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SWIMMING VERSUS VOLUNTARY RUNNING EXERCISE ON BONE
HEALTH IN OVARIECTOMIZED RETIRED BREEDER RATS

A Thesis

Submitted to the Graduate Faculty of the
Louisiana State University and
Agricultural and Mechanical College
in partial fulfillment of the
requirements for the degree of
Master of Science

in

The School of Human Ecology

by
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B.S. Louisiana State University, 2001
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LIST OF ABBREVIATIONS

°C	Degrees Celsius
μL	Microliter
21S	21% Soy diet
AA	Atomic Absorptiometry
ad lib	*ad libitum*
ANOVA	Analysis of variance
BHT	Butylated hydroxytoluene
BMC	Bone mineral content
BMD	Bone mineral density
BMI	Body mass index
Ca	Calcium
cm	Centimeter
CRB	Casein and rice bran oil group
dl	Deciliter
ERT	Estrogen replacement therapy
FSH	Follicle stimulating hormone
g	Gram
K	Potassium
kg	Kilogram
$LaCl_3$	Lanthanum chloride
LH	Luteninizing hormone
LSU A&M	Louisiana State University and Agricultural and Mechanical College
mg	Milligram
ml	Milliliter
mm	Millimeter
Na	Sodium
nm	Nanometers
O	Ovarictomized group, no exercise
OP	Osteoporosis
OR	Ovariectomized and running group
OS	Ovariectomized and swimming group
OVX	Ovariectomy
P	Phosphorus
pDEXA	Peripheral dual-energy X-ray absorptiometry
ppm	Parts per million
RT	Room temperature
Sh	Sham-operated group, no exercise
TBW	Total body weight
TGF	Transforming growth factor
WHO	World Health Organization
HCl	Hydrochloric acid

ABSTRACT

Physical activity may increase long bone calcium (Ca) content to preserve bone strength in postmenopausal women. This study determined the effect of compulsory swimming and voluntary running exercise on bone mineral density, bone Ca and phosphorus (P) content, and femoral neck and tibia strength in ovariectomized (OVX) retired breeder rats, as a model for postmenopausal women. Thirty-seven nine-month old Sprague Dawley rats were assigned randomly into one of four treatment groups for the nine-week study: OVX + running (OR; n=9); OVX + swimming (OS; n=10); OVX + no exercise (O; n=9); sham-surgery + no exercise (Sh; n=9). OR rats had free access to running wheels; OS rats were trained over one week to swim for one hour, five days a week. At sacrifice, femurs, tibias, humeri, and vertebrae were removed. Bone mineral density was analyzed using pDEXA, and bone Ca and P content were analyzed using atomic absorption spectrometry and colormetric assay, respectively. Femur and tibia strength was determined by *Q-tester*. Bone mineral density was significantly higher for all bones measured in the exercise groups compared to the sedentary groups. Mean grams (g) of Ca per dry femur weight for OS rats were higher than O rats (p=0.019). Tibias of the OR and OS rats were able to absorb significantly more energy to break load than the O rats (p=0.000; p=0.001, respectively), and energy absorption was significantly higher for the OR compared to Sh tibia (p=0.022). No other significant differences in parameters were observed among the four groups. Results of this study suggest that both types of exercise improve bone mineral density, that swim exercise may be beneficial in preserving femur Ca content, and that swim exercise and voluntary running may be beneficial in improving tibia strength in OVX rats.

INTRODUCTION

Osteoporosis (OP) is a multifactorial skeletal condition and is a primary cause for poor quality of life and increased medical expenses in those affected with this condition (23, 41). Osteoporosis is a major public health threat for an estimated 44 million Americans (16). In the U.S. today, 10 million individuals are estimated to already have the disease and almost 34 million more are estimated to have low bone mass, placing them at increased risk for osteoporosis (16). Eighty percent of those affected by osteoporosis are women (23, 41). One in five women over the age of 70 and one in three women over the age of 80 will suffer a hip fracture during her lifetime, and nearly one in five women with a hip fracture will die within 6 months of the injury (Hoshi). Death following a hip fracture is usually the result of complications from immobility. One third of women older than 65 years of age will have a fracture of the spine caused by osteoporosis (23). Chronic back pain resulting from fractures of the spine may prevent some physical activities and affect normal sleeping.

Osteopenia is a progressive condition that places patients at risk for increased morbidity and mortality if untreated. Patients with bone loss of at least 1.0 standard deviation (SD) from normal are considered osteopenic, whereas those with bone loss of at least 2.5 SDs are considered osteoporotic (2, 43). Generally, OP exhibits both reduced bone matrix and bone mineral density (20); thus, the quality of the bone remains about the same, there is just less bone present (51). Osteoporotic related bone loss is due to osteoclast activity exceeding osteoblast activity (bone resorption exceeds bone formation) (42, 2, 43). Accelerated bone loss following menopause is associated with estrogen deficiency; thus, postmenopausal women are at an increased risk of developing OP (8, 42).

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Hip fracture is perhaps the most serious type of fracture associated with OP; twenty percent of patients who suffer hip fractures do not survive one year after fracture (16). Among the important risk factors for falls, muscle weakness is one that can be improved with strength training (28). Some studies that measure mechanical properties of bone in rats use three point bending to break the femur at the middiaphysis which is easier to standardize (19, 60, 23, 35, 38). Measurement of the load needed to break the bone at the femoral neck would be more accurately applied to the age group at risk for osteoporosis, since a fall to the hip is most likely to cause a break at this site.

It has been suggested that loss of estrogen results in a reduction in muscle strength (40, 6). Mechanical factors such as muscle contraction, pressure and tension exerted by adjacent connective and muscle tissue, and gravitational force play and important regulatory role in bone development (52). Bone reacts to imposed stress by changing its structure and increasing its mass (36, 57).

Running imposes various stresses on the entire body, but are no gravitational forces acting on the limbs during swimming exercise (36). The importance of physical stress in preventing bone loss and maintaining bone mass is generally accepted (53, 47); however, there is inconclusive evidence on the effect swimming activity on bone (19). Rourke, et al. (1998) demonstrated that BMD was significantly higher in female swimmers compared to runners, indicating a beneficial effect of swimming on bone health in young females.

There are many studies on swimming and bone mass that have been conducted in younger, elite athletes, are cross-sectional, and demonstrate few skeletal benefits (1, 34, 26, 13, 7, 9, 22, 17); however, there is little information on bone mass in noncompetitive athletes.

Further, the control groups used in these studies tend to lead active lifestyles; thus, the addition of a swim program might not result in significant changes in bone status.

No relationship of swimming to BMD was identified in women aged 40-85 years (37); whereas, water exercise (such as water aerobics) was shown to be beneficial in preserving BMD in Japanese postmenopausal women (59). Activity levels are usually low in the elderly population due to fear of falling, lack of motivation, or decreased strength. Thus, introduction of a non-weight bearing form of physical activity, such as swimming, would likely appeal to this age group.

Swimming may be beneficial for the bones of young, growing rats (50, 55, 56, 36); however, there are limited data on the effects of swimming in older female rats with estrogen deficiency, which resembles the postmenopausal state (57, 36, 19). Results vary with training types, intensity, duration, and age at the onset of exercise. Older animals subjected to physical activity may exhibit a different pattern of mineralization compared with younger animals (30). Intense exercise programs that increase the production of stress hormones may have negative effects on bone formation (35, 5, 19); whereas, exercise of very low intensity may not be adequate to prevent bone loss (23).

The purpose of the present study was to determine the effect of voluntary running and non-weight bearing swimming exercise on bone mineral content of the humerus, tibia, and femur and breaking strength of the femur and tibia in OVX retired breeder rats.

CHAPER 1: REVIEW OF LITERATURE

Properties of Bone

Bone Cells and Their Function

Various bone cells are involved in the process of bone metabolism; however, the dynamic processes in bone are largely controlled by four types of bone cells: the lining cells, osteoblasts, osteoclasts, and osteocytes (20, 8). These cells are responsible for maintaining mechanical properties of bone and mediating the Ca homeostatic function of bone (20). Combined and cooperative actions of osteoblasts and osteoclasts result in a bone architecture that provides mechanical support and protection for the body.

Lining cells are fibrocyte-like, flat cells that form a membrane that completely covers free bone surfaces and insulates bone from the cells and hormones in the general circulation (20). Osteoblasts originate from mesenchymal stem cells and secrete collagen into the bone matrix (8). These cells are responsible for laying down bone, synthesizing, depositing, and orienting the fibrous proteins of the matrix, then initiating changes that enable the matrix to become mineralized (20). Hence, osteoblasts control the bone formation process.

Approximately 10-20% of osteoblasts become osteocytes during the bone formation process (8). Osteocytes are osteoblasts that have stopped matrix synthesis, becoming embedded in bone as other bone-forming cells in their proximity continue to add new layers of matrix. Osteocytes are responsible for monitoring the amount of strain occurring in their vicinity when bone is mechanically loaded and for reporting that information to the lining cells on nearby bone surfaces, possibly initiating local bone remodeling (20).

Osteoclasts are large multinucleated bone-resorbing cells (20, 8, 61). During bone resorption, osteoclasts produce and release lysosomal enzymes, hydrogen protons and free

radicals into a confined space next to the bone. These dissolve mineral and degrade bone matrix (61). Osteoclasts then release the breakdown products into the extracellular fluid around the resorption site, where circulating blood carries the by-products away. The Ca and P that are released into the bloodstream may be used to mineralize bone elsewhere in the skeleton; whereas, the protein fragments are metabolized or excreted (20). Osteoclasts eventually undergo apoptosis, leaving their excavation to be filled by osteoblasts.

Composition and Structure

Bone is connective tissue consisting of approximately 2% living cells and 95-98% nonliving material embedded within or lining surfaces of a mineralized organic matrix (61, 20). The nonliving material consists of mineral-encrusted protein matrix, called osteoid, and is responsible for the hardness, stiffness, and resiliency of bone (20). The hardness and rigidity of bone tissue provides protection for soft organs and tissues, thus enabling the skeleton to maintain the shape of the body and transmit the force of muscular contraction from one part of the body to another (53, 61).

Bone is physiologically mineralized with hydroxyapatite. Calcium (Ca) comprises 37 to 40%, phosphate, 50 to 58%, and carbonate 2 to 8% of this mineral (20). Bone mineral also contains small amounts of sodium (Na), potassium (K), magnesium (Mg), citrate, and other ions present in the extracellular fluid at the time the mineral was deposited, absorbed onto the crystal surfaces and trapped there as the water in the recently deposited matrix is displaced by the growing mineral crystals (20).

The protein matrix of bone consists predominately of type I collagen, which comprises approximately 90% of the organic matrix (20). Collagen is a long fibrous protein, coiled as a triple helix. Every third amino acid in the body of collagen is glycine, which has a compact

structure enabling the protein to coil very tightly. The side chains of various amino acids, such as lysine, project outward from the protein, allowing posttranslational formation of tight covalent bonds between collagen fibers (20). Thus, the fibers are prevented from sliding along one another when bone is stressed along the axis of the fibers.

Bone can be divided into two types of bone: cortical and trabecular. Bone consists of a dense outer shell, or cortex, and an internal, chambered system of interconnected plates, rods, and spicules called cancellous, or trabecular bone. The cortical component of bone predominates in the shafts of long bones and creates a hollow tube, while nearer the joints the cortex becomes thinner (20). The interior of long bones is made up of an extensive latticework of cancellous bone. The proportions of mineral and matrix and of Ca and P are essentially identical in cortical and cancellous bone; however, cancellous bone turns over much more rapidly than cortical bone (20). Cortical bone primarily serves mechanical and protective functions; whereas, trabecular bone primarily serves metabolic functions (8, 61).

The end segments of long bones are called epiphyses, and the shafts of bones are called diaphyses. The flared portion of the shaft merging with the region of the growth plate is called metaphysis (20). The lining cells on the outside of the bone form a tough membrane called the periosteum, and the cells on the inside surfaces of both cortical and trabecular bone are called the endosteum (20). Joints, or ends where bones meet one another, are covered with a layer of cartilage, rather than with periosteum. Cartilage is highly hydrated and lubricated by synovial fluid held there by a tough connective tissue sac called the joint capsule, ensuring that bones move on one another smoothly (20).

Anatomically, the bones of the skeleton can be classified according to their individual shapes: flat, short, irregular, and long (61). Long bones include the humerus, radius, ulna, femur,

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and tibia, and are formed through endochondral ossification. This is the process in which embryonic mesenchymal cells differentiate into chondroblasts and chondrocytes that secrete hyaline cartilage matrix and produce a cartilage model of the future bone (61).

Mechanical Measurements of Bone

Mechanical Properties

The concepts of stress and strain are fundamental to bone biomechanics. Stress is defined as force per unit area and, depending upon how loads are applied, may be classified as compressive, tensile, or shear (60). If loads are applied so that a material becomes shorter, compressive stresses are developed; however, if a material is stretched when loads are applied, tensile stresses are developed. Shear stresses are developed when one region of a material slides relative to an adjacent region (60). Compressive, tensile, and shear stresses occur in combination, even under the simplest loading schemes. Strain is defined as percentage change in length or relative deformation.

The slope of the stress-strain curve within the elastic region is called the elastic or Young's modulus and is a measure of the intrinsic stiffness of the material (60). The maximum stress a bone can sustain is called the ultimate strength, and the breaking strength is the stress at which the bone actually breaks. Strength is an intrinsic property of bone; thus, strength is independent of bone size and shape (60). Bone strength is often reported in units of force, or in terms of breaking load. Failure is defined as the number of cycles necessary to cause a specimen to lose 30% of its intrinsic stiffness (60). Mechanical testing machines often produce a plot that allows these properties of bone to be measured graphically.

Methods for Testing Mechanical Properties of Bone

Basic tools for biomechanical testing of bone include a mechanical testing machine, strain measurement transducers, and a system for recording stress and strain (60). Most mechanical testing machines are equipped with a load cell for detecting applied load to bone specimens at a variety of different rates and magnitudes. Generally, mechanical testing machines are designed to apply loads from less than one newton up to tens of thousands of newtons.

Tensile testing can be one of the most accurate methods for measuring bone properties, but bone specimens must be relatively large (60). Bending tests are useful for measuring the mechanical properties of bones from rodents and other small animals, and cause tensile stresses on one side of the bone and compressive stresses on the other. Bone is weaker in tension than compression. Bending can be applied to the bone using either three-point or four-point loading (60).

The advantage of three-point loading is its simplicity; however, it has the disadvantage of creating high shear stresses near the midsection of the bone. Four-point bending produces pure bending between two upper loading points, but requires that the force at each loading point be equal. This requirement is difficult to achieve in irregularly shaped whole bones; therefore, three-point bending is used more often for measuring the mechanical properties of rodent bones (60, 23, 19, 35, 38).

An apparatus in which bending force is transferred to the distal end of the femur by a cam mounted on a rotating vertical disk used to test the femur bending strength was described by Engesaeter, et al in 1978. In this setup, the proximal end of the femur is clamped in a hydraulic cylinder and force is applied with a constant deformation rate. To localize the fracture site in the

bone, a metal pin is used as a fulcrum and can be adjusted horizontally at the level of the disc center. Thus, the fracture is located at a site coincident with the axis of rotation. Mechanical properties of the rat femur can be tested in several ways with this apparatus, including diaphyseal, epiphysial, and metaphysis bending tests (12), depending on the location of the fulcrum.

Bone Density Measurement

The application of Archimedes' principle has been the standard method for determination of density (g/cm^3) of bones from small animals (25). The software for the determination of density (g/cm^2) with dual-energy absorptiometry (DEXA) was compared to Archimedes' principle using rat femurs. Prior to density measurements by Archimedes' principle, whole femurs were hydrated in distilled water for one hour at reduced atmospheric pressure (360 mm Hg). Mettler kit ME-33340 was used for Archimedes' principle. Femurs were weighed submerged in distilled water then weighed out of water (25). The following formula then was applied to calculate density: density=(weight of hydrated bone out of water/ weight of hydrated bone out of water -weight of hydrated bone submerged in water) x density of distilled water at a given temperature.

Following Archimedes' principle determination of density, femurs were analyzed by DEXA. Whole femurs were scanned while submerged in distilled water, and the scan area used for all femurs was the scan area of the largest femur (25). Original, dry, and ash weight was determined using the right femur of each animal.

Density values were higher with Archimedes' principle since it is a measure of true density or mass per volume (g/cm^3); whereas, DEXA bone mineral density values are calculated as mass per unit area (g/cm^2) (25). Densities determined by the two techniques were highly and

significantly correlated, and nondensity measurements from the right femurs were significantly correlated to densities. Results from Keenan, et al indicate that small animal software for DEXA yields comparable results to those from Archimedes' principle.

BMD devices such as DEXA have been used to measure bone and study the influence of physical activity on skeletal status in both rats (19, 35) and humans (44, 9, 6, 17, 46). DEXA is an appropriate technique for analysis of vertebrae in vivo and is readily available to most researchers. Determining density with Archimedes' principle is the least expensive technique, but it is not applicable to in vivo analyses (25).

Hormones and Bone

The functions of the ovaries are regulated by two gonadotropic hormones produced and secreted by the anterior pituitary: follicle-stimulating hormone (FSH) and luteinizing hormone (LH) (15). The gonads secrete the sex steroids, estrogen and progesterone. The ovaries also secrete inhibin, which is a polypeptide hormone that specifically inhibits the anterior pituitary's secretion of FSH without affecting the secretion of LH.

Cellular activities of bone modeling to increase the length, diameter, and shape of long bones in children are orchestrated by systemic hormones such as estrogen (49). Women lose bone faster than men after age 35, and this decline in bone mass is statistically related to menopause. This relationship is more strongly related to time after menopause, when estrogen levels are low, than to age (44). Estrogen deficiency increases the rate of bone resorption, which is not adequately compensated by an increase in bone formation, leading to a net bone loss (64).

Menopause

The term menopause means literally "pause in the menses" and refers to the cessation of ovarian activity and menstruation that occurs at about the age of 50 years in humans (15).

Natural menopause is a normal biological process that results in loss of menstruation, reproductive cycles, and fertility (3). Some sources suggest that a woman is considered to have reached menopause after a 12-month cessation of the menstrual cycle (29, 62, 4); whereas others define menopause as a six-month cessation of the menstrual cycle (54). During menopause, the ovaries are depleted of follicles and the secretion of estradiol and inhibin ceases (15). The secretion of FSH and LH by the pituitary is elevated because of a lack of negative feedback from estradiol and inhibin. The only estrogen found in the blood of postmenopausal women is that formed by amortization of the weak androgen androstenedione, which is secreted by the adrenal cortex and converted in the adipose tissue into a weak estrogen called estrone (15). The withdrawal of estradiol secretion from the ovaries is responsible for the many symptoms of menopause, including vasomotor disturbances and urogenial atrophy (15). Many postmenopausal women are at an increased risk for osteoporosis and cardiovascular disease (15, 3).

Factors Affecting Osteoporosis Risk in Post-menopausal Women

Osteoporosis

Osteoporosis (OP) is the cause of substantial morbidity and health expenditure and its prevalence is increasing in many countries (41). This multifactorial skeletal condition is characterized by a reduction in the amount of bone tissue per unit bone volume (42). Generally, osteoporosis exhibits both reduced bone matrix and bone mineral (20); thus, the quality of the bone remains about the same, there is just less bone present (51). Osteoporotic related bone loss is due to osteoclast activity exceeding osteoblast activity (bone resorption exceeds bone formation) (42, 2, 43). Age-related bone loss may be attributed to the process of bone

remodeling, in which slightly less bone is replaced than has been removed, leaving a small deficit (28).

Bone mass increases during puberty and reaches peak during the second to third decades of life (8). Controversy remains over the precise time at which peak bone mass is achieved, but the process seems to be at least 95% complete by the age of 17 years in females (28). Bone mass overall remains fairly stable until about the age of 50 years (28). Thereafter, gradual bone loss begins; this loss is faster in the trabecular bones and is further accelerated in women during menopause (8, 28). Osteoporosis is a primary cause for poor quality of life and increased medical expenses (23). After 50 years of age, the risk of sustaining an osteoporotic fracture is 40% (16).

Hip fracture perhaps is the most serious type of fracture associated with OP and shortens life expectancy for many. Twenty percent of patients who suffer hip fractures do not survive one year after fracture (16). More than 90% of hip fractures occur as the immediate consequence of a fall onto the hip (28). Among the important risk factors for falls, muscle weakness is one that can be improved with strength training (28).

Due to enormous health care costs of OP, such as hospitalization and medical expenses, it is important to identify subjects with increased risk for future fractures. Modifiable risk factors for developing OP include smoking, lack of physical activity, excess alcohol consumption, low calcium and vitamin D intake and thinness (20). Other risk factors are family history of the condition, white race, elderly, and premature menopause, previous history of fracture, poor eyesight, history of falls (20, 16). There is mounting evidence to indicate that genetic factors have a strong role in determining peak bone mass and influence the rates of change of bone mass at particular sites during aging; however, life-style and environment appear to interact with the

powerful genetic regulators of bone metabolism to determine net bone mass (8). This study addresses two risk factors, estrogen loss following menopause, and lack of physical activity.

Estrogen Loss

Postmenopausal bone loss is related to estrogen deficiency and usually occurs between the ages of 50 and 65 years (88, 42). Both osteoblasts and osteoclasts have estrogen receptors (16). Estrogen seems to suppress osteoclast formation and activity directly and indirectly through growth and paracrine factors (16). Cytokines, including interleukin-1 and interleukin-6, transforming growth factor (TGF), macrophage colony-stimulating factor, and granulocyte macrophage colony-stimulating factor, all of which rise with estrogen deficiency, upregulate osteoclast formation and activity (16). Loss of ovarian function increases responsiveness of bone marrow cells to cytokines. Estrogen also inhibits the proliferation and differentiation of osteoclast precursors, which are directly influenced by the presence of cytokines (16).

Even low levels of estrogen in elderly women seem to provide partial protection against bone loss. One hundred and forty-one women participated in a longitudinal study to examine bone density and its determinants (41). All women were premenopausal at baseline and had undergone menopause by the end of the ten-year study period. Estrogen replacement therapy (ERT) was used by 78.6% of the women. Mean time without estrogen was 10 ± 18 months, and this was the strongest predictor of changes occurring in bone density since baseline (41). As in other studies (10, 20, 14), women who did not use ERT had the greatest bone loss.

Muscle Strength

It also has been suggested that loss of estrogen results in a reduction in muscle strength (39, 6). Two separate studies (39,6) showed that postmenopausal women who received preventative treatment with oestrogens maintained muscle strength; whereas the women who did

not receive treatment had a significant reduction in muscle strength. These results demonstrate the important role of hormones in the postmenopausal reduction in muscle and bone strength.

Evaluations of bone mass have shown a correlation between the decline in BMD and the decline in physical activity (51) and muscle strength (6). To determine whether muscle strength is related to BMD during the postmenopausal period, a population of peri- or postmenopausal women between the ages of 44 and 87 years was examined (6). Muscle strength was evaluated by measuring the maximal isokinetic strength of the flexors and extensors of the knee and elbow of the dominant limb (6). Results showed a decline in muscle strength with age, and the absolute value of muscle strength correlated significantly with BMD. Muscle strength in the lower limb was more specific for femoral BMD. These results indicate that age-specific and site-specific physical exercise programs, designed to prevent OP, should be instigated at certain periods of life to protect sites at risk of fractures such as the hip, vertebrae, and wrist, or to have a more global action on bone mass (6).

It is important to consider, however, that this study (6) was a cross-sectional study rather than longitudinal. The comparison of subjects ages 50 and 80 years was not ideal because of lifestyle differences. Thus, further longitudinal studies examining the effect of muscle strength and BMD in postmenopausal women may provide a clearer understanding of this relationship.

The Use of Rats as a Model for Humans

"Full elucidation of the underlying biological mechanisms of the postmenopausal woman requires the use of an animal model that can be manipulated in a reproducible fashion under tightly controlled experimental conditions" (3). A key component to understanding the menopausal process is to understand the underlying molecular and cellular processes that are responsible for the exhaustion of ovarian follicles and the association of the processes with the

increased health risks in postmenopausal women. The use of an appropriate animal will allow

access to the molecular and cellular processes of the physiologic and pathophysiologic tissue-

level processes, and thus permits exploration of rational intervention and prevention strategies to

the health of middle-aged and older women (3). An animal model of postmenopausal bone loss

can be defined as a living animal in which spontaneous or induced bone loss due to ovarian

hormone deficiency can be studied, and in which the characteristics of the bone loss and its

consequences resemble those found in postmenopausal women (24).

The process of female reproductive aging, as well as the biology of pathophysiologic

processes associated with menopause in the human, differs among species; therefore, the

selection of an appropriate animal model of the human menopause is not straightforward (3).

The reproductive patterns and lifespan of nonprimate species differ so widely from humans and

other primates, emphasis is placed on middle- aged ovariectomized animals (3). Nonprimate

models, therefore, would not be used to study the reproductive aging process itself but rather the

underlying molecular and cellular processes through which ovariectomy leads to tissue-level

pathophysiology similar to that of the postmenopausal human female, with emphasis on

osteoporosis and cardiovascular disease (3).

The rat and the mouse seem to be particularly good models of estrogen protection from

OVX-induced bone loss (3). Estrus or menstrual cycles should be considered when deciding

which species to use to model the events associated with postmenopausal osteopenia. Rats

maintained under laboratory conditions will maintain estrus cycles of four to five days in

duration, and will experience regular fluctuation in gonadal steroids; thus, rats are more sensitive

to the loss of ovarian hormones (32). Characteristics of ovariectomy-induced bone loss in the rat

and postmenopausal bone loss are very similar. These characteristics include an increased rate

of bone turnover, and initial rapid phase of bone loss followed by a much slower phase, greater loss of cancellous than cortical bone, decreased intestinal absorption of Ca, some protection against bone loss by obesity, and similar skeletal response to estrogen and exercise therapy (24). Unlike humans and mice, however, glucocorticoid excess either increases or leaves unchanged the bone mass of the rat (3).

The rat model loses bone following OVX; however, the ovarian hormone deficient rat is not known to develop fractures, which are characteristic of OP (24, 3). Thus, the rat model is really a model of postmenopausal or OVX induced bone loss, rather than postmenopausal OP, and the bone loss in the OVX rat should be termed osteopenia rather than OP. Osteopenia is most evident in the central metaphyseal cancellous areas of the long bones, although other cancellous areas are affected (32).

The average lifespan of the rat is three to four years (24). The use of older animals (6-12 months of age) ensures that skeletal changes observed following OVX are due primarily to ovarian hormone deficiency, uncomplicated by continued rapid bone growth as occurs in younger animals, or age-related bone loss (3, 24). Use of the OVX, middle-aged rat for studies that model the estrogen-deficient human skeleton, particularly studies that would establish similarities in molecular mechanisms that are active during estrogen deficiency in rats and humans, continues to be encouraged (3).

Physical Activity and Bone

According to the principle known as Wolf's law, bone accommodates the loads imposed on it by altering its mass and distribution of mass (28). These changes occur only if the stress reaches a "minimal effective strain" (28, 9, 6), above which they can be viewed as an adaptation response to the stress. Exercise effects on bone mass are probably site specific and load

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dependent (9, 6). When habitual loading increases, bone is gained; however, when loading decreases, bone is lost. Habitual loading can be described as the sum of all individual daily loading events, with each event individually characterized by intensity, number of repetitions, or number of cycles (28, 50).

This effect can be demonstrated by examining the bone loss that ensues with complete immobilization. Immobilized patients may lose 40% of their original bone mass in one year (28); whereas, studies of bed rest indicate that standing upright for as little as 30 minutes daily prevents bone loss. The amount of bone gained by active individuals who increase their level of activity, however, is limited (28). Mechanical factors such as muscle contraction, the pressure and tension exerted by adjacent connective and muscle tissue and gravitational force play and important regulatory role in bone development. It has been theorized that bone lacking in one of these components will not grow (52). There is considerable variation in the magnitude and significance of the differences observed between athletes and nonathletes depending on the age, type of training, and bone site measured (53).

The acceleration of trabecular bone loss at menopause, an event associated with estrogen deficiency, has prompted several researchers to consider the role of long term exercise in women aged 40 to 60 years (53). Damilakis, et al. demonstrated a positive effect of long-term activity on bone in seventy-one healthy, postmenopausal women (9). Subjects who worked professionally on farms were compared with seventy-eight matched controls.

Farmers had significantly higher femoral BMD values than controls, but the body weight of the farmers was less than those in the age-matched controls. No significant differences in postmenopausal bone loss rate were found between farmers and controls; however, there was a trend towards greater postmenopausal bone loss rate of controls compared with farmers (9).

Postmenopausal bone loss in farmers began from a higher level compared with controls, which may have a positive influence on future fracture risk of these subjects. Results support the hypothesis that loading should cause high stress in varied patterns on bone, such as with the physical activity of farmers, to produce an anabolic effect on bone mineralization (9).

Differences in bone density of the femur and Ward's triangle over two centuries was compared following the restoration of a London church, during which skeletal material dating from 1729 to 1852 was recovered (27). Bone mineral density was examined using DEXA in only well-preserved dry femora with undamaged femoral necks. Although body weights were unknown, estimate heights were calculated from the dimensions of the long bones in the skeleton. The London subjects were matched as a group for age and height with a group of healthy white British women (27). Researchers estimated the age of menopause for this time period from which the bones came, and from this assumption, the artifacts were grouped by pre- and postmenopausal years. Present day women also were divided into two groups based on menopausal status. Comparisons of dead and living bone may be invalid since living bone includes fat and marrow elements; thus, results were expressed as a percentage of the mean value found in young normal subjects (27).

The rate of pre- and postmenopausal bone loss was significantly greater in modern-day women than in the women from two centuries ago (27). The difference in bone loss in the Ward's triangle region between the ancient samples and present-day women remained even when the assumed age at menopause was varied. Only one of the 1000 femora excavated had evidence of previous fracture (27). One of the most important factors contributing to these differences may be physical activity; although other unidentified environmental factors cannot be

excluded. The overall lifestyle two centuries ago involved considerably more activity than present day.

Thus, it was suggested that the increasing incidence of hip fracture is due to a greater loss of both pre- and postmenopausal bone density, possibly attributable to decreased levels of physical activity.

In elderly females doing little exercise, body weight and fat mass may become relatively more important determinants of BMD and sources of mechanical stress for weight-bearing bones (46). Other risk factors such as age at menopause, current exercise, and muscle strength showed no consistent relationship with bone density. The correlation coefficient between body weight and BMD is approximately equal to that between fat mass and BMD, suggesting that lean body weight is unrelated to BMD (46).

The bone mineral content of young adult athletes who performed either resistance or nonresistance exercise, and an inactive group, was measured at the radius, vertebrae, and femur using DEXA (22). The average BMC of body builders was consistently greater than the BMC of swimmers, runners, and controls at each site measured, but this difference was significant only in the femur. Results suggest that weight training may provide a better stimulus for increasing bone mineral than run and swim training in young adults (22).

Running and Bone

Running imposes various stresses, such as the pulling of muscles at different angles and directions of the contracting muscles attached to the bones (36, 56). Also, the pounding of limbs on the ground can reach values of six to twelve times the body weight (36, 56). Lastly, gravitational forces acting on the entire body may contribute substantially to the total stress exerted on the bones during running (36, 56).

Bone mineral density of the spine, proximal femur, and radius did not differ between endurance-trained and sedentary women (33); however, the BMDs of the spine and radius were significantly higher in the endurance-trained when normalized for body weight. Body weight was more closely related to BMD in the spine in the endurance-trained group than in the sedentary group. The BMD of the femoral neck correlated with body weight in the endurance-trained women only (33).

Forty-two women who could be allocated to one of three groups defined by their level of physical activity and by menstrual status participated in a study examining the effect of amenorrhea on the skeleton (18). Three groups were eumenorrheic normoactive, eumenorrheic athletes, and amenorrheic athletes. Amenorrheic athletes had significantly less total body weight and estradiol concentrations than the other two groups (18). Intense running did not significantly increase bone density over the increase associate with normal levels of activity.

One hundred and five sedentary women were assigned randomly to a calisthenics, endurance, or control group to evaluate the effects of an eighteen-month training regimen on BMD of the femoral neck and radius (21). The calisthenics training consisted of strength exercises by large muscle groups, and the endurance training consisted of walking, stair climbing, cycling, and jogging. The women were classified into two groups according to estrogen status: peri- or postmenopausal, and premenopausal (21). Femoral neck BMD in the endurance group was significantly different from that of the control group, the trend indicating maintenance of the prestudy BMD level; however, the training effect was not significant in the calisthenics group.

Positive effects on BMD of the femoral neck in the endurance group may be due to training-induced loading (21). It must be considered, however, that this methodology is only

practical for the short-term. The rapid change in physical activity naturally tend to cause temporal changes in bone mass, and thus this study may not be appropriate for evaluating the effects of continuing physical activity. Longitudinal studies in which subjects previously have been physically active may better permit evaluation of the effects of long-term regular exercise (17).

It has been shown that the rate of bone loss can be reduced by weight-loading activity (17). The effects of continued recreational exercise on the BMD of the proximal femur was investigated in pre- and perimenopausal women. (17). Twenty-two women who had participated in the same exercise for more than five years participated in the study, and were divided into three groups based on their menstrual status. All subjects kept weekly exercise records during the three-month periods before the initial and final BMD measurements. No significant differences in body weight, BMI, and body height were seen among the pre-, peri-, and postmenopausal groups of all athletes at the initial and final BMD measurements (17). In the premenopausal women, femoral BMD of the non-exercise control subjects decreased significantly compared to the athletes (17). All athletes in the perimenopausal group experienced loss of femoral BMD.

The sharp decrease in femoral BMD during the perimenopausal stage was not prevented in athletes even by long-term, vigorous weight-bearing activity. Results suggest that it is possible to achieve continuous gains in the bone mass up to the time shortly before the perimenopausal stage by engaging in vigorous weight-bearing activity (17). Study limitations must be considered when examining these results, however (17). First, subjects may not have maintained accurate exercise records. Second, the purpose of this study was to examine the

effect of continued exercise at a recreational level; thus, with the changes characteristics of recreational exercise, it is difficult to obtain an accurate measure of exercise intensity.

McDonald, et al suggested that older animals subjected to physical training may exhibit a different pattern of bone mineralization compared with younger animals (30). In order to demonstrate this effect, twenty Sprague-Dawley rats were divided into three groups according to age: seven months, fourteen months, and nineteen months (30). Rats in each age group were assigned randomly to either an exercise training group or sedentary control group. All animals that exercised were run on a motorized treadmill for thirty minutes, four days a week for twelve weeks. Femur and humerus were removed at the end of the experimental period, and femur was cut into trabecular and cortical bone (30). Calcium, Mg, and P concentrations also were analyzed in each bone.

Body weight was not affected by exercise in any of the age groups. Femoral dry weight in the nineteen-month old rats that exercised (19x) was significantly greater than that in the sedentary rats of the same age (19s). Exercise did not affect femur weight within any other age group (30). A significant age-related femur weight gain was found from seven months to fourteen months in both exercised and sedentary rats. A significant reduction in femur weight occurred in rats in the 19s group; however, rats in the 19x group did not exhibit a reduction in femur weight (30).

Seven-month old exercised rats (7x) and 19x had significantly greater femoral shaft, total femur, and humerus Ca concentrations compared with their respective sedentary controls (30). Calcium concentration of the femoral neck was not effected by exercise in either the seven or fourteen month group (30). Unlike Ca concentration, bone P concentration changed little with

age or exercise; whereas, Mg concentrations in nineteen-month old animals were lower than younger animals.

Results suggest that the older animal undergoes a total skeletal mineralization in response to exercise compared with a local adaptation in the younger animal (30). These authors have previously demonstrated an increase in the whole body Ca content of the older rat following exercise without noting any changes in the young rat (31). In the present study (30), exercise increased dry femur weight in the nineteen-month old animals, a finding that is not explained by an increase in body mass previously suggested (48). Finally, exercise was effective in increasing the bone Ca concentration in the aging rat, but could not prevent some age-dependent bone mineral loss (30).

Twenty-five three-month old female rats were used to investigate the effect of high-intensity treadmill running on the development of femoral osteopenia (35). Effects on the femurs were evaluated by DEXA, ash and Ca content, and by fracture strength of the diaphysis and neck. Rats were randomized into three groups: ovariectomy-training (OVX-T), ovariectomy-sedentary (OVX-S), and sham-operated sedentary (sham) (35). OVX rats were given a low-Ca diet and compared with sham on a normal Ca diet in order to induce a significant loss of bone with mechanical incompetence.

The weight gain was significantly higher for the OVX-S than in the OVX-T and sham groups. BMC and BMD were significantly higher in the sham group compared to the OVX-T and OVX-S groups. No significant differences existed between the OVX-S and OVX-T groups for bone mineral content or bone mineral density (35). Rats in the sham group had significantly higher mean ash weight and Ca content than OVX-S and OVX-T groups. No significant differences were found between the OVX-S and OVX-T groups (35).

Nine weeks after ovariectomy on a low calcium diet, severe femoral osteopenia was found; whereas, high intensity training had no significant effect on the development of femoral osteopenia in OVX rats on a low calcium diet (35). The fracture strength of the femoral shaft was impaired in these rats; however, there were no differences in fracture strength of the femoral neck (35). The high-intensity exercise used in this study may have provoked negative effects on bone.

A nine-week study demonstrated the positive effect of moderate-intensity running exercise on some mechanical bone parameters of OVX rats (38). Twelve-week-old rats were either ovariectomized (n=24), sham-operated (n=24), or sacrificed at study beginning to serve as baseline (n=8). Sham and OVX groups were further divided into control, slow running (R10), and faster running (R18) (38). Femora, humeri, and tibiae were removed at sacrifice, analyzed for breaking strength, ash weight, and osteoclast numbers.

Body weights were significantly greater for all rats in the OVX groups than sham rats. Rats in the OVX-R18 group gained significantly less weight than did the OVX-controls; whereas, body weight of the OVX-R10 rats did not differ from that of OVX-controls (38). Exercise had no significant effect on body weight gain in sham rats. Tibia ash weight decreased significantly as a result of OVX, in OVX-control and OVX-R18 groups, when compared to sham-control and sham-R18, respectively (38). There was no significant difference in tibia ash weight between OVX-R10 and sham-R10, however, suggesting a beneficial effect of lower exercise intensity (38). Maximal load of the femur for rats in the OVX-R10 has higher than that of the OVX-control rats; however, this difference was not evident when OVX-R18 and OVX-control groups were compared.

Both exercise programs slightly but significantly reduced the OVX-induced loss of trabecular femoral bone volume (38). Osteoclast numbers on the trabecular bone surface increased as a result of OVX, and exercise decreased this response, supporting the idea that bone resorption after ovariectomy can be inhibited by exercise (38).

Swimming and Bone

The gravitational force acting on the body during swimming is counterbalanced by buoyancy; thus, any change in the bones following swimming may be essentially related to applied stress-induced muscle contractions (36). During swimming, no limb-ground pounding is exerted and body weight-bearing action is reduced significantly.

Ovariectomy (OVX) is known to induce weight gain in the rat, which is considered protective of the skeletal system by providing increased mechanical stimulus in spite of estrogen deficiency (63). The effect that a non-weight-bearing exercise, such as swimming, has on bone status has been studied in growing animals, and may be beneficial in preventing weight gain while improving BMD, BMC, and bone strength (50, 55, 56, 52, 36). There is limited research, however, addressing the effect of swimming on bone status in older rats with estrogen deficiency, which resembles the postmenopausal state (19). The extent and location of the bone affected may vary with swim time and intensity.

Swimming was not shown to have significant effects on longitudinal growth of the rat humerus, although bone mass and BMC were positively affected during the period of slow growth and modeling (55). Twenty-eight female young adult rats were trained to swim for one hour daily five times a week for twelve weeks to determine the effects of swimming exercise on bone modeling and composition (55). Fresh, dry, and ash weights and ion content were measured on the tibia; whereas, only fresh weights were measured on the humerus (55). Bone

density, bone weight, length, and diameter also were measured on the humerus. All measurements were taken following the twelve-week exercise treatment and compared to sedentary controls (55).

Swimming did not affect the total body weight (TBW) of the rats, but the fresh tibiae and humeri weights were higher in the swim-trained rats (55). Tibia dry ash weight of rats in the swim group was significantly higher than controls, and rats in the swim group had 8% more Ca and 12% more Mg per tibia than rats in the control group (55). Swim-trained rats had higher proximal and distal humeral BD (7-11%, respectively) and BMC (12-9%, respectively). Data from this study (55) indicate that swimming exercise in rats increases long bone weight, mineral ion content, and bone density, although rats in this study were past the period of rapid growth and bone modeling. No significant changes were reported in the humeral length and diameter; however these measurements were higher in the swim-trained rats (55). Other studies have reported similar findings in both femur length (36) and humerus length and diameter (36, 56).

Rats swam one hour daily for twelve weeks and had significantly lower final body weights although there was no difference in food intake compared to the controls, indicating that swimming exercise may prevent ovariectomy-associated weight gain in rats (19). In the previously discussed study (55), 40 minutes of daily swimming had no effect on body weight; whereas, Hart, et al. suggested that 60 minutes of daily swimming is aggressive enough to lower total body weight compared to sedentary controls. This suggestion has been both supported (56) and refuted (36). In contrast, intense swimming programs may have adverse effects on bone mass (5). Rats in this study were swimming for six hours daily, and the deleterious effects on bone that were reported possibly were a result of prolonged weightlessness in the water (5).

Exercise of long duration is known to result in the elevated stress hormone corticosterone, which has negative effects on bone (19).

Swimming also improves femoral BMD and BMC (19). Bone mineral density results from Hart, et al. are supported by studies with a similar swimming protocol over a ten-week span (56, 55). Swimming for two hours a day affected the humerus more than the femur (52); however, one hour of swimming per day yielded greater effects on the femur of young adult rats (36). Nyska, et al. rationalized that while swimming, hind legs are used more extensively and forcefully while forelegs move in a circular motion used for balance. Thus, the effort of the hind legs is greater than that of the forelegs. Perhaps the two-hour swim (52) caused increased activity of the forelegs in pushing one another off or pushing each other down in an attempt to get to the edge of the tank, resulting in more pronounced humeri changes (36).

The effects of running versus swimming exercises of equal intensities were compared in female rats following a ten-week exercise protocol (52). Exercise sessions were two hours per day and five days per week. Swim trained rats swam with 2% of body weight attached to their tails. Swimming had a greater influence on bone growth but only affected the humerus. There was a significant increase in the weight and width of the humerus following swim training and the BMC was significantly higher than the control group (52). The calculated cross-sectional area of both the femur and humerus of the trained rats was significantly smaller than that of the control group, suggesting that the running and swimming exercise increased bone density. The high training intensity employed in this study and the limited training period may have been responsible for the low level of bone development observed (52).

Mechanical properties of the femur were determined by three-point break at the middiaphyseal shaft (19). Greater maximum load and stress was required to break the femur of

rats in the swim group compared to rats in the control group, although this difference was not statistically significant (19). Average cortical area and width of the femur was significantly greater for rats in the swim group. From these findings, authors of this study conclude that non-weight bearing exercise in OVX rats has mechanical benefits other than at the site where muscles originate (19). Similarly, growing rats that swam had significantly greater humeral cortical area and required more stress to break compared to sedentary controls (50). Results are indicative of the beneficial effects of swimming exercise on bone strength.

The effects of swimming the humerus and femur bone of prednisolone-induced osteoporosis was examined in fifty-six rats over a ten-week period (57). Treatment groups were sedentary (C), swim (S), sedentary plus prednisolone (CP), and swim plus prednisolone (SP). At study conclusion, it was determined that prednisolone injection decreased bone volume of the humerus and femur, and decreased dry femoral weight; however, humeral weight was not effected by the drug (57). Prednisolone administration also decreased humeral and femoral Ca content. Rats in the S group showed improved femur Ca content, but no improvement in humeral Ca content. Rats in the CP group had decreased Ca content in both femur and humeri; whereas, SP rats had improved femoral and humeral Ca content compared to CP rats (57). Humeral BMD of rats in the S group was significantly higher than sedentary controls.

Prednisolone administration significantly reduced humeral BMD; this measurement was not taken for the femur. Rats in the SP group had improved BMD to a level similar to that of the control rats. (57). Results support the notion that swimming can positively affect bone mass in elderly rats. Swimming positively affected healthy normal aged long bones, and the ability of swimming to prevent bone and Ca content loss was exhibited even in the osteopenic bones of the prednisolone-treated rats.

A study comparing the effects of swimming exercise on mechanical properties of the femur in male and female mice reported that animals that swam had significantly higher bone densities than control animals, although the body weights of the swimming groups were lower than that of the control groups (23). No effect of swimming on femoral breaking strength was observed; however, the elasticity of the femur in female mice was significantly higher than that of the control group (23). Results from this study suggest that swimming is effective in suppressing age-associated femoral bone loss in mice, and the effect of exercise in the females is greater that that in the males (23).

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**CHAPTER 2: SWIMMING VERSUS VOLUNTARY RUNNING
EXERCISE ON BONE HEALTH IN OVARIECTOMIZED
RETIRED BREEDER RATS**

Introduction

Osteoporosis (OP) is a multifactorial skeletal condition and is a primary cause for poor quality of life and increased medical expenses in the elderly (15, 26). Osteoporosis is a major public health threat for an estimated 44 million Americans, eighty percent of which are women (15, 26). One in five women over the age of 70 and one in three women over the age of 80 will suffer a hip fracture during her lifetime, and nearly one in five women with a hip fracture will die within 6 months of the injury as a result of complications from immobility (15). One third of women older than 65 years of age will have a fracture of the spine caused by osteoporosis (15).

Osteopenia is a progressive condition that places patients at risk for increased morbidity and mortality if untreated. Patients with bone loss of at least 1.0 standard deviation from normal are considered osteopenic, whereas those with bone loss of at least 2.5 standard deviations are considered osteoporotic (2, 28). Generally, osteoporosis exhibits both reduced bone matrix and bone mineral density (13); thus, the quality of the bone remains about the same, there is just less bone present. Osteoporotic related bone loss is due to osteoclast activity exceeding osteoblast activity (bone resorption exceeds bone formation) (27, 2, 28). Accelerated bone loss following menopause is associate with estrogen deficiency; thus, postmenopausal women are at an increased risk of developing OP (6, 27).

Hip fracture is perhaps the most serious type of fracture associated with OP; twenty percent of patients who suffer hip fractures do not survive one year after fracture (10). Among the important risk factors for falls, muscle weakness is one that can be improved with strength training (17). It has been suggested that loss of estrogen results in a reduction in muscle strength

(25,4). Mechanical factors such as muscle contraction, the pressure and tension exerted by adjacent connective and muscle tissue and gravitational force play an important regulatory role in bone development (36).

Bone reacts to imposed stress by changing its structure and increasing its mass (22, 36). The importance of physical stress and weight-bearing exercises in preventing bone loss and maintaining bone mass is generally accepted (33); however, there is inconclusive evidence of the effect of non-weight bearing activity on bone (12). Running imposes various stresses on the entire body; however, there are no gravitational forces acting on the limbs during swimming exercise (22).

It has not been consistently proven that physical activity has a beneficial effect in postmenopausal women. Most studies on swimming and bone mass have been conducted in younger, elite athletes, are cross-sectional, and usually demonstrate few skeletal benefits (1, 5, 8, 9, 11, 14, 16, 20); however, there is little information on the effect of swimming on bone mass in noncompetitive postmenopausal women (12). Rourke, et al. (1998) demonstrated that BMD was significantly higher in female swimmers compared to runners, indicating a beneficial effect of swimming on bone health in young females.
Postmenopausal women who engage in water exercise had positive effects on bone (37); whereas, swimming did not result in increased BMD in women aged 40-85 years (23) compared to aged matched sedentary controls. Longitudinal studies may provide a more accurate indication of how swim exercise effects bone in sedentary postmenopausal women.

Swimming may be beneficial for the bones of young, growing rats (31, 35, 34, 22); however, there are limited data on the effects of swimming in older female rats with estrogen deficiency, which resembles the postmenopausal state (36, 22, 12). Results of activity on bone

vary with training types, intensity, exercise duration, and age. Older animals subjected to physical activity may exhibit a different pattern of mineralization compared with younger animals (18). Intense exercise programs that increase the production of stress hormones may have negative effects on bone formation (21,3, 12); whereas, exercise of very low intensity may not be adequate to prevent bone loss (15).

The purpose of the present study was to determine the effect of voluntary running and non-weight bearing swimming exercise on bone mineral content of the humerus, tibia, and femur and breaking strength of the femur and tibia in OVX retired breeder rats.

Animals and Methods

Study Design

This study was approved by the Institutional Animal Care and Use Committee. Thirty-seven nine-month old retired breeder Sprague-Dawley rats (Harland Company; Indianapolis, Indiana) were housed in the animal care facility in individual stainless steel wire hanging cages, 24 cm wide x 28 cm long x 18 cm high. The room temperature was kept at 22 degrees Celsius (°C) with 60% humidity. A 12-hour light cycle was maintained (lights on at 0700 hours and lights off at 1900 hours). There were two control groups: 1, sham-operated (Sh) (ovaries left intact) and not exercised; 2, ovariectomized (OVX) control and not exercised (O). Two treatment groups were OVX that either swam (OS) or ran (OR).

Upon arrival, rats were weighed using a 500 g analog scale (Toledo Scale Company; Toledo, OH). On days two and three after arrival, rats were placed in individual cages with running wheels attached for one hour to determine their tendency to run. The number of wheel revolutions was recorded, and total revolutions run over the two-day period were calculated for each rat. Rats that ran the most were designated the OR group (n=9) and placed in individual

stainless steel cages with running wheels attached. All rats were given water and standard AIN-93M diet *ad libitum* (29). Rat weights and food intakes were recorded three times a week.

Exercise/Running and Swimming Protocol

Rats in the OR group ran voluntarily. Rats had free access to the running wheels (36 cm diameter) attached to their cages. Revolutions run were recorded three times a week. Two plastic tubs (43 cm high x 48 cm diameter) were used for swimming the OS rats. Tubs were filled each day with tap water, maintained at 30°C, to a depth of 24 cm. Five rats swam in each tub. To accustom OS rats to the water, they began swimming on day three of the study; rats swam for five minutes on that day, and ten minutes the following day. Surgery, described below, took place on days four and five, and swimming was not resumed until day ten of the study. After surgery, the rats in the OS group repeated the five and ten minute swim times, respectively. Swim time increased by ten minutes each day to a one hour time period. Rats swam for one hour daily on Monday through Friday for the remainder of the study.

Surgery and Sacrifice

On days four and five of the study, faculty and students from the LSU Veterinary School performed surgery on the rats. Rats were anesethetized by isoflourane inhalation during ovariectomy for the O, OS, and OR groups. The Sh group experienced the same surgery but the ovaries were not removed. Rats were sacrificed during the eighth week of the study via cardiac puncture after isoflurane inhalation anesthesia. All four limbs and the vertebrae were removed and frozen in saline for later analyses. Prior to analyses, bones were cleaned manually of adherent tissue

Bone Analyses

Bone density of the left side humeri, femur, and tibia, and vertebra was measured by peripheral dual energy x-ray absorptiometry (pDEXA) (Norland Medical Systems, Inc.; Trumbull, CT). Left humerus, femur, and tibia also were used for the determination of bone weight, dry weight, and ash weight. The ashing process included adding a few drops of concentrated HCl to the bone, volatilizating under a ventilation hood, and then placing in a muffle furnace (General Signal; Stamford, CT) overnight at 500°C. The bone ash was dissolved in 10 ml 10% HCl solution and sonicated (Branson Ultrasonics Corporation; Danbury, CT) for 15 minutes. Samples were then poured into labeled scintillation vials and caps were sealed with parafilm until mineral analysis.

Bone calcium (Ca) concentrations were analyzed using atomic absorption spectrometer (AA) (Perkin Elmer Corporation; Wellesley, MA). An aliquot of the bone ash sample was diluted with 0.5% lanthanum chloride ($LaCl_3$) prior to Ca analysis. A Sigma kit (Sigma Diagnostics, St. Louis, MO) was used for phosphorus analysis on the spectrophotometer (LaboMed, Inc.; Culver City, CA).

Femur and Tibia Strength Analysis

Femoral neck and tibia cortex strength was determined using a compression tester (Q-tester) (MTS Systems; Eden Prairie, MN) with a 50 pound load cell. Femurs were mounted in a one-inch copper tube filled with hot glue with the femoral head end of the bone protruding about 1.5 cm from the glue. Femoral neck diameter was measured at three points with a dial caliper (Mitutoyo Corportaion; Aurora, IL); and the average of the measurements was recorded to the nearest millimeter (mm). Femoral neck area was calculated from the measured diameter and recorded. Width was measured as the distance from the tip of the protruding femoral head to the

base of the femoral neck and recorded to the nearest mm. After measurements were obtained, the mounted femur was centered in the Q-tester and the force was applied at the femoral head until the neck broke. Tibia diameter was measured at the middiasphyseal shaft. Three-point break was used to break the tibiae at the middiasphyseal shaft.

Statistical Methods

Data were analyzed using SPSS Student Version 9.0 for Windows. (SPSS Incorporated; Chicago, Ill). Mean values of dry bone weight, ash weight, bone calcium, bone magnesium, bone phosphorus, femur breaking strength, and tibia breaking strength were calculated for each group and compared using one-way analysis of variance (ANOVA). Overall bone mineral density of the femur, tibia, humerus, and vertebra for the four rat groups was analyzed using multivariate analysis of variance (MANOVA). ANOVA was used to compare the BMD among the four groups in each of the bones measured. Post-Hoc ANOVA tests (Fisher's protected LSD and Tukey's) were used to determine which treatments, if any, were different. An alpha level of 0.05 was considered to be statistically significant. Data are presented as mean $\pm$ standard deviation.

Results

Rat Weight and Food Intake

There were no significant differences in initial or final body weight of rats. Figure 1 shows mean body weight change per week. No significant differences were found in weight change or total food intake over the study.
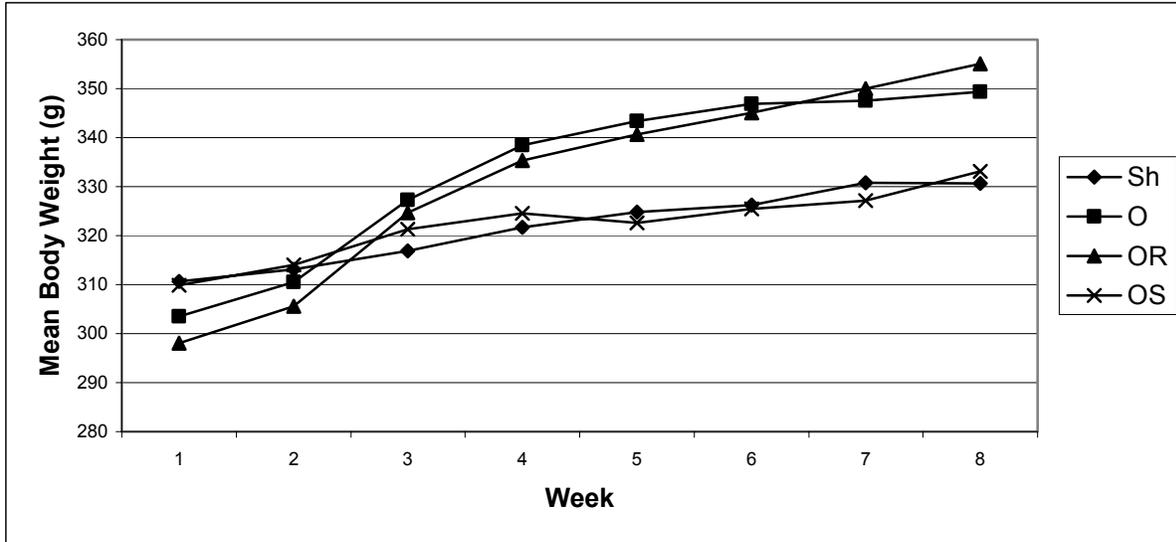
Figure 1. Weekly total mean body weights (g) for rat groups. Surgery took place during week 2.

Bone Mineral Density

Both forms of exercise were effective in improving bone density. Mean density of all bones measured was significantly greater for rats in either exercise group when compared to rats in either control group ($p < 0.05$). Rats in the swim group had the greatest density for all bones measured, and rats in the voluntary running group had the second greatest density. Mean vertebra density of the Sh group was significantly greater than the mean vertebra density of the O group. Only swim exercise was effective in increasing vertebral BMD compared to the Sh group. There were no other significant differences among groups.

Bone Mineral Density
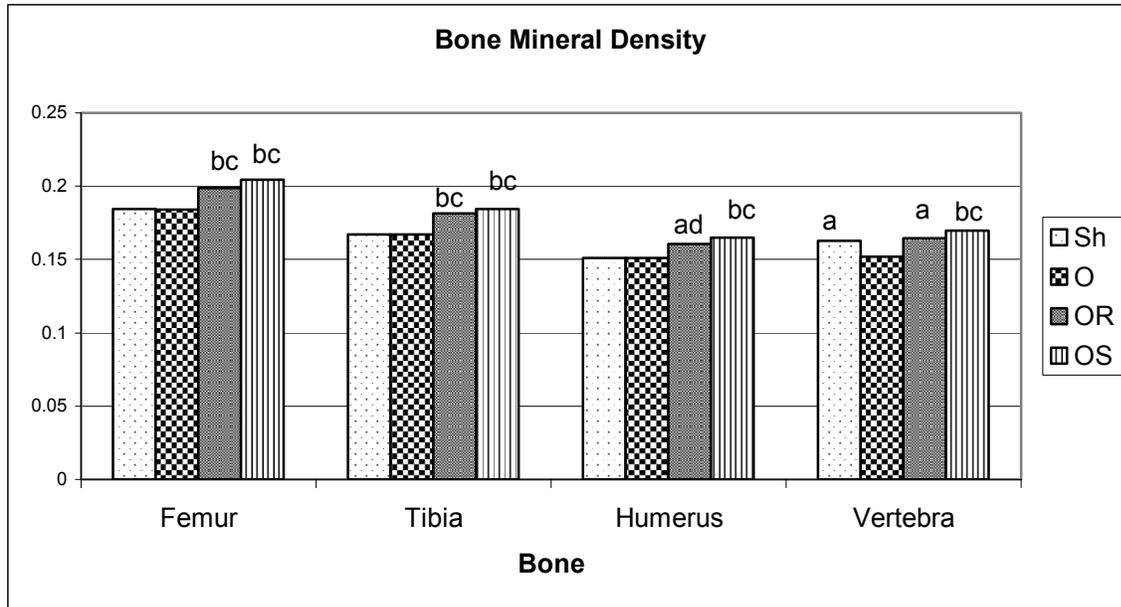
a. Significantly greater than O (P<0.05).
b. Significantly greater than O (P<0.01).
c. Significantly greater than Sh (P<0.01).
d. Significantly greater than Sh (P<0.05).

Figure 2. Mean bone mineral density of the femur, tibia, humerus, and vertebra in each group of rats

Bone Mineral Content

Mean femur Ca content was significantly greater for rats in the OS group than rats in the O group (Table 1). There were no other significant differences in the femur analyses. Mean femoral Ca and P content in the femur tended to be greater for the exercise groups than the control groups. No significant differences were found in tibia mineral analyses. Rats in the OR group tended to have higher tibia Ca and P content. There were no significant differences in the Ca and P content of the humerus.   Rats that swam tended to have higher mean humeral Ca and P content. Rats in the exercise groups (OR and OS) tended to have greater mean dry bone weights and ash weights for all three bones measured than rats that were sedentary.

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Table 1. Femur weight (g $\pm$ sd), ash weight (g $\pm$ sd), calcium content (mg $\pm$ sd) and phosphorus content (mg/dL $\pm$ sd). All values are group means.

	Sh	O	OR	OS
Dry bone, g	0.5565 $\pm$ 0.0698	0.5627 $\pm$ 0.0701	0.6014 $\pm$ 0.0570	0.6060 $\pm$ 0.0472
Ash weight, g	0.3532 $\pm$ 0.0628	0.3625 $\pm$ 0.0408	0.3830 $\pm$ 0.0428	0.3856 $\pm$ 0.0392
mg Ca/g bone	140 $\pm$ 0.008	132 $\pm$ 0.018	144 $\pm$ 0.011	146 $\pm$ 0.017[*]
P, mg/dL	5.0557 $\pm$ 0.1721	4.4055 $\pm$ 1.6754	5.1460 $\pm$ 0.2613	5.2587 $\pm$ 0.2485

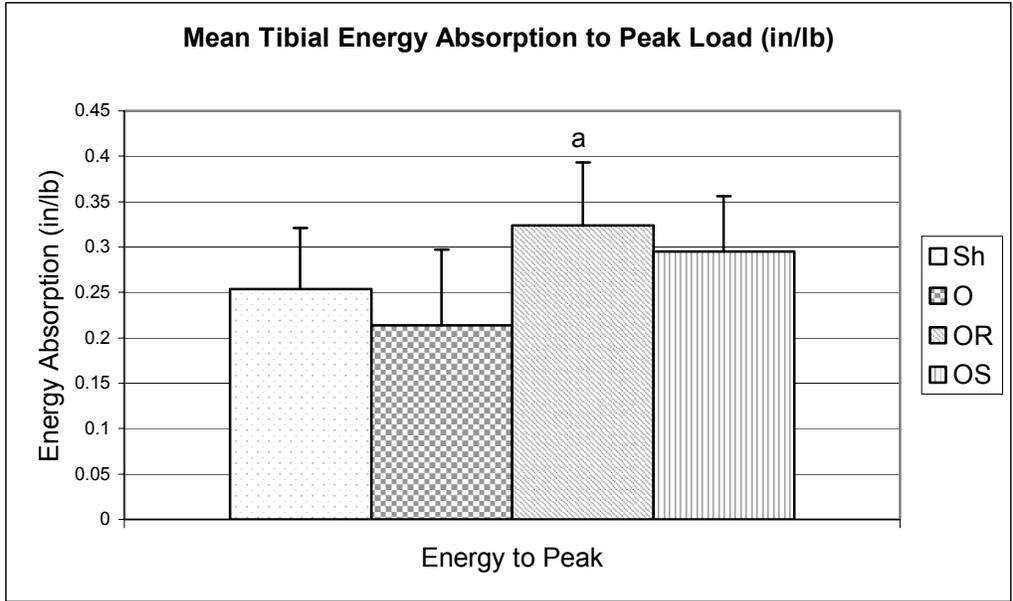[*]Significantly greater than O (p=0.019).

Tibia Strength

Swimming exercise resulted in bones with greater energy to break load compared to sedentary OVX rats. Both swimming and voluntary running resulted in greater energy to break load than both sedentary groups (Figure 3). Energy to peak load was significantly greater for rats that ran compared to sedentary OVX rats (Figure 4). Tibia break load was greatest in the rats that swam (NS).
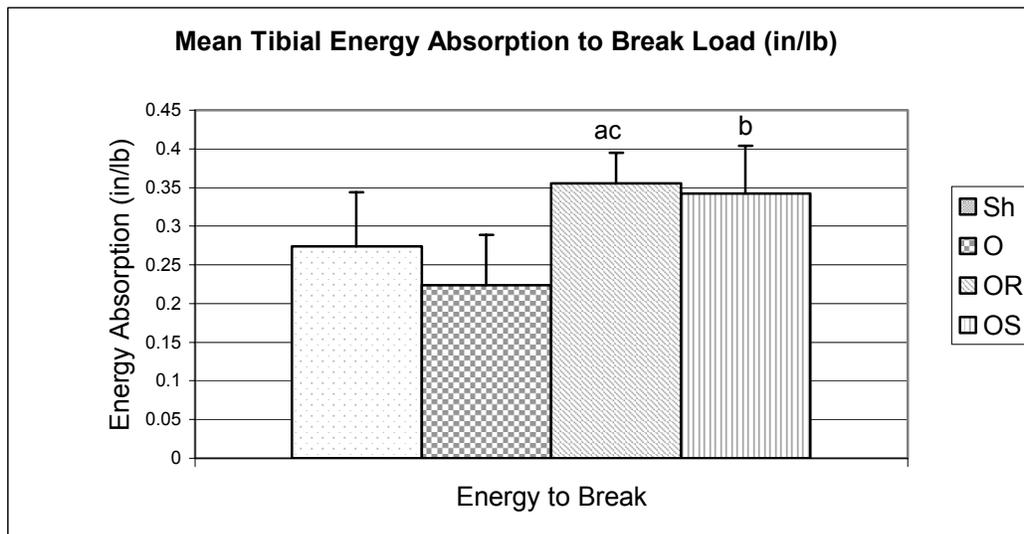
a. Significantly greater than O (p=0.000).
b. Significantly greater than O (p=0.001).
c. Significanlty greater than Sh (p=0.022).

Figure 3. Mean energy absorption to break load of the tibia in each group of rats

Mean Tibial Energy Absorption to Peak Load (in/lb)

a. Significantly greater than O (p=0.015).

Figure 4. Mean energy absorption to peak load of the tibia in each group of rats.

Femur Strength

There were no significant differences in the femoral neck strength between the treatments. Femoral neck break load, energy to break load, and energy to peak load tended to be greatest for rats that swam, followed by Sh rats (NS) (Table 2). Parameters measured were not increased in rats that ran voluntarily, and OVX did not result in a decrease in these parameters compared to sham-operation (Figure 5,6).

Table 2. Femur break load (lb $\pm$ sd), energy to break (in/lb $\pm$ sd), and energy to peak (in/lb $\pm$ sd). All values are group means.

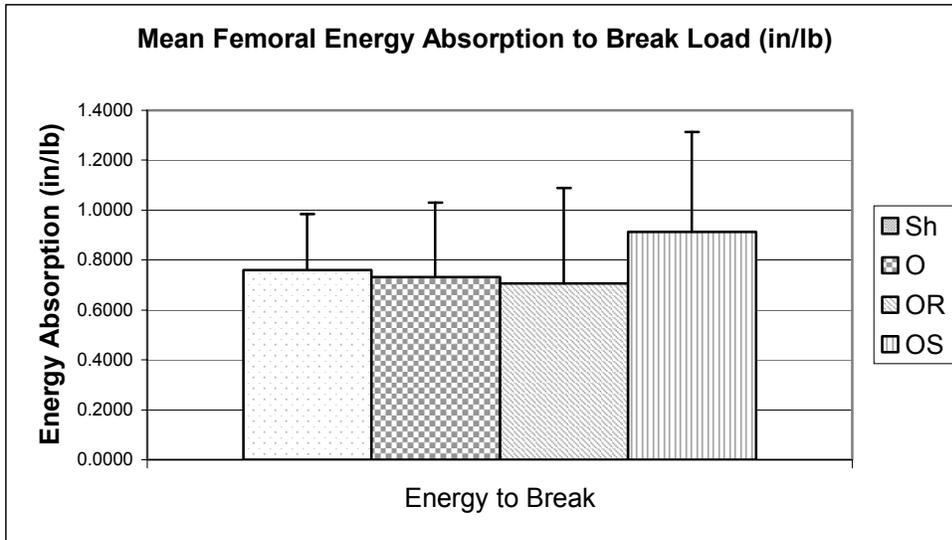|	Sh	O	OR	OS
Break Load (lb)	20.091 $\pm$ 5.479	19.808 $\pm$ 4.105	17.698 $\pm$ 7.318	23.375 $\pm$ 7.114
Energy to Break load (in/lb)	0.760 $\pm$ 0.299	0.732 $\pm$ 0.225	0.706 $\pm$ 0.384	0.913 $\pm$ 0.401
Energy to Peak load (in/lb)	0.737 $\pm$ 0.291	0.705 $\pm$ 0.217	0.653 $\pm$ 0.326	0.888 $\pm$ 0.393

Figure 5. Mean energy absorption to break load of the femur in each group of rats.
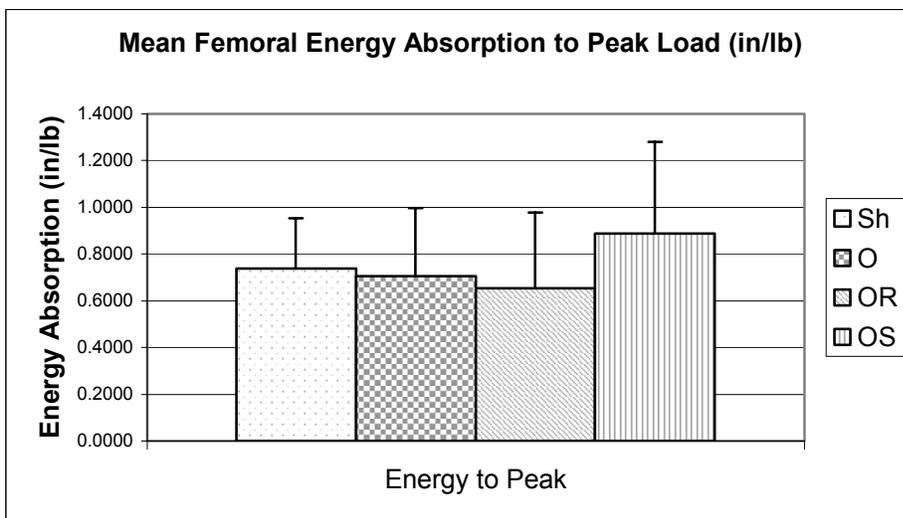

Figure 6. Mean energy absorption to peak load of the femur in each group of rats.

Revolutions Run

Mean revolutions run for the study were 50, 770, with a range from 12, 702 to 98, 634

revolutions. Figure 7 shows the variation of mean revolutions run for each study week.
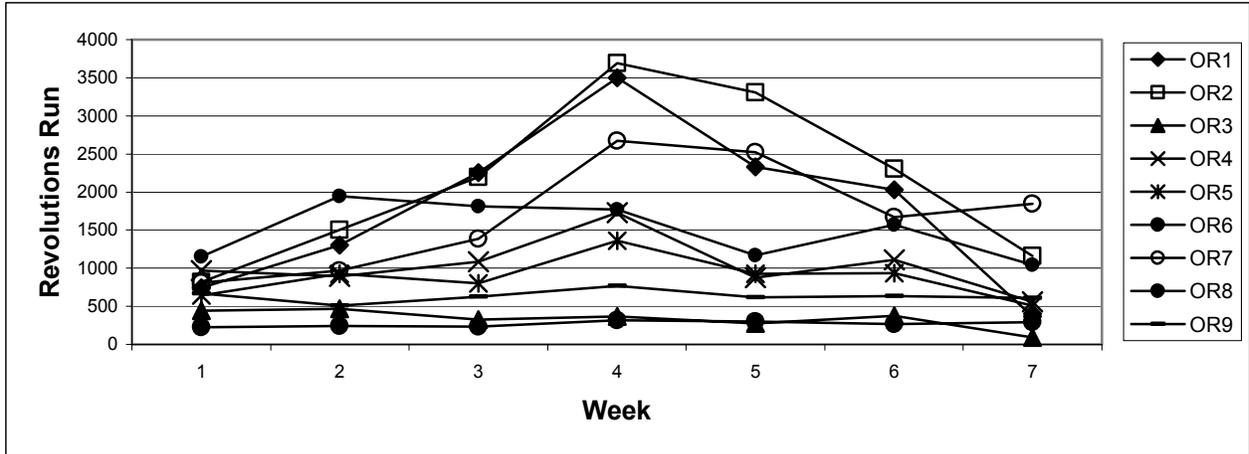
Figure 7. Weekly revolutions run for each rat in OR group.

Discussion/Conclusion

There is extreme pounding of limbs during running exercise; however, during swimming, there is no limb-ground pounding and body weight-bearing action is reduced significantly. Weight-bearing activity is prescribed commonly to maintain bone and prevent bone loss in the elderly, but weight-bearing exercise may be difficult for some older individuals; thus, swimming exercise is popular for this population. Our results support the hypothesis that swimming is effective in increasing density of the humerus, femur, tibia, and vertebra of OVX rats compared to sedentary controls that were either ovariectomized or sham-operated.

The positive affect on bone mineral density is supported by studies of rats using similar swimming protocols (12, 34, 35, 19, 15). Previous data indicate that swimming exercise in young adult rats that were not OVX and that were past the period of rapid growth and bone modeling increases long bone weight, mineral ion content, and bone density (35). Parallel to these findings, rats that swam in our study had significantly greater femur calcium content than OVX sedentary controls. Rats that swam had greater dry bone and ash weights than sedentary rats, although this difference was not statistically significant.

The most appropriate swim duration needed to improve bone status has not yet been established. The majority of previous studies used a one hour swim time (36, 31, 35, 12, 22, 35, 12); whereas, other studies deviated from this regimen (15, 32, 3). Forty minutes of swimming resulted in greater femoral density, but had no affect on breaking strength (15). Bone mineral density and mechanical properties of the femur (12, 36) and tibia mineral content (35) have been shown to significantly improve with one hour of daily swimming. In contrast, Snyder, et al (1992) found that the positive affects of two hours of daily swimming exercise were more pronounced in the humerus than the femur or tibia.

Bourrin et al. used a regimen that involved swimming rats for six hours a day, but it was detrimental to bone status. The two-hour swim time used by Snyder, et al (1992) may have caused an increase in the activity of the forelegs, which are usually used by the rat for balance in the water while the hind legs are used more extensively and forcefully. The greater change in the humerus bone may have resulted from the site-specific and load-dependent effects of exercise (8, 4).

In our study, pretrials were held prior to treatment assignment to maximize the likelihood that the OR group would be made up of rats with the greatest tendency to run. The nine rats in the OR group, however, did not run consistently throughout the study. Two rats had very little desire to run, four rats seemed to run the most frequently, and the remaining three rats fell in between the two extremes. Weekly revolutions run per rat shows that running generally declined after week four, possibly due to boredom, a natural decline in the tendency to run with aging rats, or a delayed effect of OVX. Cortright, et al reported that revolutions run peaked during week seven, then decreased for the remaining two weeks of their study. Unpublished data from our lab

showed that revolutions run by OVX rats peaked upon re-introduction to running wheels following OVX, then a decrease and plateau occurred for the remaining four weeks.

The large variability among the rats in the running group is most likely the reason that voluntary running was not more effective than swimming in improving bone status. Studies using forced treadmill running have shown beneficial affects on bone (18). Elevation of the stress hormone corticosterone with forced running is known to have negative effects on bone, which is why this type of activity was not used in this study. In contrast, a study by McDonald (1986) demonstrated that forced running significantly increased dry femoral weight and humerus Ca concentration compared with sedentary controls. These rats were older than the rats used in this study (nineteen months old and 9 months old, respectively), and may have had lower baseline weight and Ca concentration that improved to a greater extent with the onset of exercise. Rats that were similar in age to the rats in this study likewise demonstrated no difference in Ca concentration of femur and total P concentration with forced running (18).

Greater maximum load was required to break the femoral neck of rats in the swim group compared to rats in the control group, although this difference was not statistically significant. Hart, et al reported similar findings when mechanical properties of the femur were determined by three-point break at the middiaphyseal shaft. In our study, average area and width of the femoral neck was greater for rats that swam than for sedentary controls. Hart et al. (2001) found that cortical area of the femur was greater for rats that swam. Rats in the running group had the greatest mean area and width of the femoral neck compared to swimmers, OVX controls, and sham-operated controls; however, the femurs of rats that ran required the least force to break and could withstand the least amount energy prior to breaking. This suggests that while swimming

resulted in smaller femurs than running, the bone of the swimmers actually was stronger than the larger femurs of the runners.

There was extreme variability of femur strength within groups. Femurs were mounted vertically in hot-glue filled copper tube and centered in the Q-tester by visual reference. Perhaps a more standardized method of bone mounting would ensure that all bones are broken at the same angle and point on the femoral neck, thus eliminating some of the variability in results. Some studies that measure mechanical properties of bone use three point bending to break the femur at the middiaphysis which is easier to standardize (12, 38, 15, 21, 24); however, that is not a common site for fracture in the elderly. This study measured the load needed to break the bone at the femoral neck since a fall to the hip is most likely to cause a break at this site.

Hart, et al demonstrated that greater maximum load was required to break the cortical bone at the femur middiaphysis of rats that swam; whereas, results of another study failed to show an effect of swimming on femoral breaking strength (15). Our results cannot directly be compared to these studies, however, since three-point bending was used (12, 15) on the femur, and mice were used in the latter study.

Much of the research on swim exercise and bone status has been conducted in young females or perimenopausal women (1, 5, 8, 9, 11, 14, 16, 20, 22) and generally showed no beneficial effect of swimming. The control groups used in these studies, however, tend to lead active lifestyles; thus, the addition of a swim program might not result in significant changes in bone status. Bone density has been shown to be higher for females who swam compared to runners (30), but subjects were young athletes. Activity levels are usually low in the elderly population due to fear of falling, lack of motivation, or decreased strength. Thus, introduction of a non-weight bearing form of physical activity, such as swimming, would likely appeal to this

age group. Water exercise was shown to be beneficial in preserving BMD in older Japanese women (37), indicating that bone health may be improved with non-weight bearing activity in this population. Our research can be applied to sedentary postmenopausal women who become less physically active following menopause, and are more likely to benefit from swim exercise.

Results indicate that both swim exercise and voluntary running preserve bone density in OVX rats. Swim exercise was most be beneficial in preserving femur calcium content, but running exercise was most be beneficial in improving tibia strength in OVX rats. Our results also imply that swim exercise may result in smaller, denser femurs than running in OVX rats. Future studies that include larger treatment group sample sizes and standardized mechanical femoral neck breaking method are needed to clarify the effects of swim exercise and voluntary wheel running on bone health in OVX rats.

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SUMMARY AND CONCLUSIONS

Weight bearing physical activity commonly is prescribed to preserve bone mass and bone mineral content in postmenopausal women, a population at increased risk of developing this osteoporosis. The effects of non-weight bearing activity, such as swimming, in preserving bone mass and bone mineral content, however, are inconclusive. The purpose of this study was to examine the effect of swimming exercise and voluntary running exercise on bone health in thirty-seven Sprague-Dawley ovariectomized or sham-operated retired breeder rats as a model for postmenopausal women. Bone mineral content was measured in the femur, tibia, and humerus, and bone density was measured in the long bones as well as the vertebra. Breaking strength was measured at the femoral neck and tibia middiaphysis.

Femur Ca content was greater in the rats that swam compared to the OVX controls. Bone density was improved for both types of exercise compared to OVX and sham-operated sedentary controls. Ovariectomy resulted in a significant decrease in vertebral density. Rats that swam tended to have the greatest mean break load among the treatment groups. Voluntary running exercise significantly improved tibia energy absorption compared to sedentary OVX and sham-operated controls.

Results indicate that both swim exercise and voluntary running preserve bone density in OVX rats. Swim exercise was most be beneficial in preserving femur calcium content, but running exercise was most be beneficial in improving tibia strength in OVX rats. Our results also imply that swim exercise may result in smaller, denser femurs than running in OVX rats. Future studies that include larger treatment group sample sizes and standardized mechanical femoral neck breaking method are needed to clarify the effects of swim exercise and voluntary

wheel running on bone health in OVX rats. Measurement of biochemical markers of bone

turnover also would lead to a more in depth interpretation of these results.

APPENDICES

APPENDIX A
BONE ASHING PROCEDURE

1. Clean bones manually
2. Weigh and record the weight of a glass beaker
3. Weigh and record the weight of the beaker with the bone
4. Dry the bone in a vacuum oven at 60°C (temperature setting on 2.2) at 15 mmHg overnight
5. Record weights
6. Place watch glass on top of each beaker
7. Cool to room temperature in a desiccator
8. Place the beaker with watch glass lid in a muffle furnace oven at 500°C overnight then cool to room temperature
9. Place the beaker in the hood
10. Remove watch glass
11. Drop concentrated HCl over bones with a glass pipette until HCl no longer soaks into the bone
12. Volatilize HCl under the ventilation hood
13. Repeat steps 7 through 10 until bones become white
    The ash weight is the difference between the weight of the beaker containing the ash and the empty beaker
14. Place beakers with the watch glasses still on top in the desiccator for a day
15. Dissolve bone ash in 20 ml 10% HCl solution (1 part concentrated HCl and 9 parts distilled water)
    a.   Add less than half the final volume of 10% HCl to the beaker
    b.   Stand beaker in a small amount of water in the sonicator
    c.   Sonicate for fifteen minutes
16. Transfer to 25 ml volumetric flask
17. Wash the beaker with several small aliquots of fresh 10% HCl solution, adding these washes to the volume in the flask until 25 ml volume is reached
18. Decant samples into labeled scintillation vials, and seal cap with parafilm

For Calcium:
- Dilute aliquot of sample 1/1400 with 0.5% lanthanum chloride ($LaCl_3$)
- Run on an atomic absorption spectrometer at 422.7 nm

For Phosphorus:
- Follow Sigma procedure # 360-3 for inorganic phosphorus.

Calcium (1 to 5 ppm) standards are run before the samples to ensure the linear calibration of the instrument.

Accutrol is run every time to monitor inter-assay variation.

Calcium Standard

To make 100 ppm working stock:

Final ppm desired	Dilution	Amt 1000 ppm stock	Amt 0.5% $LaCl_3$	Total volume
100	1/10	20 ml	180 ml	200 ml

To make standards for AA:

Final ppm desired	Dilution	Amt 1000 ppm stock	Amt 0.5% $LaCl_3$	Total volume
1 ppm	1/100	5 ml	495 ml	500 ml
2 ppm	1/50	10 ml	490 ml	500 ml
4 ppm	1/25	20 ml	480 ml	500 ml
5 ppm	1/20	25 ml	475 ml	500 ml

Lanthanum Chloride

To make 5% working stock of $LaCl_3$:

1. Weigh 58.64 g of lanthanum oxide (La_2O_3)
2. Place in a 1 liter volumetric flask
3. Add approximately 50 ml of distilled H_2O to wet the powder
4. Add 250 ml of concentrated HCl slowly and cautiously under the hood while swirling the flask
5. Dilute to 1 L with distilled H_2O
6. Store in a dark room in a labeled 1 L storage bottle
7. Stable for 6 months

To make 0.5% $LaCl_3$:

Final % desired	Dilution	Amt 5% stock	Amt distilled H_2O	Total volume
0.5 %	1/10	100 ml	900 ml	1000 ml

Phosphorus

To make 15 mg/dl working stock:

1. Weigh 0.660 g KH_2PO_4 in a weigh boat on the analytical balance
2. Place in a 1 L volumetric flask
3. Dilute to mark with distilled H_2O
4. Store in a labeled 1 L storage bottle

To make standards for the spectrophotometer:

1. Zero the analytical balance with a 100 ml volumetric flask
2. Add the appropriate amount of 15 mg/dl working stock Phosphorus standard from the table below
3. Dilute to mark with distilled H_2O
4. Store in a labeled 100 ml storage bottle

Final mg/dl desired	Dilution	Amt 15 mg/dl working stock	Amt distilled H_2O	Total volume
1 mg/dl	1/15	6.667 g	93.333 g	100 ml
5 mg/dl	1/3	33.333 g	66.667 g	100 ml
10 mg/dl	2/3	66.667 g	33.333g	100 ml
15 mg/dl	0	100 g	0 g	100 ml

APPENDIX B
PROCEDURE FOR Q-TESTER

1. Turn computer ON

2. Turn Q-tester ON by turning red knob clockwise and pulling

3. Put in U-shaped holder

4. Place mounter into holder

5. On screen, click TESTWORKS icon for software

6. Click TEST

7. Enter Username and Password

   a. Select EDIT

   b. Click USERS and select name

      i. Make sure method access is 5

      ii. Default method is RAT BONE COMPRESSION 11

8. Put bone into holder and adjust so femoral neck is directly under the breaker

9. Click SAMPLE ID

   a. Enter file name to use (such as Shc, CE, CW, C, DAY 1, Set 1)

   b. Select OK

10. Click RUN

    a. Input WIDTH, HEIGHT, and AREA (these need to be converted to inches):

    mm/25.4 = inches

    b. Click SAVE/NEXT

11. When "Test is about to begin" appears on screen, click OK. Breaker will start to move

    down until bone breaks. After bone breaks, piece stops automatically.

12. Select FILE (to get curve and data). Then click OK when prompted.

13. Zero before next bone is tested.

14. When ready to break next bone, repeat steps 10-13

15. When ready to start new file, repeat steps 9-13.

16. Once all bones for that period have been tested: can go to SPECIMEN to review curves, DIRECTORY to find specific file.

17. Click EXIT until back to MTS screen

18. Turn off Q-tester by pushing red know

19. Shut down computer and turn off.

20. Place "IN TESTING" sign on Q-tester.

VITA

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