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THE EFFECTS OF VOLUNTARY WHEEL RUNNING AND SWIM EXERCISE ON WEIGHT GAIN, ABDOMINAL FAT, AND LEAN TISSUE MASS IN OVARIECTOMIZED RETIRED BREEDER RATS

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Abbreviations

OVX	Ovariectomy, Ovariectomized
21S	OVX, 21% soy diet, No exercise treatment group
ACSM	American College of Sports Medicine
AIN	American Institute of Nutrition
AIN-93M-MX	American Institute of Nutrition Mineral Mix
AIN-93-VX	American Institute of Nutrition Vitamin Mix
ANOVA	Analysis of Variance
BMI	Body Mass Index (body weight [kg] divided by height $[m]^2$)
^oC	Degree Celsius
C	OVX, 21% casein diet, No exercise treatment group
C14S	OVX, 7% casein + 14% soy diet, No exercise treatment group
C7S	OVX, 14% Casein + 7% soy diet, No exercise treatment group
CE	OVX, 21% casein diet, Voluntary wheel running exercise treatment group
CHD	Coronary Heart Disease
cm	Centimeter
CRB	OVX, 21% casein + rice bran oil diet, No exercise treatment group
CT	Computed Tomography
CVD	Cardiovascular Disease
CW	OVX, 21% casein diet, Swim exercise treatment group
DEXA	Dual energy x-ray absorptiometry
FFM	Fat-Free Mass
FSH	Follicle-Stimulating Hormone

g	Gram
HDL-C	High Density Lipoprotein Cholesterol
HR_{max}	Maximal Heart Rate
IU	International Unit
kcal	Kilocalorie
kg	Kilograms
L	Liter
LBM	Lean Body Mass
LDL-C	Low Density Lipoprotein Cholesterol
LH	Luteinizing Hormone
LSU	Louisiana State University and Agricultural and Mechanical College
MRI	Magnetic Resonance Imaging
NHLBI	National Heart, Lung, and Blood Institute
NIDDK	National Institute of Diabetes and Digestive and Kidney Diseases
NIDDM	Non-Insulin Dependent Diabetes Mellitus
NIH	National Institutes of Health
NoX	No exercise
RPD	Revolutions Per Day
SD	Standard Deviation
ShC	Sham-operated, No exercise treatment group
SSF	Subscapular Skinfold
µg	Microgram

VO_{2max}	Maximal Oxygen Uptake
WHR	Waist to Hip Ratio

Definitions

ad libitum	Freely
anemia	An abnormally low number of red blood cells
android fat	Fat accumulation in the abdominal area
aseptic	Sterile
gynoid fat	Fat accumulation in the gluteal-femoral area
hyperphagia	Overeating
hypertension	High blood pressure
hypertrophy	General increase in bulk of a part or organ
intramuscular	Within the muscle
ischemia	Local anemia due to mechanical obstruction (mainly arterial narrowing) of the blood supply
mesentery	A double layer of peritoneum attached to the abdominal wall and enclosing in its fold a portion or all of one of the abdominal viscera
omentum	A fold of peritoneum passing from the stomach to another abdominal organ
peritoneal	Relating to the serous sac that lines the abdominal cavity
peritoneum	The serous sac that lines the abdominal cavity and covers most of the viscera contained therein
postprandial	After eating
preprandial	Before eating
serous	Pertaining to a substance having a watery consistency
stratified	Layered into groups
subcutaneous	Beneath the skin
VO_{2max}	Maximal capacity for oxygen consumption by the body during maximal exercise; indicator of cardiorespiratory fitness

ABSTRACT

Weight gain, deleterious changes in body composition, and an increase in cardiovascular disease (CVD) risk factors are associated with postmenopausal women. Ovariectomized (OVX) rats were used as a model for menopause to examine the effects of voluntary wheel running and swim exercise on food intake, body weight, abdominal fat mass, and lean tissue mass. Thirty-seven Sprague-Dawley retired breeder rats were assigned randomly to one of four treatment groups: sham-operated (ShC); OVX, non-exercise (C); OVX, voluntary wheel running (CE); OVX, swim exercise (CW). There was no significant difference in food intake among treatment groups; there was a trend of increased food intake with increased number of revolutions run by the CE group. There was no significant difference in total weight gain or total muscle weight among treatment groups. Although not significant, rats in the CE group tended to gain less abdominal fat than rats in ShC and C groups. Rats in the CW group had significantly lower abdominal fat weights than the ShC group ($p=0.031$). The results of this study suggest that swim exercise may diminish the abdominal fat gain associated with OVX in rats. These results also imply that a higher level of voluntary wheel running increases food intake and may diminish the abdominal fat gain associated with OVX in rats.

CHAPTER 1

INTRODUCTION

Justification

Historically, the transition to menopause by women was not the widespread concern that it is today. Women born in the United States in 1900 could expect to live to an average age of 48 years (1). Menopause typically occurs at the age of 50 years (2). Most women born at the turn of the century did not live long enough to go through menopause. In contrast, a woman born in 1950 could expect to live to the age of 71 years (1), with the life expectancy increasing every decade. According to the North American Menopause Society, as of July, 2000 approximately 40 million U.S. women had reached natural menopause (3). With approximately two-thirds of the total U.S. population surviving to the age of 85 years or longer, most U.S. women will spend one-third to one-half of their lifetime in postmenopause (3).

Several deleterious consequences are associated with the transition to menopause. One of these consequences is an increased risk of developing cardiovascular disease (CVD). Cardiovascular disease is any abnormal condition characterized by dysfunction of the heart and blood vessels (4). Included in this broad category are coronary heart disease (CHD), stroke, and hypertension (HTN) (4). It is estimated that one in nine women aged 45 to 64 years and one in three women aged 65 and older have some form of CVD (5, 6). Postmenopausal women have a higher risk of CHD (7), stroke, and HTN (8-10) than premenopausal women of similar ages. The incidence of CHD increases with age in women, with the rate of increase particularly marked after menopause (6).

The loss of specific hormones after menopause contributes to women's increase of risk factors for CVD. The removal of estrogen has been correlated with an increase in cholesterol and triglyceride levels (11). Increases in low density lipoprotein cholesterol (LDL-C) and decreases in high density lipoprotein cholesterol (HDL-C) have been seen in postmenopausal women (10, 12). It has been reported that there is strong, reasonably consistent, and biologically plausible evidence suggesting that unopposed oral estrogen reduces the risk of CHD (11, 13). Estrogen replacement, however, has also been associated with an increase incidence of certain cancers, notably breast cancer (14). For this reason, options other than estrogen replacement are needed to help reduce the risk of developing CVD in postmenopausal women.

Increases in body fat and a shift in body fat distribution also increases the risk of CVD in postmenopausal women. Exercise has been shown to reduce body fat (15) and unfavorable changes in body composition (16-19), therefore making exercise a viable alternative to estrogen replacement for reducing CVD risk factors.

Ovariectomy (OVX) reduces the circulating estrogen levels in rats similar to the way menopause reduces circulating estrogen levels in humans (12, 20). Since the life span of the rat is much shorter than that of humans, the effects of reduced estrogen levels can be observed within weeks after OVX compared with months or years postmenopause in humans (21). This makes the OVX rat an excellent model for studying the effects of hormonal changes associated with menopause (21, 22).

As the "baby boomer" generation approaches menopause age, there is an increased need to reduce the CVD risk factors that are concomitant with this life transition. Preventing the weight gain, and associated body composition changes, that

further increase the CVD risk factors associated with menopause is of paramount concern. Alternatives to estrogen replacement therapy are needed to assist women in making a healthful transition to their non-reproductive years.

Objective

The objective of this study was to use retired OVX female breeder rats, as models for menopause, in order to determine the effects of swim exercise or voluntary wheel running on weight gain, abdominal fat mass, and lean tissue mass.

Hypotheses

The hypotheses of this study were:

1. That OVX in Sprague-Dawley rats will contribute to an increase in food intake, weight, and abdominal fat mass, and a decrease in lean tissue mass (LTM).
2. That voluntary wheel running will increase food intake, minimize or ablate weight gain and abdominal fat mass gain associated with OVX, and minimize the decrease in LTM associated with OVX.
3. That swim exercise will increase food intake, minimize or ablate weight gain and abdominal fat mass gain associated with OVX, and minimize the decrease in LTM associated with OVX.

Assumptions

- Ovariectomized rats are an appropriate model for menopause.
- Ovariectomy in rats produces similar hormonal changes as natural menopause does in women, which will, in turn, lead to parallel or equivalent changes in body composition.

- The sample size was adequate to detect changes in weight gain, body fat, and LTM in OVX rats.
- Pre-testing the willingness of rats to run would produce a voluntary wheel running treatment group consisting of rats that would run.

Limitations

- There was a small sample size in each treatment group.
- There may have been a diminished physiological effect of swimming on the rats due to their tendency to float. This occurred despite physical agitation to ensure continuous swimming.
- Different swim stroke patterns between humans and rats may affect different muscle groups, therefore the effects of swimming in rats may not be comparable to the benefits of swimming in humans.
- Rats assigned to the voluntary wheel running group were self-selected based on willingness to run, but there was a high degree of variability in revolutions run by each rat.
- Food spillage had to be estimated, which may have contributed to inaccurate food intake measurements. Technical errors during the study contributed to periodic inability of rats to access food and water. This also may have contributed to inaccurate food intake measurements.
- Length of study may not have been long enough to examine fully the effects of swimming and voluntary wheel running on body composition. Only 4 weeks of the 7-week study involved actual exercise participation.

CHAPTER 2

REVIEW OF LITERATURE

The physiology of menopause

The term menopause literally means "pause in the menses;" and is defined as the permanent cessation of the menses (23). The ovaries of a newborn female contain about 2 million oocytes, or immature ovum. Each ooctye is enclosed within its own ovarian follicle. The number of oocytes and follicles is finite and decreases to approximately 400,000 by puberty. During a woman's reproductive years, approximately 400 of these oocytes will be released from its follicle. This process is called ovulation.

The anterior pituitary gland secretes two gonadotropic hormones – follicle-stimulating hormone (FSH) and luteinizing hormone (LH). Luteinizing hormone and FSH promote cyclic changes in the structure and function of the ovaries. Normal serum FSH levels during the child producing years, characterized by normal menses, range from 5-20 International Units [IU] / liter [L] (24). Follicle-stimulating hormone causes the ovarian follicle to swell in preparation for the release of the ovum (*i.e.* mature oocycte). The growth of the follicle is accompanied by a rapid increase in the rate of estradiol secretion. Estradiol, the principal estrogen, initiates a surge in LH that ultimately results in the rupturing of the follicle and subsequent release of the ovum. Once ovulation has occurred, the empty follicle undergoes structural and functional changes and becomes the corpus luteum. The developing follicle secretes only estradiol; whereas, the corpus luteum secretes both estradiol and progesterone, an antiestrogenic steroid. The increased level of estradiol and progesterone exert an inhibitory, or negative feedback, effect on

FSH and LH secretion. Inhibin, another hormone secreted by the corpus luteum, also suppresses FSH secretion (2).

The cessation of menses, associated with menopause, results from the loss of ovarian follicular activity (25). The period following the cessation of menses, or menopause, is commonly called postmenopause. The generally accepted clinical criteria to define the postmenopausal state is the absence of menses for at least 12 months (10, 25-28); although, some researchers consider a lack of menses for 6 months sufficient (29-31). At an approximate age of 50 years, the ovaries are depleted of follicles and stop secreting estradiol and inhibin. The lack of negative feedback from estradiol and inhibin causes an increase in the secretion of FSH and LH by the pituitary gland (2). In addition to a lack of menses for a set period of time, a plasma FSH level >30-50 IU/L is used to define postmenopause (28-30).

Laboratory animals as models for menopause

The main focus of the overall study, of which this is an ancillary project, was to examine the effects of ovariectomy (OVX), diet, and exercise on bone density. Ovariectomized nonprimate animals are often used as models of menopause because they develop pathophysiologic changes similar to those seen in postmenopausal women (32). Mice, and especially rats, are good models for the effect of OVX and estrogen replacement on associated bone changes.

The rat was chosen over the mouse for this research because rats are the best model for the main focus of the overall study – bone density (32). The goal of this ancillary project was to examine weight and body composition changes associated with exercise. The findings of this study could then be extrapolated to postmenopausal

women. If this research had been the primary focus of the study, however, the mouse would have been a better model (32). By using the rat instead of the mouse, the efficacy of this ancillary project has not been diminished (32).

The OVX rat has been shown to undergo an increase in body weight and changes in body fat content (33, 34) similar to those experienced in women who have undergone menopause. Female rats have also been shown to undergo changes in the levels of estradiol, LH, and FSH during the transition to irregular cyclicity that are similar to those observed in women undergoing menopause (35). This makes the rat an appropriate model for the study of reproductive aging (35) and acceptable for this project.

Body composition

The human body consists of fat, water, proteins, minerals, and carbohydrates (36). The body can be divided into various compartments based on the distribution of these compounds. The way in which the body is divided depends on the purpose for analyzing the body (37) and the availability of suitable methods for estimating size in different individuals and in the same individual in different nutritional states (38).

The simplest methods for assessment of body composition use the two-compartment model, *i.e.* fat mass and fat-free mass (FFM) (39-42). Two methods of determining body composition, hydrostatic weighing and skinfold thickness measurements, are based on this two-compartment model (15). Both of these techniques determine body density, which can then be used to estimate percentages of fat mass and FFM by the use of conversion equations (38, 39).

Hydrostatic weighing determines body density based on Archimedes' principle, *i.e.* "when an object is placed in water it is buoyed up by a counterforce equal to the

water it displaces" (15). Volume of the body can then be determined by measuring the actual amount of water displaced by the submerged body or by subtracting body weight measured while the body is submerged from body weight measured while the body is on land. Body weight, or body mass, is divided by volume to determine body density.

The method of measuring skinfold thickness to determine body density is based on the principle that 50% to 70% of total body fat is subcutaneous (43) and that the measurement of several specific sites will yield a representative value for overall subcutaneous fat (15). Skinfold thickness measurements are used in generalized prediction equations to estimate total body density (41, 43). Percent body fat mass is determined from body density using conversion equations (15).

The formulas that are used to convert body density to total body fat mass assume that fat, water, protein, and mineral content have a constant density at any given time and in any given individual (39). This assumption introduces errors by not accounting for water and mineral content, *i.e.* bone density differences associated with age, gender, and ethnicity (43).

Advances in technology have made it possible to divide the body into three- and four-compartments (44). Body composition estimates from dual-energy X-ray absorptiometry (DEXA) are derived from the relative changes in attenuation of photons as dual energy radiation scans the body. Use of DEXA can divide the body into three compartments – bone, fat, and lean soft tissue (45). This division of FFM into lean soft tissue and bone reduces errors associated with the two-compartment model of body composition. Computer software allows measurements to be taken at specified regions to determine body fat distribution. Use of DEXA provides regional estimates of body

composition (40), whereas computed tomography (CT) and magnetic resonance imaging (MRI) allow for direct regional composition measurements, for example abdominal fat (43). Computed tomography and MRI can also distinguish visceral from subcutaneous abdominal fat mass (43).

Fat-free Mass

In the aforementioned two-compartment model for body composition, FFM consists of tissue devoid of all extractable fat, including water, muscle, bone, connective tissues, and internal organs (39-41). Fat-free mass is commonly used synonymously with lean body mass (LBM) although a distinct difference does exist. While FFM consists of all tissue except fat, LBM includes essential body fat (41, 42).

Fat Mass

The terms *fat* and *adipose tissue* are commonly used interchangeably, however, a distinction between the two should be made. The primary distinction between fat and adipose tissue, is that adipose tissue includes both fat and its supporting connective tissue; whereas, fat is one component of adipose tissue. As described in *Gray's Anatomy*, adipose tissue is "a distinct tissue", whereas fat is "an oily matter" (46). Adipose tissue is made up of small vesicles known as fat cells that are lodged in the meshes of areolar tissue, a type of connective tissue (46). Adipose tissue is found primarily under the skin, in mesenteries and omentum, and behind the peritoneum (47). The fat cells within the adipose tissue are filled with an oily fat comprised of the glycerin compounds olein, palmitin, and stearin, and other fatty acids (46). These compounds are primarily in the form of triglycerides (2, 41, 47).

Body fat mass consists of all extractable lipids and includes essential and nonessential body fat (39, 41). These are defined as follows:

1. Nonessential fat is all fat stored within fat cells of adipose tissue (40) and is referred to as storage fat. Storage fat insulates the body to retain heat, serves as an energy substrate for metabolism, and pads the body against physical trauma (48).
2. Essential body fat includes all other fat. It is found in cell membranes, bone marrow, spinal cord, brain, (40), muscles, nerve cells, intestines, heart, liver, and lungs (47, 48). Essential body fat is needed for normal physiological functions, including transport and storage of fat-soluble vitamins, insulation of the nervous system, and maintenance of the menstrual cycle and the reproductive system. It is also essential to growth and maturation during the developmental years (41).

Obesity, described more fully below, is defined by the National Institutes of Health (NIH) as "the condition of having an abnormally high proportion of body fat," characterized by a Body Mass Index (BMI) of greater than or equal to 30 (4). In 1947, Vague introduced a system for differentiating types of obesity based on regional fat distribution. In 1957, he coined the terms "android" and "gynoid" obesity (41). Gynoid type fat distribution is sometimes referred to as a "pear shaped" body morphology; and is the storage of fat primarily in the hips and thigh area (41, 43). Android type fat distribution is commonly referred to as an "apple shaped" body morphology; and is the accumulation of fat in the upper trunk (41, 43).

Bouchard (49) identified two distinct types of central fatness: excess subcutaneous truncal-abdominal fat (android) and excess abdominal visceral fat. Some

studies (16, 17, 27, 50) differentiate intra-abdominal fat mass into visceral and subcutaneous in a manner similar to the central fat types described by Bouchard (49).

Obesity and risk factors for cardiovascular disease

Body Mass Index is body weight in kilograms divided by height in meters squared (wt/ht^2) and is used as a practical marker to assess obesity (4). Overweight is defined as a BMI of 25.0 to 29.9; obesity is defined as a BMI of ≥ 30 (4). All cause mortality rates have been shown to increase by 50% to 100% for individuals with a BMI ≥ 30 when compared with those with a BMI between 20 and 25 (4). Obesity is associated with increased morbidity and decreased longevity from a variety of disorders and diseases (51), including but not limited to dyslipidemia (51, 52), hypertension (HTN) (52), impaired glucose tolerance and non-insulin dependent diabetes mellitus (NIDDM) (51, 52), and cardiovascular disease (CVD) (9, 51). In 1995, the National Heart, Lung, and Blood Institute (NHLBI), in cooperation with the National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK), convened an expert panel to examine evidence on risks associated with overweight and obesity, and to develop means of evaluation and treatment of overweight and obesity. This panel reported that the condition of being overweight or obese substantially raises the risk of morbidity from HTN, NIDDM, stroke, gallbladder disease, osteoarthritis, sleep apnea and other respiratory problems, and some types of cancer (4). Kessebah and Krokower (51) confirmed that obesity is the most powerful risk factor for NIDDM. Approximately 80% of individuals with NIDDM are obese; and a large proportion of individuals who are obese have NIDDM or impaired glucose tolerance (51).

Body fat distribution and risk factors for cardiovascular disease

The predominant site of body fat distribution has been found to be a stronger predictor of hyperlipidemia, glucose intolerance, NIDDM and CVD than has obesity *per se* (51). The relationship between risk factors for chronic disease and regional fat distribution has become a topic of increasing interest since it was reported originally by Vague and co-workers in 1956 (53). Male pattern distribution of body fat, *i.e.* android type body fat, was found to be associated with premature atherosclerosis and diabetes mellitus (53). Android type body fat is also seen in women, especially postmenopausal women (54, 55). There has been an increase in research on the effects of abdominal fatness and its relationship to CVD (52, 56, 57), HTN (8, 51, 52), dyslipidemia (8, 51, 52, 58, 59), impaired glucose tolerance and NIDDM (8, 51, 52, 55, 59, 60) over the last two decades.

In 1990, a workshop examining basic and clinical aspects of regional fat distribution was sponsored by the NIH to examine the data relating to variations in fat distribution and potential health risks (61). It was concluded that not only is central fat distribution associated with increased mortality, it is also associated with an increased risk of HTN and with alterations in lipoprotein and insulin metabolism (61).

Studies examining the effect of fat distribution on chronic disease have addressed the issue in both genders and in different ethnic populations (8, 56). A follow-up study using data of 7,692 men of Japanese descent who had participated in the Honolulu Heart Program, examined the relationship between central obesity and coronary heart disease (CHD) (56). This study found that central obesity, as measured by subscapular skinfold thickness (SSF), was related directly to the risk of definite CHD in men ($p<0.001$)

independent of BMI. Other risk factors found to be related to increasing SSF included an increase in concentrations of total cholesterol, glucose, triglycerides and an increase in blood pressure ($p<0.001$ for all risk factors). Another large-scale retrospective, longitudinal study of 84,910 U.S. Army veterans supported the conclusion that central adiposity is a risk factor for ischaemic heart disease and stroke mortality, independent of BMI (57).

Chang and co-workers (8) examined 329 Chinese women to determine the relationship between central obesity and CVD risk factors including abnormal blood lipid levels, elevated fasting blood glucose concentration, and elevated blood pressure. All subjects had a BMI <30. Dual energy X-ray absorptiometry, waist-to-hip ratio (WHR), and BMI were used to determine regional distribution of body fat. Central obesity was found to show a significant, independent, positive effect on fasting glucose levels, systolic and diastolic blood pressures, and serum triglyceride concentrations, and a negative effect on high density lipoprotein cholesterol (HDL-C) levels. Data from this study were adjusted for age and BMI.

Menopause and cardiovascular disease risk factors

Menopause has been identified as an independent risk factor for CVD (6, 9). It is primarily the lack of estradiol secretion by the ovaries, following natural or surgical menopause, that is responsible for the increased risk factors for CVD and metabolic diseases associated with menopause (9, 11, 47). The altered hormone status attributed to menopause is believed to contribute to changes in body weight and body composition (62, 63) that increase risk factors for CVD. A detailed discussion is beyond the scope of this thesis.

Effect of menopause on body fat

The onset of menopause often has been associated with an increase in body weight and in total body fat (10, 27, 29, 30, 31, 54). There has been conflicting evidence as to whether there is an independent effect of menopause on total body fat. Results from cross-sectional and longitudinal studies have documented an increase in total body fat associated with menopause independent of advancing age (8, 27, 30, 31, 54). Other published studies (10, 29) that have shown an increase in total body fatness in postmenopausal women compared to premenopausal women either did not adjust for age (10) or simply did not find an independent effect of menopause when total body fatness and age were used as covariates (29). In one study reporting a lack of an independent effect of menopause on body fat distribution, the age range of the women studied was 49 to 60 years (29). The change in body composition associated with menopause does not occur abruptly, but it is insidious. Menopause normally occurs at approximately 50 years of age; therefore, the body fat distribution changes may have already occurred in the women studied or may have been more pronounced in the older individuals. Changes in body weight and body composition prior to the study were not considered. These studies may not agree on the reason for the increased body fatness of postmenopausal women, but they do agree on the fact that there is an increase in body fatness (8, 10, 27, 29-31, 54).

Effect of menopause on body fat distribution

Several studies have documented an increase in abdominal fat mass in postmenopausal women (10, 27, 30, 31, 54, 65). In a cross-sectional study of 133 premenopausal and 87 postmenopausal women, Hunter and associates (10) reported that

postmenopausal women had a significantly higher percentage of both intra-abdominal fat mass ($p<0.01$) and subcutaneous abdominal fat mass ($p<0.05$), as well as a higher intra-abdominal fat to subcutaneous abdominal fat mass ratio ($p<0.01$). Postmenopausal women were significantly older (51.5 ± 10.2 yr) than the premenopausal women (36.2 ± 9.0 yr, $p<0.01$), but age was not considered a covariate. The large age range (17 – 77 years) of the women in this study leaves open the possibility that the differences in body composition may be due to age rather than menopausal status.

Whether the increase in abdominal fat observed in postmenopausal women is associated with advancing age, or menopause itself, is a topic of much debate (25). Some researchers (29, 66) have suggested that body fat distribution is not significantly affected by menopause, independent of age. Self-reported waist and hip measurements from 29,939 healthy women between the ages of 40 and 64 years were used to determine WHR (66). No independent effect of menopause on abdominal fat was found; even after statistical adjustments for age, BMI, physical activity, height, and alcohol and tobacco use (66).

A study examining 373 healthy Danish postmenopausal women using DEXA to measure android and gynoid fat regions found that changes in body fat and fat distribution in the early menopausal years are related more closely to age than to menopause. The authors of this study, however, did concede that they could not completely exclude the effect of menopause on fat mass and fat distribution (29).

Zamboni and co-workers (50) used both WHR and body fat measurements obtained from CT to compare the relationship between abdominal fatness and menopausal status. Utilizing waist and hip circumferences on 40 premenopausal and 17

postmenopausal obese Italian women, the authors concluded that menopause did not have a significant affect on WHR. Analysis of CT measurements, however, indicated a significant relationship between menopause and an increase in visceral abdominal fat ($p<0.01$), and the visceral to subcutaneous abdominal adipose tissue ratio ($p<0.01$), but a decrease in subcutaneous fat ($p<0.01$); all were independent of age. The WHR may not have shown a menopausal effect on abdominal fat in this population since the accuracy of WHR decreases with increasing levels of fatness (41). This ratio is distorted by the hypothesis that abdominal fat deposition could increase waist circumference regardless of whether adipose tissue accumulates in deep or superficial sites, whereas hip circumference is influenced by subcutaneous fat deposition only (41). This makes WHR a poor measure of visceral fat in an obese population.

A review published by Tchernof and Poehlman (25) reported that the methods used in the studies discussed above (10, 27, 31, 50, 54, 66) appear to contribute to the conflicting results that were observed. An independent effect of menopause on central body fatness was observed with DEXA or CT; whereas, studies using indirect measures, such as waist and hip circumference or WHR, showed no significant correlation between menopause and increased central body fatness (25). The conclusion of the review by Tchernof and Poehlman (25) was consistent with the findings of several studies utilizing DEXA (8, 30, 31, 54,) and CT (27, 30) technology. As the use of DEXA and CT has become more common, and several disadvantages to waist and hip circumference measures and WHR have become obvious. Waist circumference measures and WHR are indirect measures of abdominal fatness and can be affected by the quantity of subcutaneous fat, gluteal or leg muscle mass, and differing pelvic structures and laxity of

abdominal muscles typical in the elderly (41, 50, 54, 67). Variations in individual visceral fat mass (67) and reference method chosen (41) may affect the accuracy of predictive equations.

There are, however, exceptions to the analogy set forth in the review by Tchernof and Poehlman (25) that studies using DEXA or CT tend to show an independent effect of menopause on body fat distribution. Wang and colleagues (29) did not find menopause to have an independent effect on abdominal fatness even though DEXA was used. The review by Tchernof and Poehlman (25) suggested that the lack of an independent effect of menopause on body fat distribution may have been due to the fact that only postmenopausal women were studied by Wang and colleagues (25). Varied results from these studies may also be due to population differences. Some researchers examined only obese subjects (27, 50), others examined only non-obese subjects (8, 29-31, 54, 65), and still others examined both obese and non-obese subjects in the same study (10, 66). Nationalities of the subjects also varied and included North American (10, 30, 66), Chinese (8), Danish (29, 31, 65), British (54), Japanese (27), and Italian (50). The results from any given study may only hold true for that population studied and may not be comparable to others.

Effect of menopause on fat-free mass

Age *per se*, is related to a decrease in FFM (26). Sarcopenia, or the loss of muscle mass and strength caused by aging, is evident in all older persons compared to young, healthy, physically active adults (15, 30, 67, 68). This decline in FFM has also been attributed to menopause, independent of age (29, 30, 64). Two of the researchers that have reported an independent effect of menopause on the loss of FFM did not

examine if these decreases in FFM were due to a decrease in bone mineral mass or in non-bone FFM (26, 69). By using DEXA to quantify bone mass and using age as a covariate, other researchers have presented data that associate menopause with the loss of non-bone FFM and LBM (28-31).

Not all studies, however, have reported an independent menopausal effect on non-bone LBM. Ley and associates (54) reported data that confirmed a significant difference in non-bone LBM between age-adjusted men and women ($p<0.001$), but not between age-adjusted pre- and postmenopausal women. All subjects were healthy, non-obese Caucasian adults; and body composition was measured using DEXA.

Ovariectomized rats, weight gain, body fat, and lean body mass

The loss of estradiol from OVX in rats causes an increase in food intake (70-79), a decrease in exercise, and an acceleration in body weight gain (33, 70-82) in rats. Changes in body weight that occur after OVX are predominately due to changes in body fat content (33, 72, 73, 82). Other studies have shown that the increase in overall body weight from OVX was due to skin and subcutaneous tissue and whole body components, not to body fat (34). Although body fat was not increased by OVX, it was redistributed from the carcass (*i.e.* the body without skin, head, tail, extremities, and viscera [23]) to the subcutaneous fat layer (34). It has also been reported that the increase in body weight associated with OVX is due to an increase in protein mass, *i.e.* FFM, not fat mass (81).

In a study (77) examining six-month old virgin Sprague-Dawley female rats, OVX rats were reported to have an increase in body mass during the first 24 days after surgery and to remain heavier than their sham-operated counterparts for the remainder of the 35-day study. Body composition analysis of the carcasses revealed that the OVX rats

gained significantly more fat (13g) and lean mass (53g) than did the sham-operated rats. These data suggest that the weight gain associated with OVX in rats may be due to increases in both fat and LBM.

Exercise, body fat, and CVD risk factors

The NIH (4) has determined that there is strong evidence that weight loss in overweight and obese individuals reduces risk factors for NIDDM and CVD. Strong evidence also exists that weight loss reduces blood pressure (4, 83) and blood glucose levels (4, 51, 84) in overweight individuals, as well as improves dyslipidemia (4, 83, 87).

Physical activity is recommended as part of a comprehensive weight loss therapy and weight control program (4, 85). Physical activity is defined as bodily movement produced by skeletal muscles that requires energy expenditure and produces progressive health benefits (48). Exercise is a type of physical activity that requires planned, structured, and repetitive bodily movement done to improve or maintain one or more components of physical fitness, *i.e.* cardiorespiratory (aerobic) endurance, muscular strength and endurance, muscular flexibility, and/or body composition (48).

The NIH recommends accumulating at least 30 minutes or more of moderate intensity physical activity (55-70 % maximum heart rate [HR_{max}]) (85) on most, preferably all, days of the week. The American College of Sports Medicine's (ACSM) guidelines for exercise adequate to maintain cardiorespiratory fitness, as well as body composition include exercising 3-5 days per week for 20-60 minutes at 55-90% of HR_{max}.

The term metabolic fitness was introduced by Després and co-workers (86) to describe the state of metabolic systems and variables predictive of the risk of NIDDM and CVD which can be favorably altered by increased physical activity or regular

endurance exercise. These changes occur without the requirement of a training-related increase in cardiovascular fitness as measured by maximal oxygen uptake [VO_{2max}]. These variables include plasma insulin levels, glucose tolerance, insulin sensitivity, and plasma lipoprotein levels (5).

In a stringently controlled study of 5 young (25 ± 3 yrs), healthy, sedentary, overweight men, the effects of long-term exercise were examined on body composition and glucose and lipid metabolism (84, 87, 88). The 5 participants submitted to a 100-day experimental negative energy balance treatment. The subjects performed two 53-minute cycle ergometer sessions per day (mid-morning and mid-afternoon), at 55% VO_{2max} for 100 days. The treatment consisted of cycles of 6 days of exercise followed by 1 day of rest. The subjects lived in a controlled environment where daily intake was closely monitored and maintained at baseline level throughout the study. The exercise treatment induced a 1,000 kilocalorie (kcal) per day deficit. Body weight, BMI, and fat mass significantly decreased ($p<0.001$), while FFM remained unchanged from pre-treatment baseline levels. Eighty-two per cent of the body weight lost was fat mass. Subcutaneous fat decreased considerably as indicated by the reduction in the sum of 10 skin fold measurements. Compared to baseline, subcutaneous fat in the trunk region decreased by 31%, whereas subcutaneous fat in the extremities decreased by only 24%; this difference was not significant. Heart rate, systolic and diastolic blood pressure, and VO_{2max} were not altered by the treatment (88). There were, however, metabolic adaptations such as a significant reduction ($p<0.05$) in plasma low density lipoprotein cholesterol (LDL-C) and an increase ($p<0.05$) in HDL-C (87). This exercise treatment also elicited significant reductions in preprandial and postprandial levels of plasma insulin ($p<0.01$) (84). The

results of this study suggest that protracted, moderate intensity aerobic exercise training that induces a substantial weight loss causes beneficial changes in lipid and carbohydrate metabolism and improves plasma lipid transport and increased insulin sensitivity. These benefits are seen even without an increase in VO_{2max} (86). The tightly controlled environment, including diet and exercise, of this study gives credibility to the results despite the small number of subjects.

Exercise, abdominal fat, and cardiovascular disease risk factors

The benefits of exercise are related to weight loss itself and independent of body composition (15). A decrease in overall body fat also results in a decrease of abdominal fat mass (16-19). The body's preferential mobilization of fat from the abdominal area (19), specifically from visceral fat (83, 89) and abdominal subcutaneous fat (16, 17) tissue, results in an improved body fat distribution and risk factor profile. It has been reported that as the intra-abdominal visceral-to-subcutaneous fat area ratio declines in women with android type fat distribution, the improvements in glucose and lipid metabolism increase (51).

Aerobic exercise has been shown to be an excellent method for minimizing the increase in abdominal fat mass associated with menopause and age (90). Forty pre- and postmenopausal healthy Caucasian women were studied to determine the effects of regular endurance exercise on regional body fat distribution using DEXA. The women were divided into four groups: 9 premenopausal (27 ± 2 yr) and 11 postmenopausal (60 ± 2 yr) sedentary women, and 9 premenopausal (33 ± 1 yr) and 11 postmenopausal (60 ± 2 yr) distance runners. Runners were age-matched for competitive performance. The postmenopausal women exhibited a significant elevation in total body fat in both the

sedentary and endurance runner groups ($p<0.05$), but the difference was much smaller in the runners ($p<0.05$). Central body fatness did not differ between pre- and postmenopausal women in the endurance runners, but did in the sedentary women ($p<0.01$). These results support the concept that women who perform regular endurance exercise may not experience the increase in body weight and abdominal fat mass associated with menopause and advancing age and may be able to curb the associated increase in total body fat.

Aerobic exercise training resulting in a reduction in total body fat and consequently, in abdominal fat mass, has been shown to correlate with beneficial exercise-induced changes in carbohydrate and lipid metabolism (17, 19). Healthy, sedentary men and women, aged 60 to 70 years participated in a 9 to 12 month endurance exercise program (19). On average, the subjects walked and jogged or trained on rowing and cycling ergometers 45 minutes per day 4 days per week at an intensity of approximately 80% of HR_{max}. Both men and women experienced a significant decrease in total body fat content ($p<0.001$) without a reduction in FFM. Both genders also experienced a preferential loss of fat from the central, upper regions of the body ($p<0.001$) as indicated by reductions in circumferences and skinfold thickness. This decrease in total and central body fat mass was accompanied by a significant decrease in plasma cholesterol ($p<0.001$), triglyceride ($p<0.001$), and fasting glucose levels ($p<0.05$). Results of this study indicate that vigorous endurance exercise can reduce total and central body fatness, as well as improve lipid and carbohydrate metabolism in the elderly.

Strength training, as well as aerobic exercise, has been found to decrease intra-abdominal adipose tissue (18). Fourteen postmenopausal women ranging in age from 60

to 77 years were studied to determine the effects of strength training on intra-abdominal adipose tissue. The women performed 1-hour sessions of whole body strength training exercises, 3 times a week for 16 weeks while maintaining pre-treatment daily energy intake levels. There was no significant change after the exercise treatment in percent total body fat, fat mass, or FFM as measured by CT scan. There was, however, a significant decrease in intra-abdominal adipose tissue ($p<0.05$) and in intra-abdominal to subcutaneous abdominal adipose tissue ratio ($p<0.05$). Changes in HDL-C levels were significantly associated with decreases in subcutaneous abdominal fat ($p<0.05$) and in percent total body fat ($p<0.01$).

Exercise and lean body mass

Exercise sufficient to produce weight loss and corresponding improvements in CVD risk factors may or may not affect LBM (90-93). Exercise in the form of aerobic physical activity tends to spare FFM while promoting body fat mass loss (90, 93).

One hundred fifty-five overweight North American men were studied to examine the effects on fat mass and non-fat mass of moderate energy restriction compared to moderate exercise. Men that exercised maintained their normal energy intake level throughout the 12-month study. Men that dieted maintained their normal physical activity level throughout the study. After 12 months of jogging an average of 11.7 ± 8.1 miles per week, non-fat body mass changed little in the exercisers. This is in contrast to the significant decrease of non-fat body mass seen in the dieters compared to both control ($p<0.001$) and exercise ($p<0.01$) groups. Both the exercise and diet groups lost significant amounts of body fat mass (both $p<0.001$) when compared to the control group.

Exercise programs incorporating aerobic and strength training exercises also tend to promote body fat mass loss while maintaining LBM (91, 92). Comparisons of energy restriction only to energy restriction accompanied by aerobic and strength training exercise show a maintenance of LBM mass throughout significant weight loss (91, 92). Forty-six moderately obese postmenopausal North American women were studied to examine the effects of energy deficit on body composition and CVD risk factors. One group maintained a diet deficient of 700 kcal per day for 24 weeks. Another group maintained a diet deficient of 500 kcal per day and an exercise protocol that produced a 200 kcal per day deficit for the same amount of time. Compared to baseline, both groups experienced significant decreases in percent body fat ($p<0.05$) and in LBM ($p<0.05$). The amount of LBM lost by the diet and exercise group was much lower than the amount lost by the diet only group (0.2 kg versus 0.7 kg).

Exercise and body weight, abdominal fat, and fat-free mass in rats

Different modes of exercise have been shown to affect body weight in rats (76, 78, 94-96). Rats that swam for 60 minutes per day for 4 to 8 weeks, gained significantly less weight compared to sedentary controls in both male (94) and female (78) rats. Swim exercise has also been reported to either increase (94) or have no effect on (97) FFM in rats. Recent research has found that swim exercise may be beneficial in alleviating intra-abdominal obesity in rats (98).

Voluntary wheel running has been reported to decrease weight gain in male (95, 96) and female (95) rats when compared to sedentary controls. The lower weight of exercising rats was observed despite an increase in soleus muscle weights (95). This decreased relative weight was due to a decrease in body fat (95). Latour and co-workers,

however, reported that forced treadmill running did not affect body weight of OVX rats (76). The hypertrophy of muscles attributed to voluntary wheel running is contradicted in a study by Cortright and co-workers (96). Those researchers reported that daily spontaneous wheel running reduced FFM in male rats and had no effect in female rats (96). The two studies are hard to compare since Cortright and co-workers (96) reported distance run by rats in meters, while the study reporting muscle hypertrophy reported distance run in revolutions. It cannot be determined if the rats in these two studies ran comparable distances.

Purpose of this study

The purpose of this study was to investigate the effect of swim exercise or voluntary wheel running on overall weight gain, abdominal fat mass, and LBM on OVX retired breeder Sprague Dawley rats. Results were compared to an OVX, non-exercising group and to a sham operated (not OVX) group.

CHAPTER 3

ANIMALS AND METHODS

This study was approved by the Louisiana State University Institutional Animal Care and Use Committee (IACUC) (Appendix A).

Animals and Treatment Assignment

Seventy-six Sprague-Dawley 9 month-old, retired female breeder rats (Harlan Co.; Indianapolis, IN) were used in this study. Rats were weighed on arrival and were housed individually in 24cm x 28cm x 18cm hanging stainless steel wire cages. Animals were kept in the Life Sciences' animal care facility of Louisiana State University and Agricultural and Mechanical College (LSU), at 22°C, with a humidity level of 60%, and a 12-hour light/dark schedule (0700 light/1900 dark). Food and water were provided to rats *ad libitum*. A timeline showing significant events throughout the study is presented in Table 1.

Table 1. Timeline of study events.

Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7	Week 8
Rats received, assigned to treatment groups, and surgeries performed	Rats recuperate from surgeries	Swim exercise and voluntary running protocols started	Swim exercise group reach 60 minutes of continuous swimming; voluntary wheel running continues	Swimming and voluntary wheel running continues	Swimming and voluntary wheel running continues	Swimming and voluntary wheel running continues	Rats sacrificed

Following 24 hours to allow for recovery from travel, all rats were rotated through running cages for a 1-hour time period on two consecutive days to determine each animal's willingness to run. Rats were ranked according to number of revolutions run.

Nine rats with the highest number of revolutions run (at least 300 accumulated revolutions) were identified as voluntary runners. These identifications were used for later stratification.

On day four, all rats were assigned to their final treatment groups. Rats were stratified by weight, from heaviest to lightest. The heaviest and lightest animals (375 grams and 240 grams respectively) were removed from the group and maintained as "extras." The remaining animals were assigned numbers sequentially from one to eight, from the heaviest to the lightest, placing animals into groups of eight. Once assigned to these groups, random numbers were assigned to each animal in the groups, thus designating them to one of eight treatment groups (Table 2). The group with the most voluntary runners, was designated as the ovariectomized (OVX), voluntary wheel running exercise, 21% casein diet treatment group (CE). Rats in other groups identified previously as voluntary runners were exchanged for rats of comparable weights in the CE treatment group, to maintain the weight stratification and maximize the number of runners in the CE group.

Diets

After arrival, rats in sham-operated, no exercise (NoX), 21% casein diet (ShC); ovariectomized (OVX), NoX, 21% casein diet (C); CE; and OVX, swim exercise, 21% casein diet (CW) treatment groups were fed an American Institute of Nutrition (AIN)-93M diet (99) (Table 3); they were changed to a modified AIN-93M diet (Table 3) following assignment to treatment groups (day four). Only the rats in the ShC, C, CE, and CW groups were used for the ancillary study addressed by this thesis. The rats in the OVX, NoX, 14% casein + 7% soy diet (C7S); OVX, NoX, 7% casein + 14% soy diet

(C14S); OVX, NoX, 21% soy diet (21S); and OVX, NoX, 21% casein + rice bran oil (CRB) groups were part of a larger study and results from these animals are not included in this thesis.

Table 2. Treatment group nomenclature, surgery, exercise and diet treatment, and number of animals assigned to sham-operated, no exercise (NoX), 21% casein diet (ShC); Ovariectomized (OVX), NoX, 21% casein diet (C); OVX, NoX, 14% casein + 7% soy diet (C7S); OVX, NoX, 7% casein + 14% soy diet (C14S); OVX, NoX, 21% soy diet (21S); OVX, swim exercise, 21% casein diet (CW); OVX, voluntary wheel running, 21% casein diet (CE); and OVX, NoX, 21% casein + rice bran oil diet (CRB) groups.

Treatment Group	Surgery	Exercise treatment	Diet treatment	n
ShC	Sham-operated	None	21% casein	9
C	Ovariectomized	None	21% casein	9
C7S	Ovariectomized	None	14% casein + 7% soy	9
C14S	Ovariectomized	None	7% casein + 14% soy	9
21S	Ovariectomized	None	21% soy	9
CW	Ovariectomized	Swimming	21% casein	10
CE	Ovariectomized	Voluntary wheel running	21% casein	9
CRB	Ovariectomized	None	21% casein + rice bran oil	9

The diets were prepared as follows. The appropriate amount of Crisco® was melted at low heat, then the oil was added. The mixture was kept warm until added to the diet mixture. The macronutrients (sucrose, casein, cornstarch, dextrinized cornstarch, and fiber) were added to a large mixing bowl and mixed at a low speed using a Hobart mixer (model no. A-200-FD; Hobart Mfg., Co.; Troy, OH). After mixing for 10 minutes, the bowl was scraped, and mixture was mixed for another 5 minutes. The AIN-93M-MX mineral mix was crushed with mortar and pestle. Micronutrients (L-cystine, AIN-93M-MX mineral mix, AIN-93-VX vitamin mix, choline bitartrate, cholesterol, and cholic acid) were sieved sequentially through a small mesh into a medium mixing bowl. Remaining crystals were ground finely with mortar and pestle and added to the bowl.

Micronutrients were mixed by hand until a uniform distribution was achieved. Micronutrients were then added to the large mixing bowl containing macronutrients and were mixed for 10 minutes. The bowl was scraped and ingredients were mixed for another 5 minutes. The warm Crisco® and soybean oil were added to the dry ingredients in a large mixing bowl and mixed for 5 minutes. The bowl was scraped and ingredients mixed for another 10 minutes.

Table 3. American Institute of Nutrition (AIN)-93M Diet for maintenance of adult rodents (99).

INGREDIENT	AIN-93M	Modified AIN-93M
Macronutrient	g/kg diet	g/kg diet
Sucrose	100	67
Casein	140	210
Cornstarch	465.69	313.33
Dextrinized cornstarch	155	104
Fiber	50	50[a]
Micronutrient		
L-cystine	1.8	1.8
Mineral mix (AIN-93M-MX)[b,c]	35	35
Vitamin mix (AIN-93-VX)	10	10
Choline bitartrate[b]	2.5	2.5
Cholesterol	0	5
Cholic acid	0	2
Fat		
Crisco®	0	50
Soybean oil (butylated hydroxytoluene added as preservative)	40	150

[a] cellulose
[b] crush with mortar and pestle
[c] sieve

Diets were stored frozen in tightly-sealed Ziploc bags from which all excess air had been removed. Bags were labeled with diet type, date, batch number, and initials of person who prepared the diet. A sample from each batch was stored in a separate small plastic bag and labeled with diet type, date, batch number, and initials of the person who

prepared the diet. These samples were placed in a larger bag and stored in the freezer for later analysis.

Measurements of food and water intake, body weight, and revolutions run

Rats were weighed (to the nearest gram), and food consumption was quantified (full food cup – [empty food cup + spillage]; to the nearest gram), three times per week (Monday/Wednesday/ Friday). All weights were measured using a 500-gram analog scale (Toledo Scale, Co.; Toledo, Ohio). Revolutions run by each rat in the CE group were recorded five days per week.

Ovariectomy and sham surgeries

Faculty and students from the LSU School of Veterinary Medicine performed OVX and sham operations at the end of week one. Rats in the CE and CW treatment groups were operated on first to allow maximum time for recovery. All animals were anesthetized with gaseous Isoflurane (Abbott Lab, Chicago) via an induction chamber. Isoflurane inhalation was continued during surgery via an inhalation mask. Buprenorphine hydrochloride (Buprenex® by Rickitt & Colman Products; Hull, England) (0.05 mg/kg of bodyweight) was administered intramuscularly, after induction of gaseous Isoflurane, to provide postoperative analgesia. Skin covering the mid to dorsal aspects of the abdomen was clipped and disinfected with betadine to prepare for aseptic surgery. Dorsal entry to the peritoneal cavity was gained through bilateral incisions made slightly inferior to abdominal midline. Rats in the C, CE, and CW groups had their ovaries removed and the horns of their uterus returned to the abdominal cavity (ovariectomized). Rats in the ShC group had the same surgical procedure performed, however, without removal of ovaries (sham-operated). Muscle layers were sutured with

5-0 nylon suture (Ethilon™ by Ethicon, Inc.; Somerville, NJ). Skin was closed with Super Glue (Loctite, Corp.; Cincinnati, OH). Subcutaneous injections of Lactated Ringers solution (15ml) (Abbott Lab; Chicago, IL) were used for hydration following surgery. Animals were covered with towels to minimize heat loss, allowed to recover from anesthesia, and returned to their cages. Runners were separated from their running wheels for 24 hours to facilitate recovery.

Running protocol

Rats assigned to the CE group were housed individually in 15.5cm x 26 cm x 15.5 cm stainless steel wire cages adjoined to 10 cm wide, 36 cm diameter stainless steel wire running wheels (Figure 1). Rats were placed in these cages immediately following surgery but were denied access to the running wheel to promote convalescence. After 24 hours of post-surgical recovery, and throughout the remainder of the study, animals were given free access to the running wheels.

Swimming protocol

Swimming treatment consisted of rats swimming freely, without additional weights, in plastic tubs (43cm height, 48cm diameter) (Figure 2). Water was maintained at the 24cm level and at 30°C. Animals assigned to the swimming group (CW) were introduced to the water at the end of the first week (before surgery) by swimming for 5 minutes on two consecutive days. The actual swimming treatment was not started until one week after surgery (week three of study). Animals swam continuously for 5 minutes the first day of week three. Swim time was increased by 5 minutes each consecutive day until 20 minutes of continuous swimming was achieved. Swim time was increased by 10-minute increments until 60 minutes of continuous swimming was achieved. The

animals swam Monday through Friday, for 60 continuous minutes throughout the remainder of the study. After each swim session, the animals were towel-dried and returned to their respective cages.

Sacrifice

During week eight of the study, animals were anaesthetized with isoflurane for sacrifice. Animals were euthanised by cardiac puncture and exsanguinated with a 10-cc syringe fitted with a 22-gauge needle (Figure 3). Cervical dislocation was performed to ensure humane sacrifice. The heart was severed at the aorta, superior and inferior vena cava, and pulmonary veins; removed from the body, and weighed (to three decimal places). Four separate muscles were removed from the body and weighed: the soleus, gastrocnemius, quadriceps femoris, and triceps brachii (Figure 4). The soleus muscle was severed at its origin (posterior surfaces of the fibula and tibia) and insertion (calcanius). The gastrocnemius muscle was severed at its origin (lateral and medial condyles of the femur) and insertion (posterial surface of the calcaneus). Both the lateral and medial heads of the gastrocnemius muscle were removed from the body. All four heads of the quadriceps femoris muscle; rectus femoris, vastus lateralis, vastus intermedius, and vastus medialis, were severed at their origins (ilium and acetabulum, linea aspera, femur, and linea aspera, respectively) and insertion (common tendon of quadriceps femoris and patellar ligament). The triceps brachii was severed at its origin (scapula and humerus) and insertion (ulna). Soleus, gastrocnemius, quadriceps femoris, and triceps brachii muscles were removed from both right and left limbs and weighed (to nearest 0.100 gram). Abdominal fat was removed manually and weighed (to nearest

0.100 gram). All rats were inspected visually for presence or absence of ovaries. Uteri and uterine horns were weighed to confirm sham operation or OVX.

Statistical analysis

Data from this study were analyzed using SPSS® Student Version 9.0 for Windows® (©SPSS, Inc.,1999; Chicago, IL). Means and standard deviations (SD) for food intake, weight gain, abdominal fat, muscle weights and revolutions run were calculated. Mean values of food intake, weight gain, abdominal fat, muscle weights, and revolutions run were compared using analysis of variance (ANOVA). Abdominal fat weight and muscle weights were adjusted for body weight by dividing the abdominal fat or muscle weight by the rat's final body weight before sacrifice. Linear regression was used to test for linear relationships between number of revolutions run and total weight gain, total food intake, abdominal fat weight, abdominal fat weight (adjusted for bodyweight), total muscle weight, or total muscle weight (adjusted for bodyweight).

Data from rats in the CE group were divided into two groups for further analysis. These two groups consisted of rats that ran more than the mean of 50,770 total revolutions (high runners) and those that ran less than this mean (low runners). Independent t-tests were used to determine differences of mean total weight gain, mean total food intake, abdominal fat weight, abdominal fat weight (adjusted for body weight), total muscle weight, and total muscle weight (adjusted for body weight) between the "high" and "low" runner groups. In all cases, a p value ≤ 0.05 was considered significant.

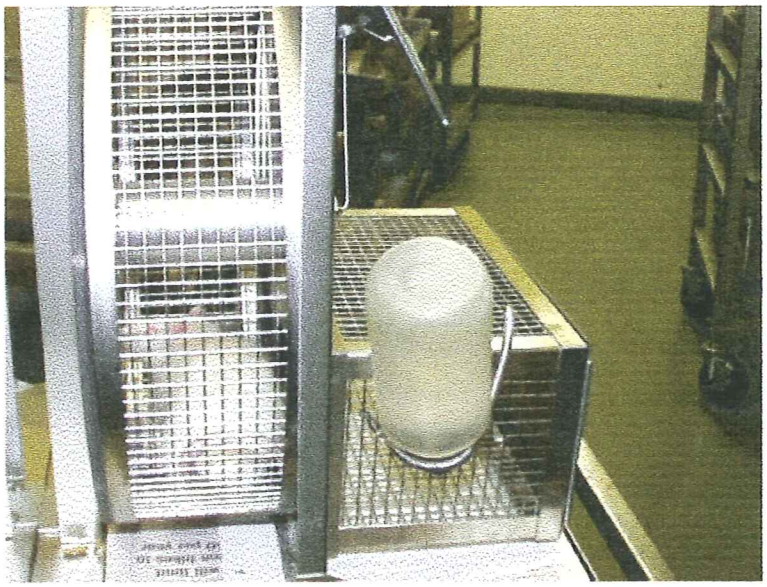

Figure 1. Running wheel cages used in voluntary wheel running exercise protocol.

Figure 2. Rats swimming during swim exercise protocol.

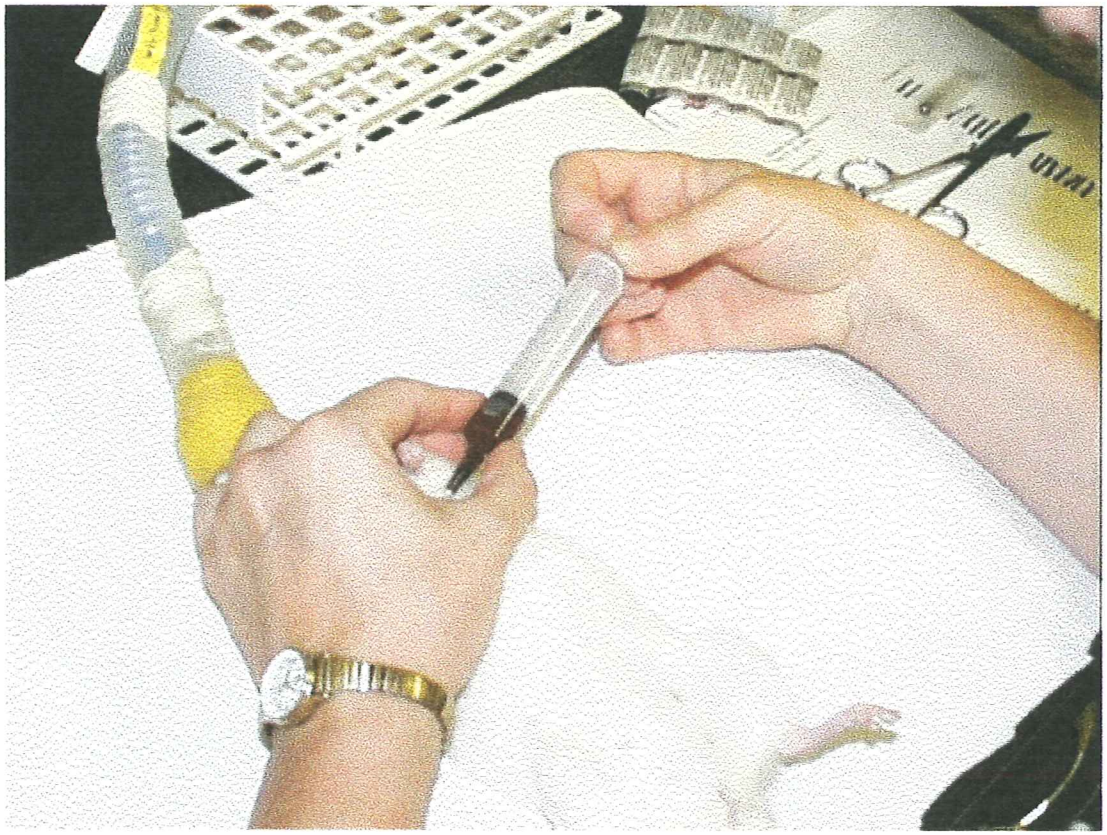

Figure 3. Rat being sacrificed by exsanguination.

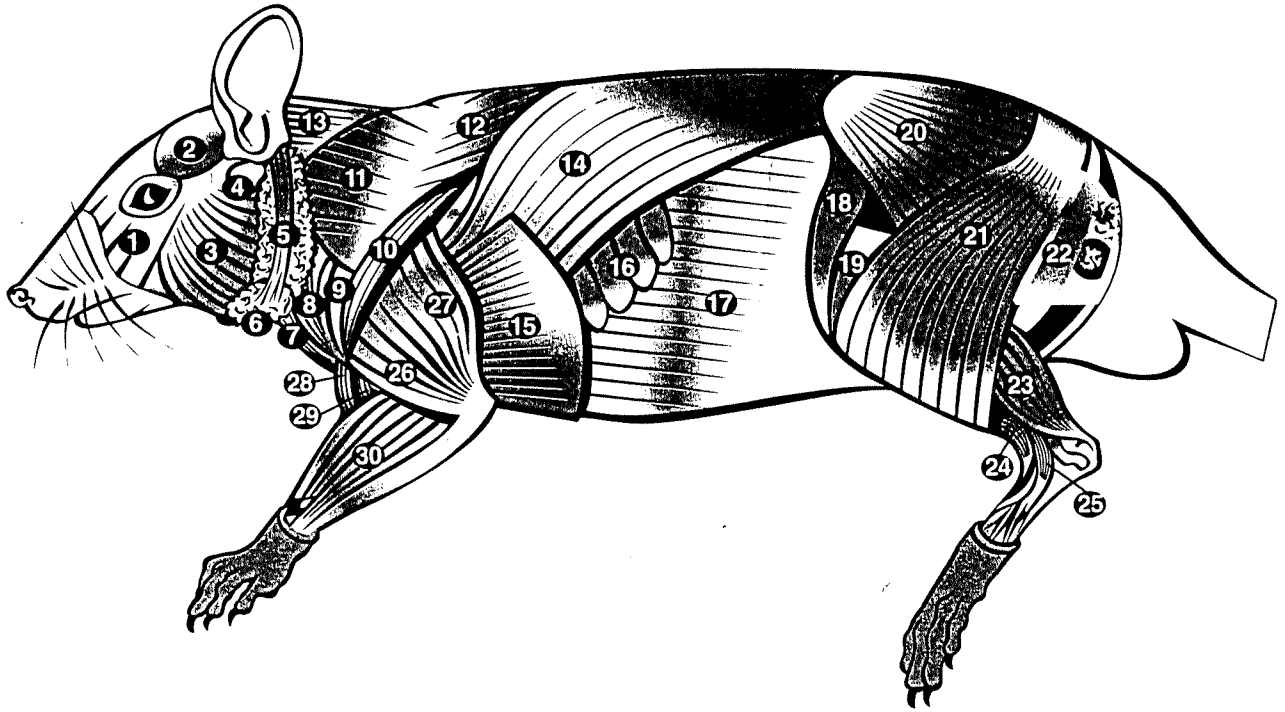

Plate 1 The superficial muscles.

1 musculi faciales
2 musculus temporalis
3 musculus masseter
4 glandula lacrimalis extraorbitalis
5 glandula parotidea with the ventral auricular muscle
6 glandula mandibularis
7 musculus sternocephalicus, pars mastoidea
8 musculus brachiocephalicus, pars occipitalis
9 musculus deltoideus, pars acromialis
10 musculus deltoideus, pars scapularis
11 musculus trapezius, pars cervicalis
12 musculus trapezius, pars thoracica
13 musculus cervicoauricularis
14 musculus latissimus dorsi
15 musculus cutaneus trunci
16 musculus serratus ventralis thoraci
17 musculus obliquus externus abdominis
18 musculus tensor fasciae latae
19 musculus quadriceps femoris, musculus rectus
20 musculus gluteus superficialis
21 musculus biceps femoris, portio cranialis et caudalis
22 musculus semitendinosus
23 musculus gastrocnemius, caput laterale
24 musculus tibialis cranialis
25 musculus extensor digitorum longus
26 musculus triceps brachii, caput laterale
27 musculus triceps brachii, caput longum
28 musculus biceps brachii
29 musculus brachialis
30 musculi extensores carpi et digitorum

Figure 4. Rat anatomy showing location of gastrocnemius (#23), quadriceps femoris (#19), and triceps brachii (#27) muscles (100). Reproduced with permission from Academic Press (Appendix B).

CHAPTER 4

RESULTS

Food Intake

Figure 5 shows mean food intake (in grams) throughout the study for rats in the sham-operated, no exercise (NoX) (ShC); ovariectomized (OVX), NoX (C); OVX, voluntary wheel running (CE); and OVX, swim exercise (CW) treatment groups.

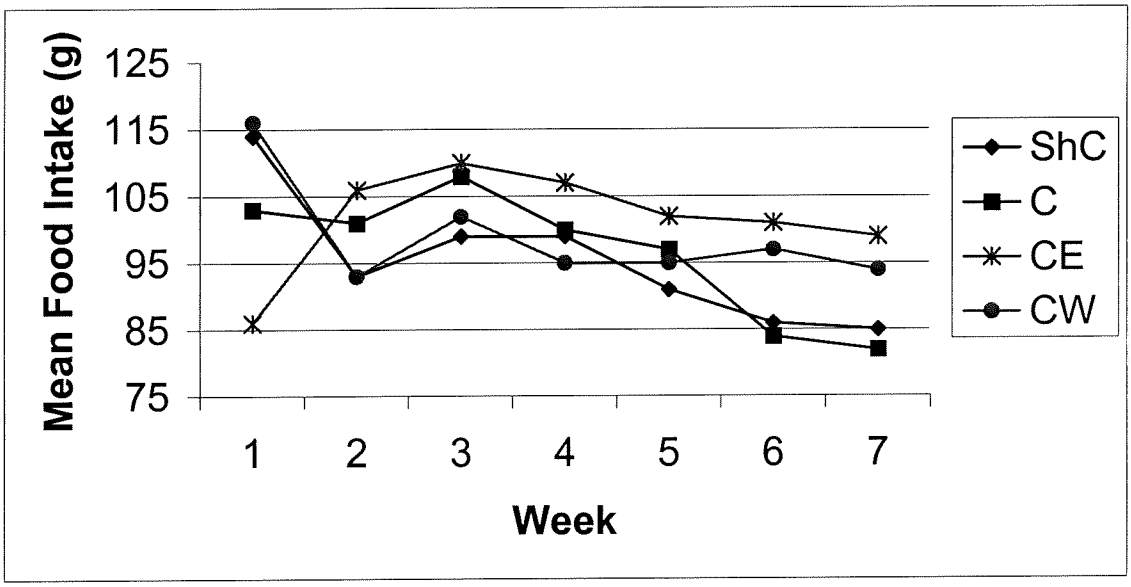

Figure 5. Mean weekly food intake for rats in the sham-operated, no exercise (NoX) (ShC); OVX, NoX (C); OVX, voluntary wheel running (CE); and OVX, swim exercise (CW) treatment groups.

Table 4 shows mean food intake (g ± SD) of each treatment group by week and at the end of study. During week one of the study, mean food intake of rats in both the ShC (114 g ± 10.8) and CW (116 g ± 10.5) groups were significantly higher than those of the rats in the CE group (86 g ± 26.8) (p=0.016, p=0.007, respectively). During the sixth week of the study, food intake of rats in the CE group (101 g ± 11.2) was significantly higher than that of rats in ShC (86 g ± 11.1) and C (84 g ± 6.0) groups (p=0.022 and p=0.011, respectively). In week seven of the study, the food intake of the rats in the CE

group (99 g ± 14.0) was significantly higher than that of the rats in the C group (82 g ± 7.2) (p=0.011). No other significant differences were determined. Numerically, the mean total study intake of the CE and CW groups were similar, as were those ShC and C groups.

Table 4. Mean food intake (grams ± SD) of sham-operated, no exercise (NoX) (ShC); OVX, NoX (C); OVX, voluntary wheel running (CE); and OVX, swim exercise (CW) treatment groups by week and end of study total.

	ShC	C	CE	CW
Week 1	114 ± 10.8[a]	103 ± 21.4	86 ± 26.8	116 ± 10.5[a]
Week 2	93 ± 6.2	101 ± 16.3	106 ± 11.0	93 ± 12.9
Week 3	99 ± 10.6	108 ± 5.9	110 ± 15.1	102 ± 11.7
Week 4	99 ± 11.8	100 ± 6.5	107 ± 12.8	95 ± 8.9
Week 5	91 ± 7.9	91 ± 7.1	102 ± 13.3	95 ± 9.6
Week 6	86 ± 11.1[b]	84 ± 6.0[b]	101 ± 11.2	97 ± 13.2
Week 7	85 ± 10.9	82 ± 7.2	99 ± 14.0[c]	94 ± 10.2
Study Total	666 ± 39.0	669 ± 43.9	712 ± 64.2	691 ± 65.7

[a] Significantly higher ($p<0.05$) than CE group.
[b] Significantly lower ($p<0.05$) than CE group.
[c] Significantly higher ($p<0.05$) than C group.

Weight gain

Figure 6 shows mean weekly weight gain of rats in ShC, C, CE, and CW treatment groups. Table 5 shows mean weight gain (g ± SD) of each treatment group by week and at the end of study total. During week one, the weight gain of rats in ShC group (15 g ± 8.4) was significantly higher than those of rats in the CE group (-1 g ± 14.3) (p=0.012). During the second week, rats in the CE group gained significantly more weight (22 g ± 6.5) than those in the ShC (3 g ± 4.8) (p=0.001) and CW (7 g ± 6.1) (p=0.001) groups. The rats in the C group gained significantly more weight (16 g ± 4.8) than those in the ShC (3 g ± 4.8) (p=0.001) and the CW (7 g ± 6.1) (p=0.005) groups.

Rats in the C group gained significantly more weight (16 g ± 3.1) than those in the ShC (4 g ± 5.6) (p=0.001) and the CW (6 g ± 3.6) (p=0.001) groups during week three. The rats in CE gained significantly more weight (16 g ± 3.1) than those in the ShC group (4 g ± 5.6) (p=0.023). During week four, the rats in the CE, ShC, and C groups gained significantly more weight (8 g ± 4.5, 4 g ± 9.6, 4 g ± 4.5, respectively) than those in the CW group (-6 g ± 7.7) (p=0.001, p=0.019, p=0.016, respectively). In the seventh week, the rats in the CE group gained significantly more weight (7 g ± 6.8) than those in the C group (-1 g ± 3.4) (p=0.026). No other significant differences were seen. Numerically, the mean study total weight gain between the C and CE groups were similar, as were those in the ShC and CW groups.

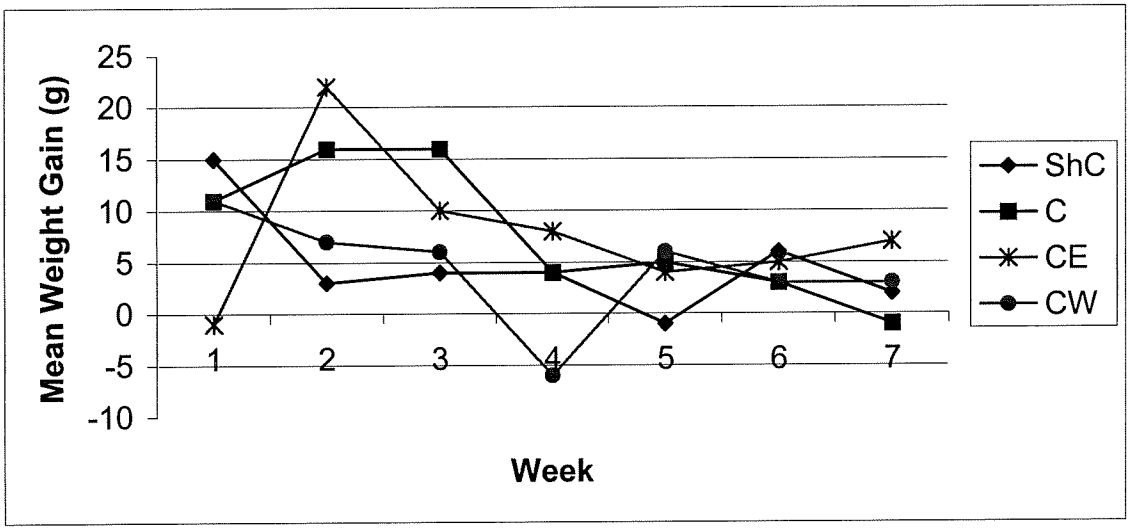

Figure 6. Mean weekly weight gain of sham-operated, no exercise (NoX) (ShC); OVX, NoX (C); OVX, voluntary wheel running (CE); and OVX, swim exercise (CW) treatment groups. Ovariectomy was performed at the beginning of week two.

Table 5. Average weight gain (grams ± SD) of sham-operated, no exercise (NoX) (ShC); OVX, NoX (C); OVX, voluntary wheel running (CE); and OVX, swim exercise (CW) treatment groups by week and end of study total.

	ShC	C	CE	CW
Week 1	15 ± 8.4[a]	11 ± 11.5	-1 ± 14.3	11 ± 7.3
Week 2	3 ± 4.8[b,c]	16 ± 4.8	22 ± 6.5	7 ± 6.1[b,c]
Week 3	4 ± 5.6[c]	16 ± 3.1	10 ± 5.9[d]	6 ± 3.6[c]
Week 4	4 ± 9.6	4 ± 4.5	8 ± 4.5	-6 ± 7.7[b,e]
Week 5	-1 ± 3.8	5 ± 3.7	4 ± 4.8	6 ± 13.4
Week 6	6 ± 8.7	3 ± 4.0	5 ± 3.7	3 ± 5.4
Week 7	2 ± 7.1	-1 ± 3.4	7 ± 6.8[f]	3 ± 4.3
Study Total	33 ± 22.1	58 ± 21.6	57 ± 20.2	36 ± 21.2

[a] Significantly higher ($p<0.05$) than CE group
[b] Significantly lower ($p<0.01$) than CE group
[c] Significantly lower ($p<0.01$) than C group
[d] Significantly higher ($p<0.05$) than ShC group
[e] Significantly lower ($p<0.05$) than ShC and C groups
[f] Signficicantly higher ($p<0.05$) than C group

Figure 7 shows mean total weight gain (g ± 1 SD) of rats in ShC, C, CE, and CW treatment groups.

Muscle weight

No significant differences in mean total skeletal muscle weight (*i.e.* weight of soleus, gastrocnemius, quadriceps femoris, and triceps brachii muscles combined) or mean individual muscle weights for the soleus, gastrocnemius, quadriceps femoris, or triceps brachii muscles were observed among treatment groups. There were no significant differences among means of individual muscle weights, or total skeletal muscle weight, when adjusted for body weight. Tables 6 and 7 show mean weight of individual soleus, gastrocnemius, quadriceps femoris, and triceps brachii muscles from the right-side or left-side of rats (g ± SD) by treatment group.

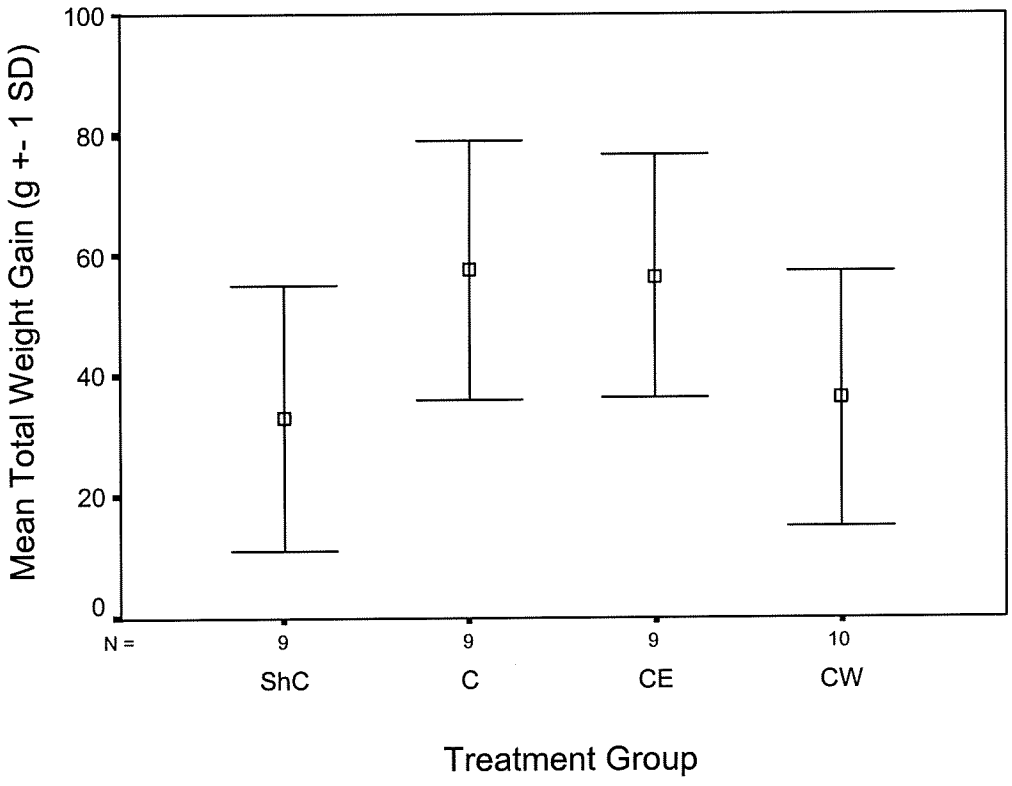

Figure 7. Mean study total weight gain (g ± 1 SD) of rats in sham-operated, no exercise (NoX) (ShC); OVX, NoX (C); OVX, voluntary wheel running (CE); and OVX, swim exercise (CW) treatment groups by week and end of study total.

Table 6. Mean weight (g ± SD) of individual muscles from right-side of rats in sham-operated, no exercise (NoX) (ShC); OVX, NoX (C); OVX, voluntary wheel running (CE); and OVX, swim exercise (CW) treatment groups.

	ShC	C	CE	CW
Soleus	0.125 ± 1.010	0.130 ± 0.019	0.138 ± 0.013	0.123 ± 0.010
Gastrocnemius	2.201 ± 0.211	2.311 ± 0.155	2.364 ± 0.153	2.272 ± 0.152
Quadriceps femoris	2.918 ± 0.317	2.979 ± 0.288	3.150 ± 0.265	3.042 ± 0.223
Triceps brachii	1.321 ± 0.119	1.414 ± 0.167	1.450 ± 0.136	1.381 ± 0.107

Table 7. Mean weight (g ± SD) of individual muscles from left-side of rats in sham-operated, no exercise (NoX) (ShC); OVX, NoX (C); OVX, voluntary wheel running (CE); and OVX, swim exercise (CW) treatment groups.

	ShC	C	CE	CW
Soleus	0.124 ± 0.010	0.129 ± 0.020	0.141 ± 0.017	0.127 ± 0.011
Gastrocnemius	2.262 ± 0.205	2.388 ± 0.283	2.380 ± 0.170	2.276 ± 0.160
Quadriceps femoris	2.989 ± 0.305	3.016 ± 0.274	3.078 ± 0.300	3.006 ± 0.290
Triceps brachii	1.338 ± 0.009	1.467 ± 0.268	1.433 ± 0.103	1.356 ± 0.126

Tables 8 and 9 show mean weight of individual right-side and left-side muscles of rats (g ± SD), adjusted for body weight, by treatment group. There were no significant differences between left- and right-side muscles of each treatment group.

Table 8. Mean weight (g ± SD) of individual muscles from right-side, adjusted for body weight, of rats in sham-operated, no exercise (NoX) (ShC); OVX, NoX (C); OVX, voluntary wheel running (CE); and OVX, swim exercise (CW) treatment groups.

	ShC	C	CE	CW
Soleus	0.388 ± 0.0353	0.356 ± 0.0527	0.389 ± 0.0333	0.370 ± 0.0483
Gastrocnemius	6.667 ± 0.5024	6.600 ± 0.2872	6.667 ± 0.5852	6.840 ± 0.2913
Quadriceps femoris	8.833 ± 0.8047	8.522 ± 0.8151	8.878 ± 0.7546	9.160 ± 0.3949
Triceps brachii	4.022 ± 0.5166	4.044 ± 0.4419	4.089 ± 0.3689	4.1700 ± 0.3198

Table 9. Mean weight (g ± SD) of individual muscles from left-side, adjusted for body weight, of rats in sham-operated, no exercise (NoX) (ShC); OVX, NoX (C); OVX, voluntary wheel running (CE); and OVX, swim exercise (CW) treatment groups.

	ShC	C	CE	CW
Soleus	0.388 ± 0.0353	0.378 ± 0.0440	0.040 ± 0.0500	0.040 ± 0.047
Gastrocnemius	6.856 ± 0.4245	6.811 ± 0.6972	6.700 ± 0.5477	6.850 ± 0.2635
Quadriceps femoris	9.056 ± 0.6729	8.611 ± 0.6790	8.678 ± 0.8941	9.040 ± 0.5440
Triceps brachii	4.056 ± 0.2788	4.178 ± 0.7276	4.033 ± 0.2449	4.090 ± 0.5173

Mean total skeletal muscle weight of rats by treatment group is shown in Table 10 and Figure 8.

Table 10. Mean total combined skeletal muscle weight of soleus, gastrocnemius, quadriceps femoris and triceps brachii muscles (g ± SD) of rats in sham-operated, no exercise (NoX) (ShC); OVX, NoX (C); OVX, voluntary wheel running (CE); and OVX, swim exercise (CW) treatment groups.

	ShC	C	CE	CW
Total[a]	13.253 ± 0.942	13.836 ± 0.823	14.137 ± 0.948	13.581 ± 0.879
Total[b]	0.041 ± 0.002	0.039 ± 0.001	0.040 ± 0.003	0.040 ± 0.002

[a] unadjusted for weight (g)
[b] adjusted for body weight (g)

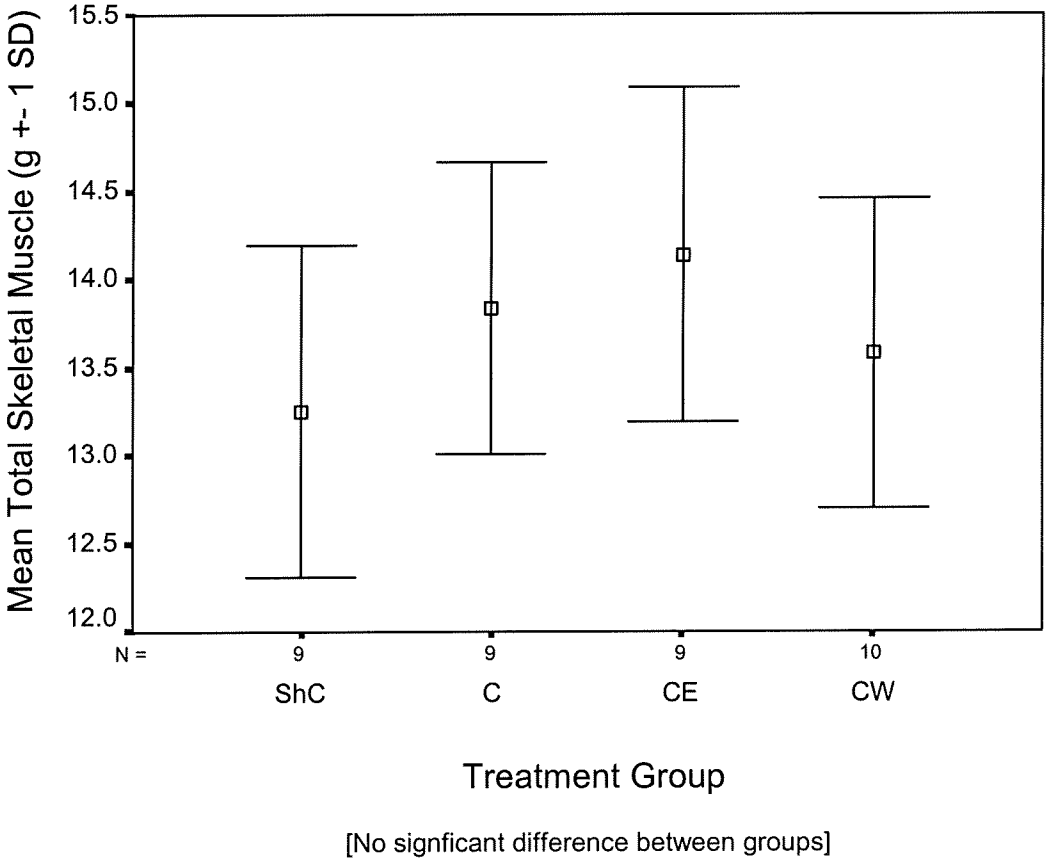

Figure 8. Mean total skeletal muscle weight (g ± 1 SD) of rats in sham-operated, no exercise (NoX) (ShC); OVX, NoX (C); OVX, voluntary wheel running (CE); and OVX, swim exercise (CW) treatment groups.

Abdominal fat

The mean total abdominal fat mass of rats in ShC, C, CE, and CW groups is presented in Table 11 and Figure 9. The average weight of the abdominal fat of the rats in the ShC group (14.335 g ± 3.927) was significantly higher than that in the CW group (9.770 g ± 2.235, p=0.031). The average weight of abdominal fat of rats in the C and CE groups was 13.437 g ± 2.572 and 12.057 g ± 4.506, respectively. There was no significant difference between the other groups.

Table 11. Mean total abdominal fat weight (g ± SD) of rats in sham-operated, no exercise (NoX) (ShC); OVX, NoX (C); OVX, voluntary wheel running (CE); and OVX, swim exercise (CW) treatment groups.

	ShC	C	CE	CW
Total[a]	14.335 ± 3.927	13.437 ± 2.572	12.057 ± 4.506	9.770 ± 2.235
Total[b]	0.043 ± 0.011	0.038 ± 0.007	0.034 ± 0.011	0.029 ± 0.010

[a] unadjusted for weight (g)
[b] adjusted for body weight (g)

Running exercise group

Figure 10 shows the total revolutions run by each rat in the CE group. There was no linear relationship between the number of revolutions run by each rat and total weight gain (r = 0.351), total food intake (r = 0.619), total abdominal fat mass (r = 0.175), total abdominal fat mass adjusted for body weight (r = 0.092), total muscle mass (r = 0.583), or total muscle mass adjusted for body weight (r = 0.068). There was no linear relationship between rats that ran more or less than the mean revolutions that the rats ran (50,770 revolutions) and total weight gain, total abdominal fat mass, total abdominal fat

mass adjusted for body weight, total muscle mass, or total muscle mass adjusted for body weight. Rats that ran more than the mean revolutions run had a significantly higher total food intake than the rats that ran below the mean revolutions run (p=0.041). Figure 11 shows the weekly revolutions run by rats in the CE group.

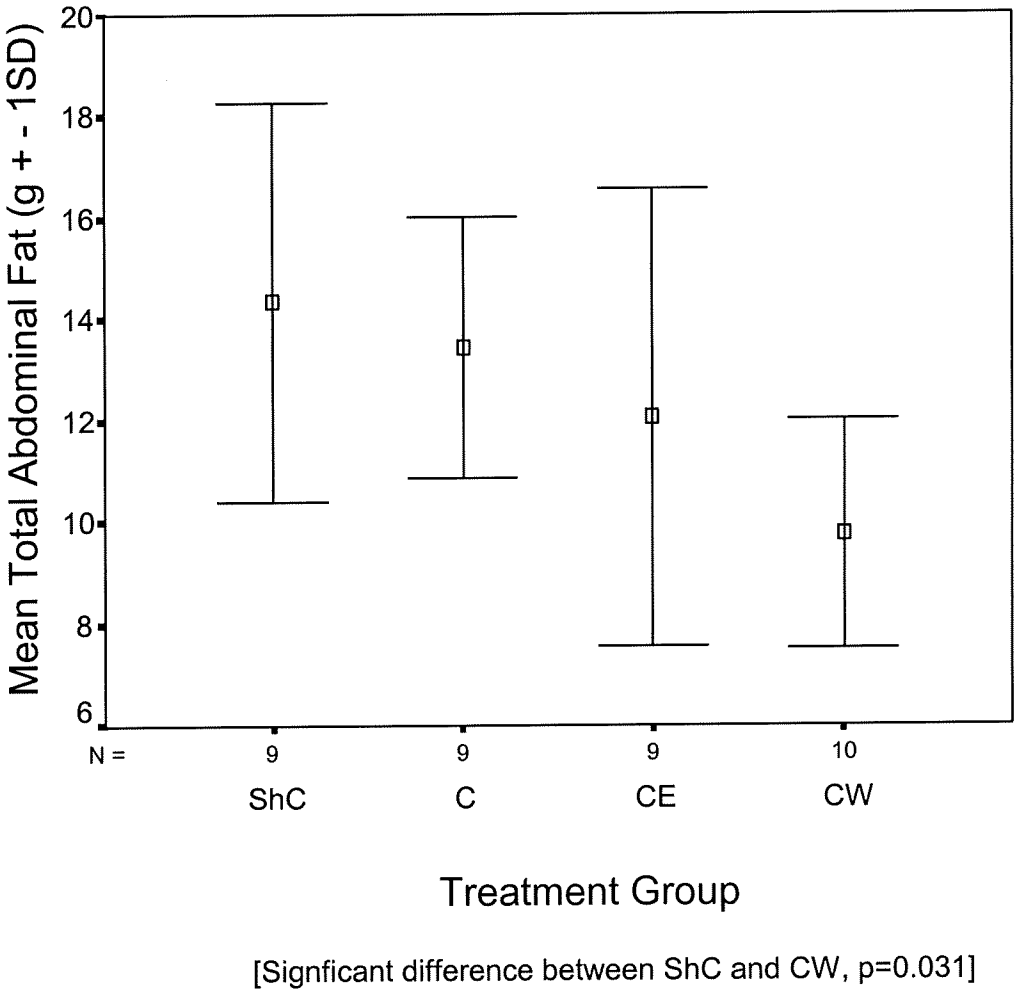

Figure 9. Mean total abdominal fat weight (g ± 1 SD) of rats in sham-operated, no exercise (NoX) (ShC); OVX, NoX (C); OVX, voluntary wheel running (CE); and OVX, swim exercise (CW) treatment groups.

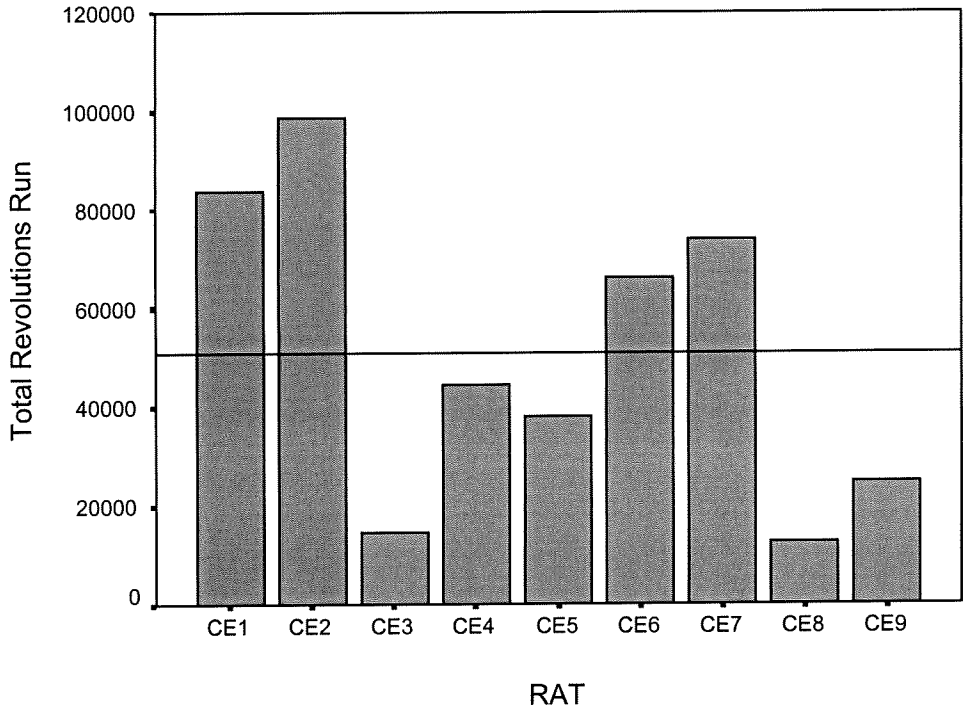

Figure 10. Total revolutions run by each rat for CE group. Line indicates group mean revolutions run (50,770).

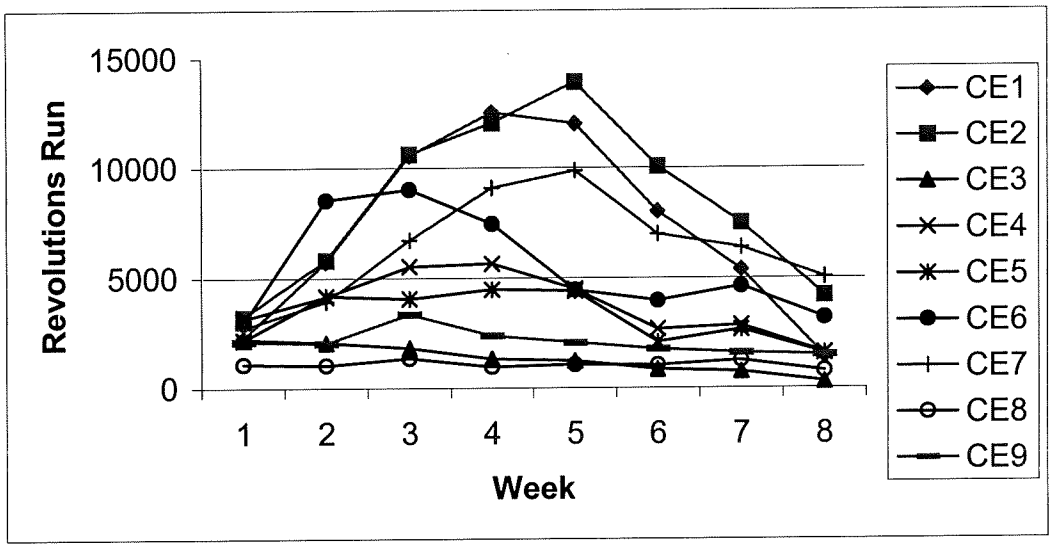

Figure 11. Weekly revolutions run by each rats in CE group.

CHAPTER 5

DISCUSSION

Our study produced several unexpected results. There were no significant differences in total food intake or total weight gain among treatment groups. There were no significant differences in average individual soleus, gastrocnemius, quadriceps femoris, or triceps brachii muscle weights among treatment groups, nor were there significant differences in total combined skeletal muscle weights among treatment groups. There was a significant difference in mean total abdominal fat between the sham-operated, no exercise (NoX) treatment group (ShC) and the ovariectomized (OVX), swim exercise group (CW), but not in any of the other treatment groups. There was extreme variability in the number of revolutions run by the rats in the OVX, voluntary wheel running (CE) treatment group. There was no linear relationship between the number of revolutions run by each rat and total weight gain, total food intake, total abdominal fat mass, total abdominal fat mass adjusted for body weight, total muscle mass, or total muscle mass adjusted for body weight. There was also no linear relationship between rats that ran more or less than the mean revolutions run by the CE treatment group. Rats that ran more than the mean revolutions run, however, did have a significantly higher total food intake than the rats that ran less than the mean revolutions run by the CE treatment group.

Food Intake

The results of our study did not support the hypothesis that OVX in Sprague-Dawley rats would contribute to an increase in food intake. It has been reported previously that ovariectomized (OVX) rats experience an increase in food intake (70-77)

during the first 28 days after surgery. The rats in our study did not follow this pattern. Neither weekly, nor total study, food intake differed between the ShC or the OVX, NoX treatment group (C). The lack of an increase in daily food intake by all OVX groups during the first 28 days after OVX was surprising. Although counter to many studies reporting an increase in food intake post-OVX (70-77), our finding does agree with the results of a study by Roy and associates (80) that examined food intake of OVX rats over a 44-day period. At the end of the 44-day period, the rats had gained body weight, but their food intake had not significantly increased. This suggests that variables other than OVX induced hyperphagia contribute to post OVX weight gain (80). Unlike the rats in the study by Roy and associates (80), the rats in our study did not show a significant difference in mean total weight gain. This would seem to be consistent with the lack of significant increase in mean total food intake.

The rats in our study were changed from an American Institute of Nutrition (AIN)-93M (99) diet (containing 40 grams per kilogram [g/kg] of fat) to a modified AIN-93M diet (containing 200 g/kg of fat) on day four after arrival (day one of week one of study). The effect of the modified AIN-93M diet cannot be compared to the effects of the AIN-93M diet since food intake records were not kept before day one of week one of the study. The trend of decreasing food intake during the first week, with the exception of CE group, may have been an adjustment period. Upon arrival, the rats were dehydrated and had been deprived of food during shipping. Once the rats adapted to travel or adjusted to the novel diet introduced during week one, food intake decreased. The rats had been fed a pelleted chow prior to arrival at our lab. The sharp increase in food intake by CE group between weeks one and two can be attributed largely to the inadvertent

inaccessibility of food to one rat in the CE group during week one. The rat resumed a relatively normal eating pattern during the second week, which resulted in a marked increase of food intake when compared to week one.

Our results did not support the hypothesis that voluntary wheel running and swim exercise would increase food intake in OVX rats. Findings from previous studies examining the effect of exercise on food intake are inconsistent and appear to be dependent upon the type of exercise and the intensity of that exercise (12, 76, 81, 95, 96, 101, 102). In studies using running as the mode of exercise, those that involved forced treadmill running have reported either no significant effect on food intake (76, 81), or a moderate decrease in food intake, although the effect was not significant (102). Forced treadmill running ensures a specific level of intensity for a specific amount of time. These studies cannot be compared directly to those using voluntary running, such as our study, since voluntary wheel running does not use specific intensity or duration of exercise.

Mean food intake of the CE group during weeks six and seven of our study was significantly higher than that of rats in the C group. Mean total food intake, however, did not differ statistically between the two groups. This is in agreement with previous findings from our lab. Bush (12) reported that, overall, rats in exercising and non-exercising treatment groups consumed remarkably similar amounts of total energy throughout a 12-week study. Other studies (95, 96), however, have reported a significantly higher food intake of voluntary wheel running rats compared to non-exercising control groups. The lack of agreement between these studies may be due to inconsistent intensity and duration of exercise associated with voluntary wheel running.

In fact, rats that ran more than the mean revolutions run in this study did have a significantly higher food intake than did rats that ran less than the mean revolutions run, suggesting a positive relationship between the amount of exercise performed and the amount of food ingested. This positive relationship between revolutions run and food intake has been reported previously by Tokuyama and associates (95). A sample size of more than 9 rats in our CE group, as well as a forced running protocol, may have produced a stronger relationship between energy expended and food intake.

Type of diet may also affect the food intake of exercising rats. Cortright and co-workers (96) reported a significantly higher food intake of voluntary wheel running rats when compared to non-exercising control groups. The rats in that study were fed standard, pelleted rat chow (Purina #5008). The rats in our study had a modified AIN-93M diet that had a soft, moist powdery texture, when compared to a standard pellet rat chow.

An exercise protocol using non-weighted swimming as the mode of exercise, similar to the protocol used in this study, has been shown to cause no significant difference in total food intake (22). This is consistent with the results of our study. In contrast, however, Wilterdink and colleagues (94) have shown a significant increase of food intake in rats after six weeks of swim exercise. Rats in the swim protocol of our study may have shown also a significant increase in food intake after six weeks of swimming; however, the rats in our study were sacrificed after four weeks of swim exercise. A study longer than four weeks may need to be conducted to elicit an increase in food intake due to swim exercise.

Weight Gain

The results of this study did not support clearly the hypothesis that OVX would increase weight in Sprague-Dawley rats. Weight gain during the first 28 days after OVX has been observed in several studies (71, 72, 75, 81) of both weanling (73, 74, 76) and adult (70, 77, 78, 80, 82) rats. The lack of a significant difference in body weight of OVX and ShC rats at the end of our study conflicts with those studies. Although no significant difference among groups was observed, rats in the CW group tended to have a lower total study weight gain than did the rats in CE and C groups. This is in line with the hypothesis that swim exercise would minimize or ablate the weight gain associated with OVX in rats. Weight gain of the CW group was similar to that of the ShC group. This agrees with previous findings that swimming eliminates the weight gain associated with OVX (78, 94). In contrast, the weight gain of the CE group was similar to that of the C group. This opposes the hypothesis that voluntary wheel running would minimize or ablate weight gain associated with OVX in rats. This is probably the result of the extreme variability in the number of revolutions run by individual rats in the CE group. Since neither intensity nor duration of running was controlled during our study, the effect of exercise from voluntary wheel running on weight gain cannot be determined. Female rats that maintained approximately 11,000 revolutions per day (RPD), and male rats that maintained approximately 4,000 RPD, have been shown to gain significantly less weight than rats in a sedentary control group (95). Although male rats used the running wheel consistently less than female rats throughout that experiment, both groups maintained a relatively constant level of exercise throughout the study. This suggests some degree of consistency must be maintained in order to reduce weight gain in rats by wheel running.

Rats that received a swim protocol, similar to the one used in our study, were found to weigh significantly less than sedentary rats in as little as two weeks and were able to maintain a significantly lower body weight for the remainder of the 8 weeks of swim exercise (78, 94). Parallel to these findings, rats in the CW group in our study gained significantly less weight than rats in the C group during the first and second weeks of swimming (weeks three and four of the study) and less than ShC and CE groups in the second week (week four of the study). This trend did not, however, remain consistent throughout the remainder of the study. The ability of the rats in CW to defend against weight gain early in the swim protocol may have been the result of more effort expended during swimming in the early weeks of our study. As time progressed, the rats expended less energy as they became accustomed to swimming and learned how to float.

Muscle weight

Neither OVX, nor exercise, had an effect on muscle weight of the rats in our study. These findings do not support the hypotheses that OVX would decrease lean tissue mass in rats, and that voluntary wheel running and swim exercise would minimize the decrease in lean tissue mass associated with OVX in rats. These surprising findings contrast with published results that suggested OVX in rats increases fat-free mass (FFM) (81). In a study that used forced treadmill running, the FFM gain associated with OVX persisted, even with exercise (81). In another study that examined the effects of voluntary wheel running on both male and female rats, loss of FFM in male, but not in female, rats was reported (96). Fat-free mass contains bone, connective tissue, muscle, and internal organs. It is impossible to determine if the increase or decrease of FFM reported by these two studies was due to bone, connective tissue, muscle, internal organs,

or the combination of all of these components since they were not divided for analysis (81, 96). The lack of a significant difference of muscle weights among treatment groups in our study suggests that voluntary wheel running or swim exercise neither increases, nor decreases, lean tissue in OVX rats. Oscai and colleagues (97) reported similar findings from a study that used swimming as the mode of exercise. After 21 weeks of swim exercise, the muscles of rats directly involved in the swimming, *i.e.* limb and girdle muscles, did not hypertrophy. The sparse amount of literature on the affects of swimming on muscle size makes a comparison of our results to previous findings difficult.

Hypertrophy of the soleus muscle that resulted from voluntary wheel running has been observed in both male (95, 103) and female (95, 104) rats. With the use of a voluntary wheel running protocol similar to the one used in our study, Tokuyama and co-workers (95) reported higher soleus muscle weights of exercising male and female rats compared to sedentary rats. In contrast to the high level of running wheel activity and consistency exhibited by these rats, the rats in our study were relatively inactive and inconsistent. This may have contributed to the lack of hypertrophy of the soleus muscle observed in our study.

Abdominal fat

Studies (72, 73, 81) have produced conflicting results about whether weight gain associated with OVX in rats is due to an increase in abdominal fat mass, or an increase in protein mass. Our study revealed no significant difference in total weight gain between treatment groups. There was, however, a significant difference in abdominal fat mass between the CW and ShC groups. This is concordant with the hypothesis that swim

exercise would minimize or ablate the abdominal fat mass gain associated with OVX. Fat mass was lost by the CW group, but total body weights between the CW and ShC groups were comparable. A possible explanation for this may be a difference in bone density. Since there was no difference in muscle mass between the two groups, a change in body composition must have occurred in tissue other than fat mass or muscle mass. The results of the bone density assessment of this study, which is not addressed in this thesis, revealed that total femur, midshaft, tibia, and humerus bone density of the rats in the CW group were significantly higher than that of the rats in ShC group. This higher density of bone may have offset the total body weight as fat mass was lost. There did not appear to be a correlation between the loss of abdominal fat and the gain of total muscle weight. It should be noted, however, that only four different muscle groups were removed from the rats. It is possible that there was an increase in weight of muscles other than those examined in our study

Total abdominal fat mass has been shown to be less in rats who perform daily swim exercise (78, 94). This parallels the findings in our study. Rats in the CW group had a significantly lower total abdominal fat weight than did the rats in the ShC group. Though not significant, total abdominal fat weight of the rats in the CE group was lower than the rats in the ShC group, but similar to that in the C group. This only partially supports the hypothesis that voluntary wheel running would minimize or ablate the increase in abdominal fat mass associated with OVX. This may be due to the extreme variability in the number of revolutions run by rats in the CE group. It should be noted, however, that the range of fat mass for the CE group was large in comparison to the range of other treatment groups. No correlation between revolutions run and abdominal fat

mass was seen. The large range of revolutions run may have contributed to the large range of fat mass observed in the rats in this group. This lack of consistency in revolutions run by the CE group may have contributed to the differences of results between our study and previously reported findings. Tokuyama and colleagues (95) reported significantly less body fat content in rats that consistently ran in wheels compared to sedentary controls. This suggests that a relatively consistent amount of exercise must be maintained in order to decrease abdominal fat in rats.

The decline in revolutions run by rats in the CE group toward the end of the study (weeks five through eight) may have been due to the decreased novelty of this form of exercise, a possible natural decreasing tendency to run by aging rats, or a delayed effect of OVX. There is conflicting findings about the tendency of rats to run over time (12, 95, 96). It has been reported previously (95) that voluntary wheel running, by both male and female rats, rapidly increased in the first 10 days of exposure to this form of exercise, then decreased and reached a plateau and remained relatively constant for the remainder of the 50-day study. A previous study in our lab (12) showed that revolutions run by OVX rats was highest upon re-introduction to running wheels (two weeks post OVX), then decreased and remained relatively constant for the next four weeks. In a study by Cortright and co-workers (96), rats achieved a peak in revolutions run during week seven, then a decrease and plateau for the remaining two weeks of the study. It is difficult to compare our results to these since male (95, 96), female (12, 95, 96), weanling (96), adult (95), and retired breeder rats (12) were used in these studies.

Several factors may have contributed to the unexpected results in our study. The number of rats in each treatment group was relatively small. A larger sample size for the

CE group may have reduced the large range of revolutions run by the group. The amount of time the animals were subjected to exercise protocols may have obscured the effects of swimming and voluntary wheel running in our study. Exercise protocols longer than 4 weeks (animals did not start exercising until week three of our study) may have increased the effects of exercise on the rats. A longer study would have also allowed for a longer period of time for animals to adjust to our lab, exercise protocols, and novel diets, and for us to identify animals with a sustained tendency to run. Examining the sustained tendency to run by rats in the CE group after OVX may have also contributed to a more consistent exercise pattern which may have led to a stronger relationship between revolutions run and changes in body weight, abdominal fat, and lean tissue. The variability in final muscle and abdominal fat weights may be the result of more than one person dissecting the rats at sacrifice. This may have resulted in inconsistencies of excision techniques. Experience gained at dissecting muscles and abdominal fat during multiple days of sacrifice may have contributed to varying excision techniques, even by the same person. This, however, was controlled for by continuous rotation of treatment groups throughout the sacrifice and dissecting procedures (*e.g.* ShC1 followed by C1, CE1, CW1, etc.). Measuring the rats from nose to tail at the beginning of the study and at sacrifice may have helped to discern between naturally occurring growth and OVX induced growth in this study.

Conclusions

The results of this study suggest that swim exercise may diminish the abdominal fat gain associated with OVX in rats. Our results also imply that a higher level of voluntary wheel running increases food intake and may also diminish the abdominal fat

gain associated with OVX in rats. Future studies that include larger treatment group sample sizes and longer duration of exercise protocols would be needed to clarify the effects of swim exercise and voluntary wheel running on weight gain, abdominal fat, and muscle mass in OVX rats.

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APENDICES

APPENDIX A

LOUISIANA STATE UNIVERSITY
Institutional Animal Care and Use Committee

ANIMAL WELFARE ASSURANCE # A3612-01
LICENSE# 72-3
MULTIPLE ASSURANCE# M1128

January 19, 2000

Dr. Maren Hegsted
Department of Human Ecology

Dear Dr. Hegsted:

Protocol #00-003, entitled "Effects of Swimming and Varying Levels of Dietary Soy Protein on Bone Density and Coronary Heart Disease Risk Factors in Ovariectomized Retired Breeder Rats" lists you as the Principal Investigator.

I am happy to inform you that your protocol was **approved** by the IACUC during our regularly scheduled meeting held on January 13, 2000. This approval is valid for 3 years, and authorizes the use of 72 rats. **Please note that DLAM representative signature was omitted on the first page of the protocol. Also, Dr. Baker's name should have been listed on the last page. Send a new "signed" front page, and last page that includes Dr. Baker's name.**

When ordering animals for this project, please provide a copy of this letter to DLAM along with your order. This will help keep better track of the animals being used by various investigators. Thank you!

Sincerely,

Philip Elzer, Ph.D.
Interim Chairman

jdb

Revised OCTOBER 1998

LSU PROTOCOL FOR ANIMAL CARE AND USE

Instructions for submission: MUST BE TYPED! (Use additional sheets if necessary and attach to this form.) SUBMIT ORIGINAL plus 12 COPIES TO IACUC OFFICE (Rm 1502 SVM).

Protocol# 00-003
Research office control#_____
Date received _____
Action: Approval date_____
Deferral date_____
Disapproval date_____

1. PRINCIPAL INVESTIGATOR: **Maren Hegsted** Phone: Off:**388-1518** Home:**769-3097**
E-Mail Address: **mhegste@unix1.sncc.lsu.edu**

Co-PRINCIPAL INVESTIGATOR: **Sheri Melton, West Chester University**

2. PROJECT TITLE: Enter the name of your project/course# in the block below.

Effects of swimming and varying levels of dietary soy protein on bone density and coronary heart disease risk factors in ovariectomized retired breeder rats

3. ANIMAL SPECIES: **rat** Strain: **Sprague Dawley**

Total number of animals needed: 72 Maximum number needed at one time: 72
Number of animals to be placed in each group: 9
Animal housing and veterinary care have been coordinated with DLAM office, or LSU Agricultural Center Unit: Yes X No_____
Name and initials of representative contacted: (May be printed, then initialed) **Laurie Henderson** LMH

LOCATION OF ANIMALS:

☐	DLAM Vivarium	X	Life Sciences Vivarium	☐	SVM Barns
☐	SVM Fish Building	☐	LAES (Site:_____)	☐	Other_____

4. ABSTRACT PLAN OF RESEARCH/TEACHING:

Provide a brief layman's description of the project in the block below. (This information will help the animal care staff to understand any conditions encountered while caring for your animals.)

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This study will determine if there is a dose-response to increasing levels of soy protein on bone and heart disease parameters. In addition, the additive effect of swim exercise will be compared at each level of dietary soy. Seventy-two rats will be housed in individual stainless steel cages and allowed 3-5 days recovery from travel prior their being ovariectomized by LSU veterinarians. Rats will be assigned to one of eight treatment groups as follows: 1) 21% casein diet; 2) 21% casein diet + swim; 3) 14% casein + 7% soy diet; 4) 14% casein + 7% soy diet + swim; 5) 7 % casein + 14% soy diet; 6) 7% casein + 14% soy diet + swim; 7) 21% soy diet; 8) 21% soy diet + swim. Swim training will begin after recovery from surgery, 3-5 days. The 36 rats in the swimming groups will be gradually introduced to swimming, 5 minutes/day the first day, 10 minutes/day the second day, etc until they are swimming 75 minutes/day 5 days/week. After swimming the rats will be towel dried and returned to their cages. Rats will have ad libitum access to the AIN-93M modified diet and water. Food intake and weight will be recorded 3 times each week for the 6 week treatment period. Twelve days prior to sacrifice, rats will be injected subcutaneously with calcein, a fluorescent marker for calcium at 0.1 mg/100 g rat weight. Ten days later (2 days prior to sacrifice) the rats will be injected with a second fluorescent marker, tetracycline at 0.1 mg/100 g rat weight. These markers will allow determination of bone formation rate at specific bone sites. Six weeks after surgery the rats will be anesthetized with isoflurane and killed by exsanquination via cardiac puncture. Heart, liver, uterus, abdominal fat, and specific bones and muscles will be removed for later analysis.

Revised OCTOBER 1998

5. INVESTIGATOR'S STATEMENT: **Assurances for the humane care and use of vertebrate animals.**

By signing this form, we agree to abide by the Policy for the Care and Use of Animals of Louisiana State University, or that of the LSU Agricultural Center. This project will be in accordance with the NIH "Guide for the Care and Use of Laboratory Animals" (except as explained in the accompanying Protocol), and the Louisiana State University or the LSU Agricultural Center Animal Welfare Assurance on file with the U.S. Public Health Service. I further assure the Committee that: **1) I will abide by all federal, state, and local laws and regulations governing the use of animals in teaching and research; 2) the investigators and technicians are adequately trained to perform the research techniques required in these studies; and 3) the fewest number of animals required to produce significant results are being used in this study.**

Maren Hegsted — **Professor** — 12-20-99

Principal Investigator — Title/Rank — Date

Maren Hegsted

Typed Name of Principal Investigator

Sheri Melton — **Assistant Professor** — Date

Co-Principal Investigators — Title/Rank

Sheri Melton

Typed Name of Co-Principal Investigators

D. M. Baker — Associate Professor — 12/20/99

Surgeon (if applicable) — Title/Rank — Date

David Baker

Typed Name of Surgeon

6. SPECIAL HUSBANDRY REQUIREMENTS:

Do your animals have special needs to be addressed by DLAM? __X__ Yes _____ No _____ N/A
If you indicate **No**, your animals will be cared for according to the standard operating procedures of DLAM.

Temperature range:(°F) ____ Humidity (%) ____
Light cycle (hours light/hours dark): **7 a.m. to 7 p.m. light**
*Caging: Type: wire-bottom hanging cages Size:_____ Filter tops?:_____
Bedding/Litter: Type:_____ Autoclaved?:____ Changes/Week:_____
Water: Sterile:___ De-ionized:___ Acidified:___ Tap: X Other:_____
Diet: Special feeding requirements: researchers will feed powdered diet

*** During weeks 2 & 5, each rat will be placed in a metabolism cage overnight for the collection of urine.**

7. HAZARDOUS MATERIALS:

Zoonotic or recombinant, radioactive, or carcinogenic agents will be **PRESENT IN THE ANIMAL**:

Zoonotic/Recombinant Agents Yes___ No_X__ Agent(s):	Radioisotopes? Yes___ No_X__ Isotope(s):	Carcinogens? Yes___ No_X__ Compounds(s):

Note: If zoonotic (infectious to humans) or recombinant organisms are to be used, this protocol request must be submitted to the Biohazardous Materials Safety Committee for approval **PRIOR TO CONSIDERATION** by the IACUC. Similarly, if carcinogens are to be used in the animal room, submit the proposal to the Carcinogen Use Committee for prior approval. **P.I. MUST PROVIDE** health and safety measures for animal technicians and facility maintenance personnel. In Standard Operating Procedure (SOP) form, describe any precautions, procedures, or personal protection required in handling animals or waste containing listed agents or compounds, or in working in or around the animal room (including air handling system), and **attach a copy of your SOP(s) to this protocol proposal.**

8. SUMMARY OF PROCEDURES:

Your response in this section should provide the reader with a complete description of how every animal to be used in this project is to be treated during every phase of the study. Your target audience is a faculty member from a discipline unrelated to yours. Do not use jargon. Describe the experimental design of the study, and a clear definition of the various treatment groups. Describe each procedure to which any animal in any group may be subjected. Include a list of any physical, chemical or biological agents **(name, dose, route, frequency)** that may be administered.

72 retired breeder female rats (9 months old) will be purchased from Harlan Sprague Dawley Co. for the summer 2000. After a short adjustment period of 3-5 days all rats will be ovariectomized by veterinarians from the LSU School of Veterinary Medicine.

Ovariectomy Procedure: Rats will be anesthetized via Isoflurane inhalation in an induction chamber, followed by transfer to the surgical table and fitting with an inhalation mask and continued Isoflurane inhalation. Following induction, a single injection of buprenorphine (0.2 mg/kg SQ) will be given to provide preoperative analgesia. The hair over the mid to dorsal aspects of the abdomen will be removed with a clipper, followed by application of a depilatory agent ("Nair"), and finally a disinfectant (betadyne). The following surgical procedure will be performed aseptically. A small dorsal midline incision will be made halfway between the middle of the back and the base of the tail. Entrance to the peritoneal cavity will be made by small bilateral incisions made 2/3 down the side of the body wall. The ovaries will be removed by sharp dissection and the horn returned to the abdominal cavity. No hemostasis is required. The muscle incisions do not require suturing unless they have been inordinately large, where a single suture will be placed. Skin will be closed with "superglue". The rats will be returned to their cages and monitored until ambulatory. Daily observations by caretakers will determine if the rats need additional analgesia. Rats showing signs of pain (lethargy, anorexia, rough coat) will be given buprenorphine (0.2 mg/kg SQ bid) for as long as needed.

Rats will divided into 8 treatment groups in a 2X2 factorial assignment of treatments with 4 levels of soy protein (0, 7, 14, or 21% soy) for one variable and swim exercise for the second variable.

1) 21% casein diet
2) 21% casein diet + swim
3) 14% casein + 7% soy diet
4) 14% casein + 7% soy diet + swim
5) 7% casein + 14% soy diet
6) 7% casein + 14% soy diet + swim
7) 21% soy diet
8) 21% soy diet + swim

All rats will have free access to modified AIN-93M powdered diet with food intake recorded 3 times a week. After recovery from surgery rats will be trained to swim by the researchers in plastic barrels with gradual increments in swimming time starting at 5 minutes. Twice during the study, in weeks 2 and 5, each rat will be placed in a metabolic cage overnight for the collection of rat urine to measure markers of bone resorption. Six weeks after initiation of swimming the study will be terminated. Fluorescent bone markers (0.1 mg/100 g rat weight) will be injected subcutaneously, calcein at 12 days and tetracycline at 2 days prior to sacrifice date when 14 or 15 rats/day will be killed over a one week period. Rats will be anesthetized using Isoflurane gas and the anesthesia instrument in the Life Sciences animal facility. They will be killed by exsanguination using cardiac puncture with blood collected for cholesterol, triglyceride, osteocalcin, and alkaline phosphatase analysis. Heart, liver, uterus, abdominal fat, and selected muscles and bones will be removed for measuring weight or for later analysis of factors related to heart disease and osteoporosis. Bones will be cleaned of soft tissue and either frozen for measurement of density, ash, and mineral content or placed in formalin for later dehydration and histomorphometry to calculate bone formation rate.

9. TYPE OF PROJECT:

_X_ Type A - Pain or distress will not be induced; animals will only be used for injections, collections, or procedures causing nothing more than minor discomfort

___ Type B - Pain or distress will be relieved by appropriate therapy

___ Type C - Drug intervention for pain or distress would interfere with the protocol. **(If this block is checked, specific justification MUST be provided).**

___ Type D - Hazardous agents (Zoonotic/Carcinogenic/Radioisotopic) - Item #7.

10. Check "Yes" or "No" to each of the following questions. **On a separate page, provide an explanation for any "Yes" answers that are not included in the above summary.** Provide justification for **why** the action is needed, and include information such as **who** will perform procedures, **how** they will be performed, frequency, duration, drugs to be used, dosages, routes of administration, etc. Not all this information may be needed for every "Yes" answer. The information you provide in this section is very important in highlighting specific points of your study that are important considerations for the IACUC in their review process.

Yes	No		Individual(s) Responsible
___	_X_	Will animals be restrained?	
___	_X_	Will animals be fasted?	
___	_X_	Are neuromuscular blocking agents to be used?	
_X_	___	Will surgical procedures be employed?. Ovariectomy by veterinarians at LSU	Dr. David Baker
		Survival __X___ Multiple_____ Terminal_____ If survival, who will be responsible for recovery of the animals?	Dr. Maren Hegsted
_X_	___	Are any ANESTHETIC, ANALGESICS or TRANQUILIZERS to be used?	Isoflurane
___	_X_	Do you anticipate any adverse effects of the experimental procedures on the animals (e.g., pain, discomfort, reduced growth, fever, anemia, etc.)?	
___	_X_	Is death an endpoint in your experimental procedure? **Note:** Death as an endpoint refers to acute toxicity testing, assessment of virulence of pathogens, neutralization tests for toxins, and other studies in which animals are not euthanized, but die as a direct result of the experimental manipulation.	
___	_X_	Are there emergency treatments by the DLAM veterinary staff that would not be allowed?	
___	_X_	Are you using wild or exotic species for which permits are necessary? (Attach copy)	
_X_	___	Will animals be euthanized during or at the close of the study?	Dr. Hegsted
___	_X_	Will animals be used for antibody production?	
___	_X_	Will complete Freund's adjuvant be used?	
___	_X_	Will incomplete Freund's adjuvant be used?	
___	_X_	Will other adjuvants be used; specify _____?	
_X_	___	Will blood be collected? How often Once, end of study Volume as much as possible, to exsanguination by cardiac puncture	Dr. Hegsted

11. ANIMAL MANAGEMENT:

Individual (or groups of) animals are identified by: Rat numbers placed on cages

Check all applicable blanks:

Care of Sick Animals	Disposal of Dead Animals	Pest Control
_X_ Call investigator	_X_ Call investigator	_X_ Call investigator
___ Clinician to treat	___ Necropsy	___ Pesticides OK
___ Euthanasia	___ Disposal	___ No Pesticides

12. DISPOSITION OF ANIMALS:

What will be done with any animals **not** euthanized at the conclusion of the project? **N/A**
___ DLAM/LAES has permission to reassign animals to another IACUC-approved protocol.
___ Transfer animals to another IACUC-approved protocol (Protocol#_____)

13. NARRATIVE STATEMENT:

Federal regulations mandate that you provide written, narrative statements for all projects: 1) that the activities do not unnecessarily duplicate previous experiments. In this statement, include literature sources used to make such a determination (e.g., Databases, workshops, expertise in the field, etc.), **2)** that you have considered alternatives to procedures producing more than momentary or slight pain or distress, **3)** describing the methods you used to determine that alternatives to such procedures were not available (Databases, years and words searched , date of search, etc.). Put your statements in the block below:

1) This is part of an ongoing project to examine dietary and exercise factors that can reduce osteoporosis and other consequences of loss of estrogen in women. The ovariectomized rat is the required pre-clinical animal model for testing osteoporosis treatments. Previous work has indicated that swimming may have beneficial effects on preventing bone loss in this model and in postmenopausal women but the data are contradictory. A recent human study reported at the American Society for Bone and Mineral Research meeting (1999) showed an improvement in postmenopausal women with regular swim exercise. If further evidence supports swimming as an appropriate exercise to reduce bone loss this would provide an exercise method that is more acceptable to some elderly women with arthritis and other medical problems that make weight bearing exercises difficult. Soy protein contains the phytoestrogens, genistein and daidzein which can bind to estrogen receptors and may have estrogen-like activity. The data on benefits of soy in reducing osteoporosis are limited. Five studies have reported some benefit but none have looked at a dose-response to soy and how it affects bone formation rate. If this study supports previous research it will provide a quantitative basis for testing soy alternatives to estrogen replacement in postmenopausal women.

2) There is no pain involved beyond that caused by an injection or during recovery from ovariectomy surgery. Animals will be allowed recovery time after surgery before swim exercise will begin.

3) Since the ovariectomized rat model is the FDA approved pre-clinical trial for osteoporosis treatment there are not any other alternatives unless human studies are conducted. Human trials will be necessary at a later date but the animal model is used to examine bone histomorphometry, bone breaking strength, bone ash and mineral content. None of these can be measured in human studies.

Sources of information for these statements include articles located through the medical database Medline, the agricultural database Agricola, information obtained from the American Society for Bone and Mineral Research annual meeting (Oct 1999) and personal research experience.

14. INVESTIGATOR TRAINING:

In accordance with Federal Regulations, all personnel conducting animal-based research must attend a Rules and Regulations Course and verify their training, experience and skills in the care and use of the animals and techniques they are responsible for.

List all persons involved in animal care and use for this study below.

	REGULATIONS AND TRAINING COURSES							
			Date	Species		Date	Training/	
Name	Rules/regs		Attended	Wet Lab*		Attended	Experience**	
	Yes	No		Yes	No		Yes	No
Maren Hegsted		X			X			X
Michael Keenan	X		3-9-99	X			X	
Karen Jones	X		3-9-99	X			X	
Sheri Melton		X			X			X
Carol O'Neil	X	X	6-1-99	X	X			X
Rhonda Prisby	X	X	6-1-99	X	X			X
David Baker	X			X				

* Exemption based on previous experience with the study species may be obtained by written request
**Training/Experience in assigned procedures for this protocol.
Who will train individuals for participation in protocol procedures? **Dr. Stout will be contacted for wet lab training of undergraduate students and all who did not attend rules and regulations class will attend class within six months**

Personnel participating in the project that have not attended the Rules and Regulations Course or the applicable Species Wet Lab, will have six (6) months from the start of the project to complete them. Rules and Regulations Courses will be held the first Tuesday of every month from 11:00 am until Noon in room 1212C. The Wet Labs will be held on the same day beginning at 1:00 pm in the DLAM facility. Please call Dawn Best-Desjardins at 346-3145 to sign up for these courses.

(DO NOT WRITE BELOW THE ASTERISKS. FOR COMMITTEE USE ONLY.)

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IACUC Action/Notes