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Original article

Effects of short-term swimming exercise on bone mineral density, geometry, and microstructural properties in sham and ovariectomized rats

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Abstract

Little information exists about the effects of swimming exercise on bone health in ovariectomized animals with estrogen deficiency, which resembles the postmenopausal state and age-related bone loss in humans. This study investigated the effects of swimming exercise on tibia and femur bone mineral density (BMD), geometry, and microstructure in sham and ovariectomized rats. Forty 3-month-old female rats were divided into four groups: sham operated-sedentary control (Sham-control), sham operated with swimming exercise group (Sham-Swim), ovariectomy-sedentary control (OVx-control), and ovariectomy and swimming exercise (OVx-Swim) groups. Swimming sessions were performed by the rats 90 minutes/day for 5 days/week for a total of 8 weeks. At the end of the study, tibial and femoral proximal volumetric total BMD, midshaft cortical volumetric BMD, cross-sectional area, and cross-sectional moment of inertia (MOI), and bone microstructural properties were measured for comparison. Data were analyzed using one-way analysis of variance (ANOVA). The Sham-Swim group exhibited significantly ($p < 0.05$; one-way ANOVA) greater values in bone geometry parameters, that is, tibial midshaft cortical area and MOI compared to the Sham-control group. However, no significant differences were observed in these parameters between the Ovx-Swim and Ovx-control groups. There were no significant differences in femoral BMD between the Sham-Swim and Sham-control groups. Nevertheless, the Ovx-Swim group elicited significantly ($p < 0.05$; one-way ANOVA) higher femoral proximal total BMD and improved bone microstructure compared to the Ovx-Sham group. In conclusion, the positive effects of swimming on bone properties in the ovariectomized rats in the present study may suggest that swimming as a non- or low-weight-bearing exercise may be beneficial for enhancing bone health in the postmenopausal population.

Keywords: Bone cortical area; Bone microstructural properties; Bone mineral density; Bone moment of inertia; Swimming exercise

Introduction

Bone is made up of microscopic crystals of phosphates and calcium contained within a matrix of collagen. It protects vital organs and the bone marrow, and the rigidity that it provides permits locomotion and the support of loads against gravity. All healthy humans gain bone mass during growth early in life. After the attainment of maximum bone mass, by about 30 years of age, bone mass remains constant until about the age of 40–45 years, after which it begins to decrease in both men and women.[1–3] This gradual decline in bone mass can lead to serious consequences, particularly in females following menopause. Adult females generally have less bone mass than adult males, and immediately after menopause they lose it

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more rapidly than men of a comparable age do.[4] Consequently, women are more prone to developing serious osteoporosis.[5–8]

Hormones are key regulators of growth and maintenance of skeletal tissue, and gonadal hormones such as estrogen, progesterone, and testosterone in particular have an important impact on bone physiology. It is also generally known that insufficient levels of gonadal hormones predispose the human skeleton to bone loss and osteoporotic fractures.[9–12] Estrogen is the dominant hormone in skeletal regulation, and its level is reduced with advancing age and during menopause.[13,14]

As mentioned by Kanis,[8] Manolagas et al,[9] Syed and Khosla,[10] and Frenkel et al,[11] a decrease in bone mass is usually associated with estrogen deficiency in postmenopausal osteoporosis, in which estrogen deficiency increases bone turnover and induces an imbalance between resorption and formation, a relative deficit in bone formation, and eventually accelerates skeletal losses and increases fracture risk.

Osteoporosis is characterized by low bone mass and microarchitectural deterioration of bone tissue, with a consequent increase in bone fragility and susceptibility to fracture.[6,7,15,16] The recognition of osteoporosis as a major health problem among a growing number of elderly people around the world has resulted in widespread efforts to determine ways to reduce the rate of bone loss. These could be achieved through proper diet,[17] estrogen therapy,[18,19] and regular exercises.[20–23]

It is generally accepted that rats are excellent animal models for studying bone health due to the similarity in terms of morphometry and structure with human bone. Ovariectomy of the animal, which resembles the postmenopausal state in humans, has been used for bone morphometry analysis of bone fragility and is considered to serve as a highly predictive model of fracture risk in humans.[24,25] This commonly used protocol to induce menopause in animals has been reported to result in bone loss due to estrogen deficiency.[26–29] Ovariectomy is not limited to mature and aging rats as young female rats can also be used for ovariectomy. A previous bone study by Honda et al,[26] reported that 12-week-old young female rats were used to mimic the postmenopausal population via ovariectomy. Similarly, in another study by Estai et al,[28] a group of young rats were ovariectomized for the induction of an estrogen deficiency condition to mimic the postmenopausal population.

Forces generated through mechanical loading during exercise promote osteogenesis; therefore weight-bearing exercises are conventionally prescribed for enhancing bone health. Nevertheless, the high force magnitude elicited by high mechanical loading may cause detrimental effects on osteoporotic bones. Numerous previous studies on the effects of swimming, which is a non- or low-weight-bearing exercise, on bone health have been conducted in young animals.[30–34] However, to date, little information exists about the effects of swimming exercise on bone mineral density (BMD), geometry, and microstructural properties in ovariectomized animals with estrogen deficiency, which resemble the postmenopausal state and age-related bone loss in humans. It was hypothesized that the ovariectomy process carried out in the present study can elicit negative effects on bone properties because estrogen deficiency could cause imbalance in bone resorption and formation, and a relative deficit in bone formation, resulting in accelerated bone loss.[9–11,26,27] Additionally, swimming exercise as a non- or low-weight-bearing exercise, which is an alternative to moderate- and high-weight-bearing exercises, was believed to be a type of less strenuous exercise for older population with bones that are less strong compared to young bones. Therefore, the present study was carried out to compare the effects of an 8-week period of short-term swimming exercise on tibial and femoral BMD, geometry, and microstructural properties in sham and ovariectomized 3-month-old rats. It is hoped that the results of the present study can be extrapolated to postmenopausal women particularly for preventing and/or reducing bone loss.

Methods

Animal groups

In this study, forty 3-month-old female Sprague Dawley rats, weighing between 250 g and 300 g, were used. The rats were housed at normal room temperature with adequate ventilation and a normal 12-hour light/dark cycle, and free access to water and chow (Gold Coin, Port Klang, Malaysia) throughout the experiment. They were divided into four main groups: sham operated-sedentary control (Sham-control, $n = 10$); sham operated with swimming exercise (Sham-Swim, $n = 10$); ovariectomy-control (OVx-control, $n = 10$); and ovariectomy and swimming exercise (OVx-Swim, $n = 10$) groups. The body weight of each rat was recorded at the following stages: preovariectomy, postovariectomy at 4 weeks (i.e., the initial body weight prior to intervention), and after intervention (the final body weight).

Ovariectomy

Bilateral ovariectomy (OVx) was performed using a ventral approach under anesthesia. The rats were anesthetized with an intramuscular (IM) injection of Ketapex 0.1 mL and Xylazil 0.03 mL. Gas anesthesia (isoflurane) was used if the animals woke up during the surgical procedure. The animals were placed in dorsal recumbency to make a midline coeliotomy incision. A 1-cm incision was made between the umbilical scar and the pubis. The ovaries were removed using a forceps. The ovarian vessels and Fallopian tubes were ligated individually and the ovary and periovarian fat were removed. The skin incision was closed with wound clips. The same procedure was followed for the sham operated group (Sham) except for the removal of the ovaries. The rats were randomly allocated to nominated groups after the ovariectomies were performed. The intervention was started 4 weeks after ovariectomy to ensure healing and clear estrogen residues.

Swimming exercise

Swimming exercise was performed by rats 90 minutes/day for 5 days/week for a total of 8 weeks with the water temperature maintained at room temperature.[35] All of the exercise