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Effect of Aquatic Exercise on Bone Metabolism in a Rat Model of Postmenopausal Osteoporosis

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The effectiveness of aquatic exercise was evaluated for preventing bone loss after menopause. Eight-month-old Wistar strain female rats were divided into a sham group and an ovariectomized (OVX) group. After 15 days, the OVX group was further divided into two groups: a non-exercise (control) group and an aquatic exercise (swimming) group. During this experiment (170 days), all the rats had *ad libitum* access to a solid diet (1.2% Ca, 0.96% P) and drinking water. The bone mineral density (BMD), intestinal Ca absorption, and weight of the skeletal muscles were all reduced by ovariectomy. The BMD value and muscle weight for the OVX swimmng group were, however, significantly higher than those of the OVX control group. Compared with the OVX control group, the decrease in intestinal Ca absorption of the OVX swimming group was significantly alleviated for 6 months, and the energy metabolism accelerated due to the aquatic exercise. These results suggest that aquatic exercise would be effective for preventing a disturbance to bone metabolism in estrogen deficiency.

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Keywords: aquatic exercise, bone mineral density, calcium absorption, muscle weight, ovariectomy.

INTRODUCTION

Osteoporosis is one of the most serious public health problems for elderly people. Bone loss with aging is affected by multiple factors,[1] such as heredity, estrogen deficiency, lack of physical activity, unbalanced nutritions, and life-style. It is a well known fact that when the bone mineral density (BMD) or bone volume falls below a certain level, there is a high fracture risk and improvement of this condition becomes difficult. Consequently, it is essential to prevent osteoporosis. To obtain both a high peak bone mass when young and to prevent bone loss with aging are necessary for preventing osteoporosis. A moderate level of exercise is one of the most important factors for preventing and treating osteoporosis,[2] as well as a sufficient intake of calcium (Ca).[3] It has been reported that weight-bearing exercise in particular is effective for increasing BMD.[4] On the other hand, bone loss results from the removal of mechanical loading caused by bedrest,[5,6] weightlessness,[7] paralysis,[8,9] and immobilization.[10,11] Voluntary exercise has recently been found effective for the prevention or treatment of osteoporosis. However, in many cases, elderly people have back pain and/or joint pain, that make it difficult or impossible to do weight-bearing exercise such as walking, running, weight lifting and tennis. To compensate this problem, aquatic exercise, even though it is a non-weight bearing and does not put mechanical stress on the back and legs, can be done by people with back pain and joint pain. We have reported that aquatic exercise (swimming) was effective for the bone strength and mineral content of the ovariectomized rat.[12] Moreover, consistent participation in water exercise has been reported as an important factor for preventing bone loss.[13] However, the mechanism for improving bone metabolism through aquatic exercise is still unclear. In this study, the BMD value, mechanical bone strength, intestinal Ca absorption, and energy metabolism were examined in an attempt to clarify the effect of aquatic exercise on bone metabolism in a rat model of ovariectomized osteoporosis.

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MATERIALS AND METHODS

Experimental animals and protocol

Eight-month-old Wistar-strain female rats were

used. The rats were divided into two groups: an ovariectomized (OVX) group and a sham operation (sham) group. After 15 days, the OVX group was further divided into non-exercise (control) and aquatic exercise (swimming) sub-groups. The exercise group practiced aquatic exercise (swimming) in a water bath for 40 min/day at noon, six times a week for 170 days. The water bath comprised a stainless steel tank (52×147×60 cm) with a water temperature 28±1℃. During this experiment, all the rats were allowed *ad libitum* access to a solid diet (Table 1: 1.2% calcium (Ca), 0.96% phosphorus (P); Funabashi Co. F-2) and drinking water. They were kept in separate cages (15×25×19.5 cm) in an animal laboratory under the following conditions: temperature, 23±1℃, humidity, 50±5%; fluorescent lighting, for all animals from 7:00 a.m. to 7:00 p.m., with darkness from 7:00 p.m. to 7:00 a.m.

Biochemical assays of the serum

At the end of this experiment, all the rats were deprived of food overnight (7:00 p.m.-9:00 a.m.). The next day, after inducting anesthesia with ether, a blood sample was taken from the abdominal aorta of each rat, these blood samples being centrifuged at 2,500 rpm for 15 min to collect the serum.

The level of serum Ca was measured by atomic absorption spectrophotometry with a Shimadzu AA-640-12 atomic absorption spectrophotometer. Phosphorus was determined by the Fiske-SubbaRow method,[14] and total protein was measured by the Biuret method.[15]

Measurement of the wet weight of skeletal muscles

During the subsequent dissection, the right quadriceps femoris and right triceps surae of each rat were isolated. After washing them in a saline solution, the wet weight of each was measured.

Table 1. Composition of the experimental diet

Constituent	%
Moisture	7.0
Protein	20.8
Fat	4.5
Fiber	3.4
Ash	5.7
Ca	1.2
P	0.96
Mg	0.26
K	0.52
Na	0.19
Fe	0.03
Nitrogen-free extract	58.6

Measurement of the bone mineral density

The lumbar spine, right femur, and right and left tibiae of each rat were isolated by dissection, and all the muscle and connective tissue was carefully removed. Thereafter, the bone mineral density (BMD) values of the fourth and fifth lumbar vertebrae (L4 and L5), the whole femur, and the whole tibia were measured by dual-energy X-ray absorptiometry (DXA) with a Hologic QDR-1000 X-ray bone densitometer as previously reported.[16] All measurements were taken in the ultra-high-resolution scan mode (rat mode, Version 2.0 software), a detector collimator with a single slit being fitted to the X-ray generator, as previously reported,[16] to suit the low density in small animals.

An analysis of tibial BMD and femoral BMD was carried out as previously reported,[17] because of the differing bone structure in different areas of the tibia and femur. Tibial proximal metaphysis, and femoral proximal and distal metaphyses are examples of trabecular bone, while tibial and femoral diaphyses are examples of cortical bone.

Measurement of breaking energy

After measuring the BMD values, the breaking energy at the center of the femoral diaphysis was evaluated by test previously reported[18] under the following measurement conditions: sample clearance, 1.0 cm; plunger speed, 100 mm/min; load range, 50.0 kg.

Balance study

Mineral balance was evaluated at intervals six times during the study to determine the intestinal Ca absorption, Ca accumulation, and urinary phosphorus (P) and creatinine (Cr) excretion. For each evaluation, feces and urine were collected over two 24-h periods. Urine was collected under acidic conditions by using 1 ml of 6 N hydrochloric acid, thus preventing Ca precipitation and putrefaction. The first evaluation was carried out on the last 2 days before starting the exercise period (0 time balance). After starting the exercise period, the mineral balance was evaluated once a month (1, 2, 3, 4 and 5 month balance). All the collected urine was centrifuged at 2,500 rpm for 15 min, as quickly as possible, to extract the supernatant. For the fecal determination, all daily feces were burnt to ash at 550-600℃ for approximately 18 h, and the resulting ash was dissolved in 1 N nitric acid. The urinary and fecal Ca

excretions and the urinary P excretion were measured by using the same method as that for the biochemical assay of the serum, while Cr excretion was measured by the Folin-Wo method.[19]

Statistical methods

The Student's *t*-test was used to analyze differences between the OVX group and the sham group, $p<0.05$ being considered statistically significant within each experiment. The *t*-test was also used to analyze the differences between the control group and the aquatic exercise group.

RESULTS

The body weight gain, food intake and food efficiency during the experimental exercise period are shown in Table 2. The initial body weight was approximately 290 g in each group (sham group: 289.4 ± 4.4 (mean ± SE), OVX control group: 291.0 ± 2.5, OVX swimming group: 292.0 ± 3.2). However, the body weight gain of the OVX control group was significantly higher than that of the sham group ($p<0.01$), while the body weight gain of the OVX swimming group was significantly lower than that of the control group. In respect of food intake, there was no significant difference between the sham and OVX control groups, nor between OVX control group and the OVX swimming group. The food efficiency of the OVX control group was significantly higher than that of the sham group ($p<0.001$), and that of the OVX swimming group was significantly lower than that of the OVX control group ($p<0.001$). The levels of serum Ca, P, and total protein did not differ among the three groups (data not shown).

The BMD values for the femur, tibia and lumbar spine, and the breaking energy of the femur are shown in Figs. 1-4. The BMD values at each site for the femoral proximal and distal metaphyses, femoral diaphysis, tibial proximal metaphysis and diaphysis, and the lumbar spine for the OVX control group were significantly lower or tended to be lower than those for the sham group. On the other hand, the BMD values at each site for the OVX swimming group were significantly greater than those for the OVX control group.

There was a significant difference between the final body weight of the sham group and that of the OVX control group, and between the final body weight of the OVX control and OVX swimming groups; however, the breaking energy, which represents the mechanical bone strength, of the OVX control group was slightly lower than that of the sham group, while that of the swimming group was higher than that of the OVX control group (Fig. 4). In this experiment, there was no difference in the bone length, width and depth among the three groups.

The intestinal Ca absorption is shown in Fig. 5, the values for all the groups decreasing during the 6-month period. However, in the OVX-swimming group, the decrease in intestinal Ca absorption was significantly alleviated when compared with the OVX control group. The variation in the results of the Ca accumulation, rate of Ca accumulation, and rate of Ca absorption was almost the same as that of the intestinal Ca absorption.

Figure 6 shows the urinary Cr excretion, the value for the OVX swimming group being significantly higher or tending to be higher than that for the OVX

Table 2. Body weight gain, food intake and food efficiency

	Body weight gain (g/day)	Food intake (g/day)	Food efficiency
Sham	0.38±0.04 **	13.5±0.3	0.03±0.002 ***
OVX			
Control	0.52±0.02	13.7±0.2	0.04±0.001
Swimming	0.20±0.03 ***	14.3±0.6	0.01±0.001 ***

Mean±SE. **$p<0.01$, ***$p<0.001$.

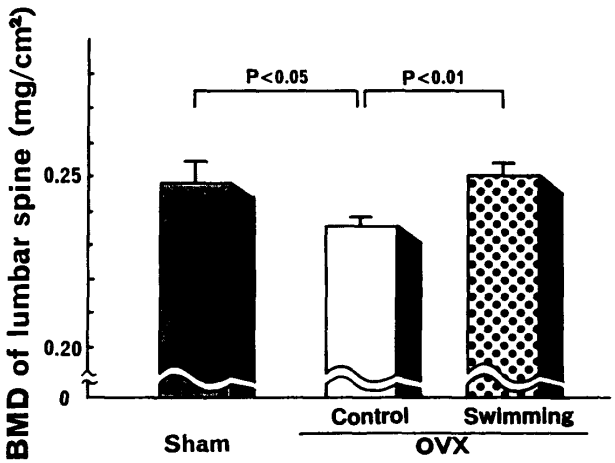

Fig. 1. Bone mineral density of the lumbar spine

The fourth and the fifth lumbar vertebrae of each rat were isolated by dissection, and the muscle and connective tissue were carefully removed. The bone mineral density (BMD) was then measured by dual-energy X-ray absorptiometry. Vertical bars indicate the standard error.

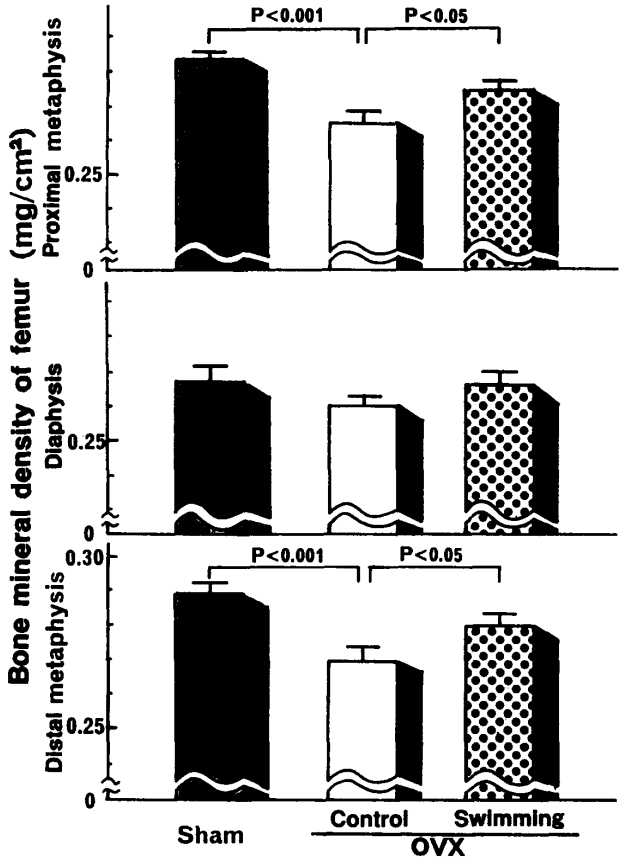

Fig. 2. Bone mineral density of the femur

The right femur was isolated by dissection, and the muscle and connective tissue were carefully removed. The bone mineral density (BMD) was then measured by dual-energy X-ray absorptiometry. Vertical bars indicate the standard error.

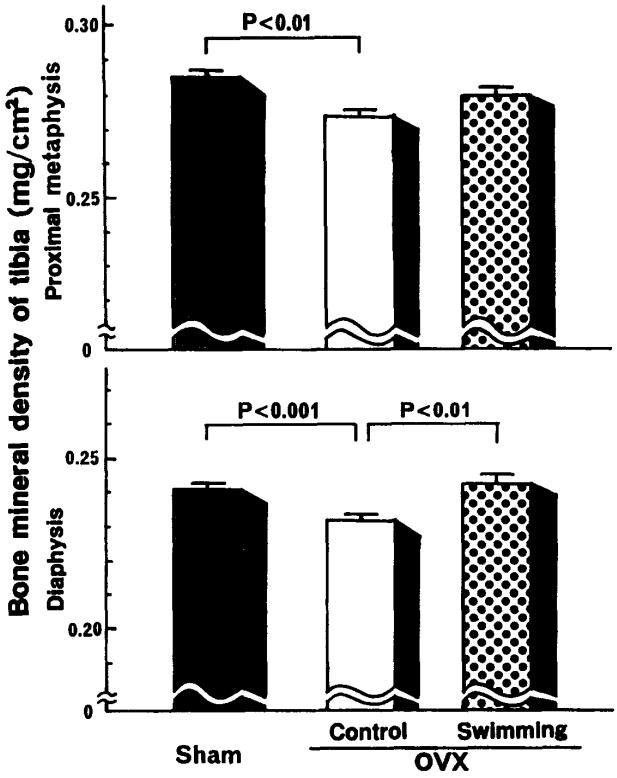

Fig. 3. Bone mineral density of the tibia

The right and left tibiae were isolated by dissection, and the muscle and connective tissue were carefully removed. The bone mineral density (BMD) was then measured by dual-energy X-ray absorptiometry. Vertical bars indicate the standard error.

control group after 3 months. The urinary P excretion is shown in Fig. 7, and was also significantly higher for the OVX swimming group than for the OVX control group. The skeletal muscle weight of the OVX control group was reduced by ovariectomy, while the muscle weight of the OVX swimming group was slightly or significantly higher than that of the OVX control group (Fig. 8).

DISCUSSION

Osteoporosis is one of the involutional disorders resulting in bone loss and/or fracture. The bone mass decreases rapidly after menopause because of the reduced estrogen secretion at menopause. Due to an increase in the number of elderly people in Japan, osteoporosis is becoming more and more of a major public health problem. Accordingly, it is necessary to prevent, or at least delay, osteoporosis. It is a well-known fact that a moderate level of physical activity,[2] especially weight-bearing exercises,[4] is effective for increasing bone mass. Aloia *et al.* have reported that total body Ca increased as a result of moderate exercise in postmenopausal women, and that there was a positive correlation between the bone mineral content (BMC) and the daily physical activity of elderly people.[20] BMD in women aged between fifty and sixty, who exercise regularly, was also reportedly greater when compared with those people who are not used to doing regular exercise.[21] On the other hand, substantial bone loss could be caused by a sedentary lifestyle and/or immobilization. Moreover, astronauts during space flight can lose total body Ca,[22] and their skeletal muscles and bones become atrophied because of the loss of mechanical loading due to the lack of gravity in space. However, a decrease in bone mass could be prevented by an increased Ca intake and by carrying out physical exercise in space.[22] This result suggests that physical exercise with mechanical non-loading could be effective for preventing bone

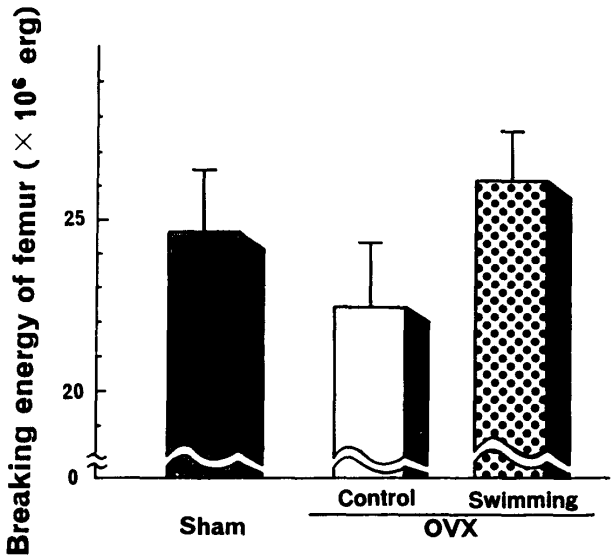

Fig. 4. Breaking energy of the femoral diaphysis

The right femur was isolated by dissection, and the muscle and connective tissue were carefully removed. The breaking energy at the center of the femoral diaphysis was then evaluated. Vertical bars indicate the standard error.

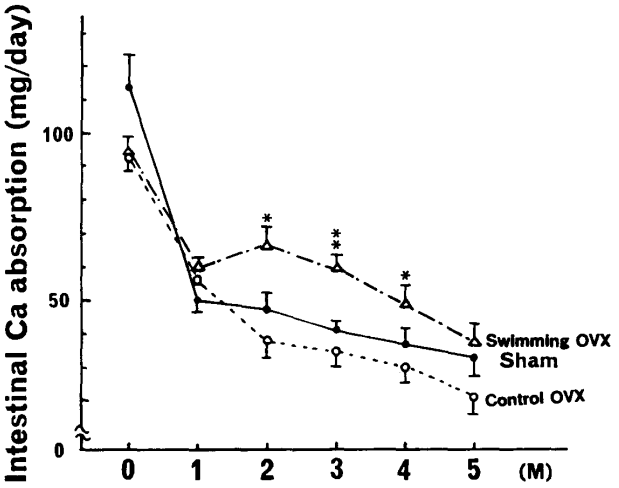

Fig. 5. Intestinal calcium absorption over 6 months

Six balance evaluations were carried out at intervals during the experiment. The first was carried out during the last 2 days before starting the exercise period (0 time balance). After starting the exercise, the balance was evaluated once a month (1M, 2M, 3M, 4M and 5M balance). The key to the symbols used in Fig. 5 is as follows: ●, sham group; ○, OVX control group; △, OVX swimming group. *Significantly different from the OVX group, $p<0.05$. **Significantly different from the OVX group, $p<0.01$. Vertical bars indicate the standard error.

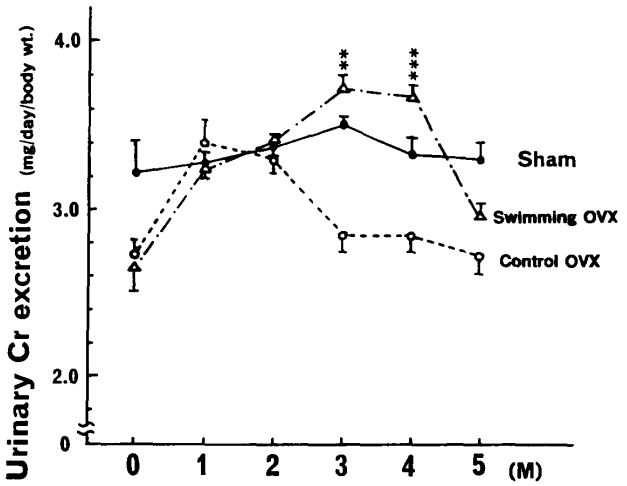

Fig. 6. Urinary creatinine excretion over 6 months

Six balance evaluations were carried out at intervals during the experiment. The first was carried out during the last 2 days before starting the exercise period (0 time balance). After starting the exercise, the balance was evaluated once a month (1M, 2M, 3M, 4M and 5M balance). The key to the symbols used in Fig. 6 is as follows: ●, sham group; ○, OVX control group; △, OVX swimming group. **Significantly different from the OVX group, $p<0.01$. ***Significantly different from the OVX group, $p<0.001$. Vertical bars indicate the standard error.

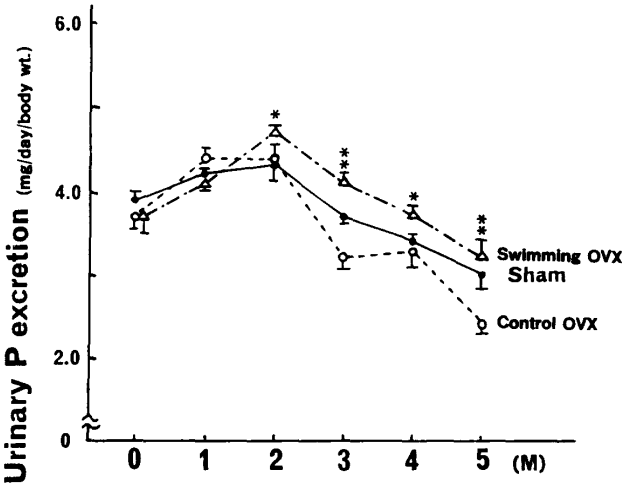

Fig. 7. Urinary phosphorus excretion over 6 months

Six balance evaluations were carried out at intervals during the experiment. The first was carried out during the last 2 days before starting the exercise period (0 time balance). After starting the exercise, the balance was evaluated once a month (1M, 2M, 3M, 4M and 5M balance). The key to the symbols used in Fig. 7 is as follows: ●, sham group; ○, OVX control group; △, OVX swimming group. *Significantly different from the OVX group, $p<0.05$. **Significantly different from the OVX group, $p<0.01$. Vertical bars indicate the standard error.

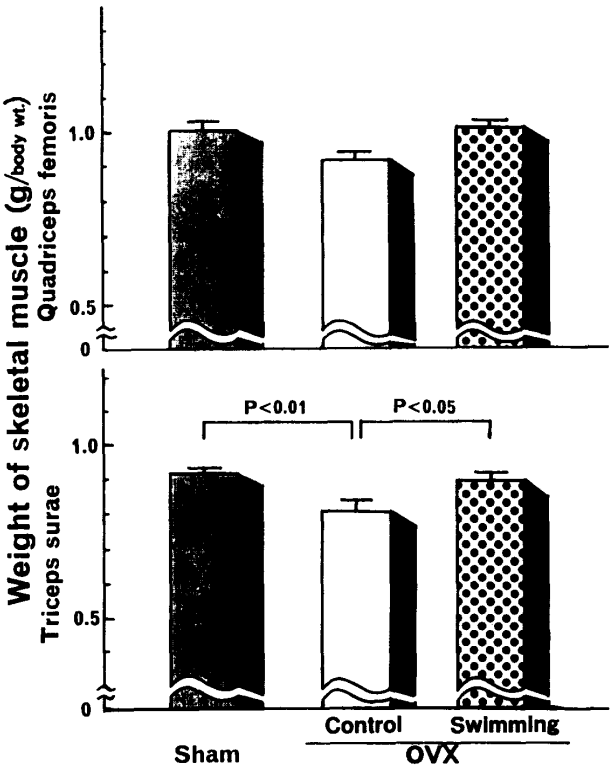

Fig. 8. Weights of the skeletal muscles

The right quadriceps femoris and right triceps saurae were carefully removed by dissection, and the wet weight of each was measured. In this study the final body weight of each group was significantly different. Therefore, the results are presented as the muscle weight per body weight. Vertical bars indicate the standard error.

loss. A great deal of research, which has evaluated whether exercise is effective for preventing osteoporosis especially in postmenopausal women, has been reported.[23,24] Orwoll *et al.* have reported that the BMD values for the radius and lumbar spine in male swimmers aged 40 to 80 was significantly greater than that of the non-swimming control group, and also that the BMD values for female swimmers tended to be greater than those for the control group.[25] We have reported that consistent aquatic exercise was one of the important factors for increasing daily physical activity, improving the subjects' awareness of health and fitness in daily life, and preventing bone loss.[13] In addition, we have also suggested that aquatic exercise was effective for increasing the mechanical bone strength and the mineral content of the femur in ovariectomized rats.[12] Furthermore, the effects of swimming on bone growth, on the development of the long bone, and on the increase in bone mass, mineralization and hydration properties have been reported in young and adult female rats.[26,27] However, details about the effect of aquatic exercise and the mechanism for the improvement of bone metabolism through aquatic exercise have not been evaluated. Therefore, in this study, the BMD value mechanical bone strength, intestinal Ca absorption, Ca accumulation, energy metabolism, and weight of the skeletal muscles were examined to clarify the effects of aquatic exercise on bone metabolism in osteoporotic rats.

The BMD values for the lumbar spine, femur and the tibia, and the breaking energy of the femur were found to decrease after the rats had been ovariectomized. However, such a decrease in the BMD values and breaking strength in the OVX swimming group was prevented by 6 months of aquatic exercise. These results indicate that aquatic exercise, which is a non-weight-bearing exercise, could be effective for preventing a decrease in the BMD value and bone strength. It is possible to say that the movement in water helps to alleviate bone loss in elderly people with back pain and/or joint pain.

As shown in Fig. 5, the intestinal Ca absorption of the rats in the aquatic exercise group was significantly higher than that of the non-exercise control group. We have reported that a decrease in intestinal Ca absorption and Ca retention in old rats within OVX and sham groups was alleviated by using the voluntary treadmill running exercise.[28] Moderate physical activity thus improved the negative Ca balance in old rats. Based on this result, aquatic exercise as a means of increasing daily physical activity would be effective for enhancing intestinal Ca absorption and Ca accumulation.

Urinary Cr and P excretion increased significantly or tended to increase in the aquatic exercise group (Figs. 6 and 7). It has been reported that urinary Cr and P excretion was increased by voluntary running exercise, and that the increase was concurrent with the increase in distance run.[17] In addition, in the present experiment, the food efficiency of the OVX swimming group was significantly lower than that of the OVX control group. These results of increasing urinary Cr and P excretion and the low level of food efficiency indicate that the energy metabolism was elevated by doing aquatic exercise. These results are similar to those reported in the previous paper.[17] It has been reported that the BMD value, bone strength, Ca content in bone and plasma Ca level were each decreased under the condition of restricted energy intake.[29] It is possible that proper energy metabolism

is necessary for normal Ca metabolism; that is, an acceleration in the energy metabolism might have a positive effect on Ca metabolism. Further investigation is required to clarify the connection between energy metabolism and Ca metabolism.

The level of urinary Cr excretion depends on the body weight and skeletal muscle volume. Consequently, the lower level of Cr excreted by the OVX group in comparison with the sham group suggests that the rats in the OVX group had atrophied muscles. Moreover, the weight of the skeletal muscles in the OVX control group was significantly or slightly lighter than that of the sham group. It has been found that skeletal muscle became atrophied due to ovariectomy.[30] Therefore, it is possible to say that the skeletal muscle volume was reduced by ovariectomy from the results of this experiment. It has also been reported that skeletal muscle became atrophied with a loss of mechanical loading.[30] On the other hand, it has been reported that running exercise was effective for increasing the weight of the quadriceps femoris and soleus.[30,31] In this study, the weight of the skeletal muscle also became heavier by doing aquatic exercise. Moreover, Cr excreted by the OVX swimming group in the final phase was higher than that by the OVX control group. Based on this data, a reduction in the volume of the skeletal muscle was alleviated by aquatic exercise, even though aquatic exercise is mechanically non-loading. It is known that the skeletal muscle and bone mass become atrophied due to the loss of mechanical loading that is caused by immobilization of the extremities,[30,32] and that there was a positive correlation between the BMD value and the power of the skeletal muscle.[33] Accordingly, the increase in weight of the skeletal muscle could have been effective for preventing bone loss in the aquatic exercise group.

These results indicate aquatic exercise to be an effective form of physical activity for maintaining the BMD value, bone strength, and skeletal muscle, and for enhancing intestinal Ca absorption and accelerating energy metabolism. It could be that the enhanced intestinal Ca absorption, accelerated energy metabolism, and the greater volume of skeletal muscle were factors contributing to the higher BMD values and bone strength in this study. Experimental animals such as rats are not congruent with human beings. However, it is thought that aquatic exercise, which is a mechanically non-loading exercise, could be regarded as valuable and effective physical activity for increasing the BMD value and/or preventing bone loss in elderly people.

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閉経後骨粗鬆症モデルラットの骨代謝に対する水中運動の効果

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本研究では，水中運動が，閉経後の骨量減少の抑制に効果的であるか否かを検討した．8カ月齢Wistar系雌ラットに卵巣摘出術または偽手術を施した．術後15日目に，卵巣摘出群は，非運動群と水中運動群に分けた．なお，本研究期間中（170日），ラットには固形飼料と水道水を自由摂取させた．その結果，骨密度，腸管からのカルシウム吸収，および筋重量は卵巣摘出により低下した．しかし水中運動群の骨密度および筋重量は非運動群に比べ有意な高値を示した．腸管からのカルシウム吸収量においては，いずれの群も飼育期間中減少したが，水中運動群は非運動群に比べ，その減少が有意に抑制された．また，水中運動によりエネルギー代謝が亢進された．以上より，水中運動は，閉経後の骨代謝の改善に効果的であることが示唆された．

キーワード：水中運動，骨密度，カルシウム吸収，筋重量，卵巣摘出．