD of the long bones.[40] To obtain standard swimming exercise intensities, in some studies, weights have been attached to rats' tail.[35] When comparing to swimming with well designed running protocols, this is logical to make comparisons feasible. However, not involving a running group, but including only free rats in cages, our study did not methodologically necessitate this technique. From another point of view, it can be argued that using free form of swimming may be more appropriate for clinical application, since recommendation of swimming at constant intensities obviously will not be realistic for the patient group of interest. With this design of study, it can be asserted that swimming, in anyway, is beneficial for recovery of bones. Findings suggest that swimming can also be a clinical adjunct to prevent the attenuation of adaptive bone response caused by large number of strain in weight bearing form reported by Umemura et al.[6] As a limitation, examination of bone mechanical properties and histomorphometric indexes were not included in this study. However, previous studies showed that changes in these parameters were nearly always parallel to BMD.[30,35,37] Casting caused weight loss in animals. Moreover, weight gains during mobilization were more rapid in no exercised FM group compared with the swimming animals, suggesting that the thought of body weight is an additional indicator for monitoring and adjusting the training intensity may be valid for also lower level exercise intensities.[35]

In summary, our study showed that swimming exercise had a significant rehabilitative effect on BMD loss associated with immobilization. Our study also suggest that swimming can be recommended at least as an adjunct form of exercise especially after bone mineral loss due to immobilization.

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