INFLUENCE OF SERUM LEPTIN ON EXERCISE DEPENDENCE, DIETARY INTAKE, AND SATIETY IN COMPETITIVE FEMALE RUNNERS AND INACTIVE FEMALES

A THESIS

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DEDICATION

I dedicate this to my late father, William L. Barber, PE, MBA, JD. He was my biggest cheerleader in life. My dad was always there to help with homework and provide words of encouragement. When I started running, he showed up to every race with a hot cup of coffee and flip flops (no matter the weather), always with a huge smile on his face. We shared a love of black coffee and current events, and he always knew what to say to make me feel better. My dad was the smartest man I knew. I miss him every day and would like to honor his memory by dedicating my thesis to him.

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From the intellectual support, guidance, and kindness I have encountered throughout this journey, I will gladly pay it forward. Thank you all.

ABSTRACT

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INFLUENCE OF SERUM LEPTIN ON EXERCISE DEPENDENCE, DIETARY INTAKE, AND SATIETY IN COMPETITIVE FEMALE RUNNERS AND INACTIVE FEMALES

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Leptin is derived primarily from adipocytes to impact appetite, energy expenditure, and reward-seeking behaviors; and may contribute to compulsive-type behaviors. This study examined the relationship of leptin at fasting, postprandial, and post exercise with diet, subjective satiety, and exercise dependence (ED) between female runners (FR) and inactive females (IF). Mean age of subjects 23 years and body mass index (BMI) of 21.5 kg/m^2. Across time on the day of the study, significant leptin differences ($P < 0.001$) were seen in FR ($n = 14$; BMI: 19.9 ± 1.3) compared to IF ($n = 16$; BMI: 23.0 ± 2.8), with no significant differences within groups. Fat mass (FM) was lower ($P < 0.000$) and lean mass (LM) was higher in FR ($P < 0.001$) than IF. FR had an average LM of 75.5% in comparison to IF at 65.6%. In conclusion, FR had significantly lower leptin than IF across time. Additionally, FR had a significantly lower BMI, FM, and body weight than IF. However, FR scored significantly higher in mean overall ED and consumed a significantly higher percentage of carbohydrates and fiber than IF.

TABLE OF CONTENTS

Page

LIST OF TABLES

Table

LIST OF FIGURES

Figure

LIST OF ABBREVIATIONS

ADP	air-displacement plethysmography
AF	activity factor
AI	adequate intake
ANCOVA	analysis of covariance
ANOVA	analysis of variance
ANS	autonomic nervous system
BMI	body mass index
DIT	diet-induced thermogenesis
DXA	dual-energy x-ray absorptiometry
ED	exercise dependence
EDTA	ethylenediaminetetraacetic acid
EE	energy expenditure
FFM	fat-free mass
FM	fat mass
FR	female runners
HW	hydrostatic weighing
IF	inactive females
LM	lean mass
LepR	leptin receptor
NAc	nucleus accumbens
NDSR	Nutrient Data System for Research

PI	principal investigator
RDA	Recommended Dietary Allowance
REE	resting energy expenditure
SD	standard deviation
SF	skinfold
SPSS	Statistical Package for the Social Sciences
STAT3	signal transducer and activator of transcription-3
TBM	total body mass
TEE	total energy expenditure
TH	tyrosine hydroxylase
TWU	Texas Woman's University
VAS	visual analog scale
VTA	ventral tegmental area

CHAPTER I

INTRODUCTION

Leptin is an adipokine that primarily regulates energy homeostasis and appetite.[1] Leptin is also a hormone produced primarily by adipose tissue and maintains long-term energy homeostasis by sensing the amount of triacylglycerol stored in adipocytes through a negative feedback loop.[2] A direct correlation exists between leptin circulating in the blood and stored triacylglycerol. This hormone also regulates short-term energy by dietary intake through satiety signals. Leptin crosses the blood brain barrier and binds to leptin receptors (LepR) in the hypothalamus to generate downstream signals that promote satiety following food intake.[3]

Leptin also influences the reward, or hedonic, system in the brain by indirectly impacting the release of dopamine from the Mesolimbic Dopamine System.[4] Leptin can reduce food reward and may modulate the Mesolimbic Dopamine System via neural pathways and LepR signaling.[4] Dopamine neurons are predominately produced in the ventral tegmental area (VTA).[5] LepR is expressed on dopamine neurons[3,6-9] and signal within the neuron via signal transduction.[10] VTA dopamine neurons project to regions of the brain including the nucleus accumbens (NAc)[4] along the Mesolimbic Dopamine Pathway. The NAc is dopamine sensitive[5] and thought to encode positive and negative memories of rewarding experiences, and attribute them to the environmental stimuli associated with obtaining that experience. A recent study measured dopamine release in the NAc of rats performing behavioral tasks and found that dopamine signals encoded negative and

positive reward prediction errors.[11] A positive prediction error occurs when more reward is experienced than expected, while a negative prediction error signifies depressed activity when less reward is experienced, and fully predicted rewards reflect baseline activity.[12] The study used operant decision-making tasks that provided two lotteries, each providing probabilistic rewards. Before the rats began the behavioral tasks, the dopamine levels in their NAc increased rapidly from these unsignaled rewards (e.g. food pellets).[11] These findings support the theory that changes in dopamine levels of the NAc could encode negative and positive reward in a reinforced learning task.[11] Therefore, a change in dopamine concentrations by LepR signaling could affect behavior.

Leptin deficiency is known to reduce tyrosine hydroxylase (TH), which is the rate-limiting enzyme for the biosynthesis of dopamine, thus also impacting dopamine concentrations.[3,7] In contrast, presence of leptin may also decrease dopamine concentrations through LepR signaling.[8] Various pathways in LepR signaling can coordinate the modulation of energy homeostasis.[13] One such pathway of LepR signaling involves the inhibition of the activation of the signal transducer and activator of transcription-3 (STAT3) located in the dopamine neurons of the VTA.[3] Inhibition of STAT3 prevents the Mesolimbic Pathway from delivering dopamine neurons from the VTA to the NAc.[3] However, despite a loss of STAT3 in dopamine neurons, a study found that mice lacking STAT3 in dopamine neurons expressed greater voluntary running. It has been hypothesized that decreased leptin levels heighten the stamina and rewarding effects of running for the hunting and gathering of food.[3] Interestingly, the

restoration of STAT3 reversed these effects.[3] This suggests that decreased leptin levels could impact behavior and reward of voluntary running as a survival mechanism in an effort to regain energy homeostasis.[3]

Physical activity is influenced by leptin, both positively and negatively in humans and animals. During fed states, leptin deficiency increases voluntary running in humans and rodents.[3,14-19] In contrast, when food is limited, leptin inhibits locomotor activity and the desire to run in rodents.[3,16,20,21] These mechanisms have been hypothesized to describe leptin's role in maintaining energy homeostasis. Fernandes et al.[3] found that leptin signaling suppressed the rewarding effects of running without influencing hedonic feeding or the anorectic characteristics of leptin in mice.

A study found that excessive exercise training is associated with low amounts of body-fat adjusted leptin concentrations in men.[1] In women, excessive exercise is associated with hypothalamic amenorrhea, and also low leptin levels and body-fat percentage.[1] Leptin may affect exercise dependence, which is defined as patterns of exercise that are obsessive or in excess, and potentially result in negative consequences (e.g. inability to reduce exercise amounts, overuse injuries, and interference with work and family).[1,22-25] The two forms of exercise dependence are primary and secondary. Primary uses exercise for fulfillment without presence of an eating disorder; whereas secondary uses exercise as a mechanism to lose weight and is usually accompanied by an eating disorder.[24] The prevalence of exercise dependence differs between males and

females. Males are more likely to experience primary exercise dependence, while females more commonly exhibit secondary exercise dependence.[24]

Modolo et al.[26] reported a 28% prevalence rate for exercise dependence in women. The Exercise Dependence Scale-21[27] identifies exercise dependence as having at least three of the following criteria: (1) Tolerance: having either the need for increased exercise to achieve the desired effect or experiencing a diminished effect with continued rate of exercise; (2) Withdrawal: manifestation of either the characteristic exercise withdrawal symptoms (e.g., fatigue, anxiety) or the compensation of exercise taken to avoid or relieve such symptoms; (3) Intention Effect: exercising more rigorously or for longer periods of time than intended; (4) Lack of Control: chronic failure to decrease or control exercise despite desire or attempts; (5) Time: large quantities of time being dedicated to exercise activities (e.g., physical activity vacations); (6) Reductions in Other Activities: exercise impedes occupational, social, or recreational activities; and (7) Continuance: continuing to exercise despite knowledge of physical injury or psychological impediment related to exercise (e.g., running while injured).[27] The exercise dependence scale was developed according to criteria from the Diagnostic and Statistical Manual-IV (the most current version available at the time the scale was created).[25,28] The scale measured for substance dependence, and was expanded and validated by five exercise dependence studies using a cumulative of 2420 subjects.[25] These studies revealed that at-risk individuals reported higher levels of vigorous exercise, self-efficacy, and perfectionism than nondependent individuals.[25]

A relationship between leptin and dietary intake also exists. One study took blood samples from subjects of a normal weight every 30-60 minutes for a 24-hour period to measure differences in serum leptin levels between a group consuming high-fat, low-carbohydrate meals, and a group consuming low-fat, high-carbohydrate meals. Results showed high-carbohydrate meals cause greater postprandial levels of leptin than high-fat meals in women.[29,30] However, it has also been shown that diets high in fiber lead to decreased leptin concentrations in human subjects and in cats.[29,31-33] A high fiber intake could also help control leptin levels in healthy adults.[29,34] Therefore, leptin response might vary according to carbohydrate type.[29] A review examining leptin concentrations and dietary intake revealed that high concentrations of dietary fat increased leptin levels in animals [32,35] with some studies differentiating between fat type.[29] One study found that in women, consumption of linoleic acid positively correlated with serum leptin, while arachidonic acids showed a negative correlation with leptin[29,36] However, dietary carbohydrates were found to cause higher levels of postprandial leptin in women than fat.[29,30] In regards to high-protein diets, results varied. One study showed that a high-protein, low-calorie diet increased leptin sensitivity in adults.[29,31] In contrast, another study showed no effect of a high-protein diet on serum leptin levels in animal subjects.[29,37] With regard to overall energy intake, energy restriction decreased serum leptin levels independent of weight loss,[38] and that a high-energy intake over time can lead to leptin resistance in adults.[29,38] One study using healthy men found that an energy-

restricted diet decreased fasting leptin levels by 39.4%, but it is not clear if leptin is reduced by this amount in females.[29,38]

The existing literature does not clarify the interrelationship of leptin on exercise dependence and dietary intake, especially in female runners (FR). It is also unknown if leptin affects satiety in this population. Thus, this study seeks to understand the relationship among leptin concentrations, dietary intake (macronutrients and energy), subjective satiety, and exercise dependence in healthy females.

CHAPTER II

REVIEW OF LITERATURE

Leptin is a cytokine that is produced by and has a positive relationship with adipose tissue. Concentrations of serum leptin are usually greater in obese than lean individuals due to the long-term adipose tissue stores.[39] One of leptin's primary roles is as an adipose-tissue modulator. When the body has adequate fat stores and thus adequate leptin levels, the desire to eat is decreased and energy expenditure (EE) is enabled through the channels of neuroendocrine axes and the Autonomic Nervous System (ANS),[39] which results in satiety. Inversely, when fat stores are diminished, the decreased leptin levels cause appetite to increase and EE to decrease.[39] Under normal conditions, this serves as a means of conserving energy when stores are depleted. These inverse mechanisms are what make leptin a modulator of energy homeostasis.

Appetite is hormonally regulated as a metabolic function to preserve energy balance; however, eating can be activated by either metabolic necessity or hedonic drive.[40] Hedonic refers to the pleasantness or unpleasantness associated with different sensations, such as taste. The NAc, located in the hypothalamus of the brain, plays a role in modulating the reward center as opioid signaling in the NAc shell influences the hedonic drive. This motivational drive is then activated via dopamine signaling.[40] Studies have shown activated LepR to reduce the release of dopamine in the NAc. Inversely, reduced LepR activity increased dopamine in the NAc as well as energy intake with a

preference to sucrose.[8,40,41] There are multiple pathways of LepR signaling that can coordinate the modulation of energy homeostasis.[13]

LepR signaling may also have an impact on locomotor activity, which affects physical activities such as running. Once leptin binds to LepR, several major signaling pathways are galvanized.[13] In rodents, leptin has been shown to modulate energy intake and locomotor activity by regulating dopamine in the VTA of the brain.[8] Administration of leptin causes phosphorylation of STAT3 in VTA dopamine neurons and thereby causes the firing rate of those dopamine neurons to decrease.[8] Fernandes et al.[3] found that reduced leptin levels during food restriction in rodents increased physical activity by reduced LepR-STAT3 signaling. Although loss of STAT3 prevented the Mesolimbic Pathway from delivering dopamine neurons from the VTA to the NAc, the rewarding effects of running were enhanced.[3] It has been suggested that despite a lack of food, energy reserves are maintained to increase behavior associated with food acquisition.[3,42,43] Fernandes et al.[3] further speculated that the "runner's high" associated with reward, could have evolved to promote stamina. Once food is procured, the energy expended will be replenished. This act may provide broader evidence of leptin's role as an energy homeostasis regulator.

Several studies have been conducted to determine the relationship between exercise and leptin, but the outcomes are conflicting. One study on men found that after an hour of moderate exercise leptin concentrations dropped 18% after 24-hours and 40% after 48 hours; but not after a short, intense treadmill session defined as increasing

workload every two minutes until volitional exhaustion.[44,45] Another study on women reported exercise of >60 minutes may have 48-hour delayed leptin effects post activity.[44,46] A review examining leptin and endurance exercise[44] found the effects of chronic exercise on leptin to be inconsistent. Some found no change in serum leptin[44,46,47], other studies showed changes solely in regards to adiposity,[44,48-53] while others revealed a reduction in leptin regardless of changes in adiposity.[44,54,55,56,57] One study revealed that 120-minutes of treadmill running at mixed intensities of fast to moderate in a fasted state produced a large energy deficit and temporarily decreased hunger. However, no effect was found on overall energy intake, and leptin levels were unchanged pre and post exercise.[58]

Exercise dependence generally results in higher levels of exercise; therefore, a relationship between leptin and exercise dependence should be explored. Exercise dependence is defined by the Exercise Dependence Scale-21[27] Manual as presenting with three of seven criteria: tolerance built to exercise, withdrawal symptoms, planned versus actual exercise performed, lack of control, time spent, reductions in other activities, and continuance despite injury.[27] Exercise dependence can be divided into two categories: primary and secondary. Primary occurs when a person over exercises for psychological fulfillment, while secondary abuses exercise due to an underlying eating disorder or body dysmorphia.[59] A study on men of normal body mass index (BMI; 18.5-24.9kg/m^2) revealed that exercise dependence is associated with low leptin levels, even after adjusted for body-fat percentage.[60] Other studies that did not adjust for body-fat also found lean

men to have low leptin levels and attributed it to physical[60,61] and psychological stress.[60,62] The association between excessive exercise and leptin in men seemed to be related to primary exercise addiction. The cross-sectional study also revealed that hypoleptinemia may be connected to the pathogenesis of exercise dependence in men of a normal BMI; however, results did not clarify if leptin acts as the mediator or is the result of the dependence.[60] Longitudinal studies may be necessary to determine pathogenesis of leptin on exercise dependence.[60] Current data on the impact of leptin on exercise dependence is limited.

A more abundant amount of research exists on the relationship between leptin and body composition. Being that leptin is an adipose-sensitive hormone, concentration levels may be expressed differently among the population. Women have about 10% higher body-fat percentage than men when comparing similar BMI.[63] Both sexes experience increases in adiposity with age, but women still have a greater body-fat percentage throughout the life span.[63] Since females have higher body-fat percentages than men, they therefore have higher leptin concentrations due to leptin being predominately produced by adipose tissue. There is a positive correlation between serum leptin and total fat mass (FM) in children and adults.[64]

As leptin is positively correlated with adiposity, weight status effects leptin concentrations. A study that found serum leptin levels to be significantly lower in lean women than obese women, also found that leptin positively correlated more for obese women than lean.[65] A recent study by Kitaoka et al.[66] found serum leptin levels had a

direct relationship with adiposity, as is consistent with the literature, and that exercise training decreased adipose tissue and thereby leptin levels. Another study revealed that obese persons had higher adiposity and circulating leptin than individuals of a normal BMI.[67] Therefore, low FM correlates with lower leptin levels. Exercise can also affect leptin concentrations. Studies have frequently observed hypoleptinemia in athletes and associate it to an exercise-induced energy deficit[45,68] and/or reduced FM.[68-70] However, while chronic exercise has been associated with low leptin levels regardless of FM,[68,71] hypoleptinemia has also been observed in subjects performing acute exercise where the duration was not long enough to effect FM.[68,72-74]

Body composition varies between sexes, as previously mentioned, and athletic status. Physical activity affects BMI, percent-body fat, and muscle mass.[75,76] A study comparing female athletes to non-athletes found female athletes to have significantly lower percent-body fat than non-athletic females.[75] A lean body involves a higher amount of muscle mass over adipose tissue and can be advantageous in sports involving speed. While the body-fat percentages of athletes vary by sex and sport, the estimated minimal level of body fat required for optimal health in females is 12%.[77] The American Council on Exercise defines essential percent body fat for women as 10-13% and 14-20% specifically for female athletes.[78] A lower percent body fat can produce athletic benefits related to speed, power, and endurance. However, too low of body fat poses health risks such as sports injuries, amenorrhea, iron-deficiency anemia, and premature osteoporosis.[79,-81] Note that studies have shown a rise in leptin levels during the luteal

phase of menstruation in sedentary normal-weight women without change to FM.[82-85] In amenorrheic elite athletes, leptin was found to be significantly lower than cyclic elite athletes (women who successfully compete in their respective sports) and recreationally active cyclic women (who exercise regularly, but do not compete), despite elite athletes having similar body fat.[82] The cyclic athletes with normal menstrual cycles were also shown to have increased leptin in the luteal phase of their cycle.[82]

For athletes, body composition is important for performance and a few methods exist to measure lean mass (LM), FM, and total body mass (TBM) depending on cost and accessibility. One study compared the four most popular methods: hydrostatic weighing (HW), dual-energy x-ray absorptiometry (DXA), skinfold (SF), and the air displacement plethysmograph (ADP). The long-time gold standard in measuring body composition has been HW; however, HW requires a highly-trained technician and only measures TBM. Body density and body-fat percentage can be obtained after water displacement measurements through a specialized calculation.[79] The SF technique uses caliper measuring in combination with a body-density equation based off densitometry to assess body-fat percentage. It is the least complex, inexpensive method and does not require a lab, but only body density and body-fat percentage (via a specialized calculation) are able to be measured. Dual-energy x-ray absorptiometry measures FM, LM, segmental LM, segmental FM, and body-fat percentage using x-ray technology.[79,86] It is an expensive method, but is being increasingly used in body composition analyses as a validation tool.[87] The BOD POD uses ADP to measure TBM, FM, fat-free mass (FFM), and body

density.[79] It is easy to operate and less expensive than DXA.[88] However, compared to DXA, ADP has been found to overestimate percent body fat by up to 13.2% in underweight subjects with a BMI <18.5 kg/m^2.[88] There was less discrepancy in subjects with a normal BMI (18.5-24.9 kg/m^2); however, ADP body fat results were still significantly higher than DXA.[88] The variation in results could be explained by ADP using a two-compartment method to measure the body: FM versus FFM. Note that FFM is composed of other items in addition to muscle including bone, connective tissue, vasculature, water, etc. However, DXA uses a three-compartment method that also incorporates bone density thereby accounting for one more variable than ADP.[88] The addition of bone density as a measurement makes DXA a more desirable method than ADP to measure body composition.

Measuring body composition can be a useful tool in assessing athletic performance and progress. Another aspect of athletic performance to monitor for is exercise dependence. A study on male and female college students aged 18-25 found the majority of participants at risk for exercise dependence to be runners and walkers. The author contributed these findings to the addictive nature of running and its history of positive addiction.[59,89] The euphoric feeling associated with rigorous running occurs through the indirect hyper-stimulation of dopamine in the VTA and NAc, which is caused by decreased activity of gamma-amino butyric acid (GABA) neurons that hinder dopamine.[90-92] Leptin may play a role through leptin signaling, which involves the activation of STAT3 in dopamine neurons of the VTA.[3] In a study by Fernandes et al.,[3]

mice lacking leptin and therefore STAT3 in dopamine neurons had greater voluntary running. When leptin was present, the rewarding effects of running were diminished.[3]

Addiction, in this case runner's high, occurs after repeated exposure to the experience causing a neuroadaptation.[90] Young women, high achievers, and high-performing athletes are at greater risk of developing exercise dependence with females also being more prone than males to develop eating disorders (3% vs. 1%, respectively), which would also affect their leptin concentrations.[59]

However, determining the prevalence of exercise dependence varies in the literature from a reported 3-42%.[60,93-97] A review found the variation was due to the wide range of terminology used to describe exercise dependence (e.g. exercise addiction, compensatory exercise, obligatory exercise, etc.) and the many tools available to measure it.[93] Numerous studies have revealed a high prevalence of eating disorders in endurance athletes, such as marathon runners.[98] The literature suggests that secondary exercise dependence is more prevalent in females than males, although not much research exists on quantifying the prevalence between sexes. An older study found exercise dependence in marathon runners to be significantly higher in women than men.[93,99] However, more recent literature determined there was not a sex difference in exercise dependence of competitive runners.[94] Despite conflicting results, there is much discussion in the literature of sex differences between primary and secondary exercise dependence. In the population of female college athletes, Greenleaf et al.[100] found 2.0% to have distorted eating, 25.5% to be symptomatic, and 72.5% asymptomatic. Most of these participants

reported using exercise to control their weight with 25.5% exercising two hours a day to burn additional calories.[100] Most athletes incur sufficient exercise from training; therefore, additional exercise to burn calories may be unhealthy.[100] Athletes should strive for balance between overall energy expelled and energy consumed.

In planning for an event, endurance athletes typically follow a training schedule consisting of three macrocycles: preparatory, competitive, and transition phases.[101] The preparatory phase consists of high-volume training at moderate intensities to improve endurance, with volume decreasing and intensity increasing towards the end of the phase. One week prior to the event, volume and intensity decrease to allow for optimal recovery. During the competitive event, athletes engage in the highest intensity of exercise. Post competition, a low-volume, low-intensity training is resumed to prepare the athlete for the next event's preparatory phase. Training is usually combined with a nutrition regimen that has specifications for macronutrient intake during the preparatory and competition phases.[101] In the exercise community, food intake may be affected by exercise-induced anorexia; a short-lived effect observed after intense exercise.[102] While the mechanism for this occurrence in humans is not fully understood.[102]

Nutrition should be individualized according to height, weight, activity level, and sex. Furthermore, an athlete's energy requirements will vary from day-to-day due to the volume and intensity of different phases in the training and competition cycles.[103,104] In a joint position statement, the Academy of Nutrition and Dietetics, Dietitians of Canada, and the American College of Sports Medicine note that basal metabolic rate (BMR) may

be calculated using the Harris-Benedict[105] or Cunningham[106] equations along with an appropriate activity factor (AF) for competitive athletes.[103,104] Macronutrient recommendations include: 6-10 gm/kg/d of carbohydrates for endurance program (e.g.1-3h/d moderate-high intensity exercise)[107], 1.2 to 2.0 gm/kg/d protein intake, and 20-35%[108] of total energy intake from fat.[103] Pre-event, foods that are low-moderate in protein and low in fiber and fat are generally recommended to avoid gastric upset and delayed gastric emptying.[103,109] According to the Dietary Guidelines for Healthy People 2015-2020, daily consumption recommendations for sedentary females ages 18-30 are 1800-2000 calories[110], 46 grams (gm) of protein (10-35% of total calories), 130gm of carbohydrates (45- 65% of total calories), and 25.2-28.0gm fiber with 20-35% of calories coming from fat[111]. General fiber recommendations are based off the adequate intake (AI) of 14gm per 1,000 calories.[111] All individuals ranging from athletes to sedentary persons need adequate nutrition to sustain normal daily metabolic functions.

Athletes require adequate energy intake to achieve optimum athletic performance.[103] Low energy intake results in a negative energy balance. This imbalance leads to endocrine function disruption, weight loss, and the breakdown of adipose tissue and muscle mass to be used as fuel for bodily functions. Compromised immune function, loss of musculoskeletal function, and a decrease in strength and endurance are all effects of deteriorated muscle mass. Additionally, malnutrition is also a consequence of low energy intake. Inadequate intake of nutrients can lead to metabolic dysfunctions in the

body secondary to nutrient deficiencies and a decreased resting energy expenditure (REE).[103] Therefore, it is imperative that adequate nutrition is achieved.

To maintain weight, energy balance must be regulated with respect to energy intake versus total energy expenditure (TEE). Total energy expenditure can be defined as the amount of energy burned from physical activity in addition to an individual's REE. Resting energy expenditure is dependent on metabolically active tissue and can be described as the amount of energy needed in an awake, post-absorptive state while remaining thermoneutral for having not exercised in the past 12 hours.[112] It should be noted that REE can be up to 10% higher than BMR due to BMR capturing individuals completely at rest; however REE is a more practical measurement[103] in a lab setting. TEE is measured by multiplying REE by an AF appropriate for the level of physical activity performed. The higher the AF, the more energy expended as a result of the activity.

Equations to calculate REE are individualized to include height, weight, age, and sex. Examples of these predictive equations include the Cunningham and Mifflin St. Jeor equations. Other various methods may also be used to calculate REE such as ADP. With ADP, a regression equation is used to estimate REE; it is not directly measured as in indirect calorimetry.[113] Metabolic factors that affect REE, but are harder to calculate include diet-induced thermogenesis (DIT), which is a postprandial response to meal digestion and the corresponding assimilation of its nutrients.[114] In this process, heat is produced to create enough energy post meal to digest, absorb, and assimilate the nutrients of the meal. An increase in body temperature increases metabolic rate and the process of

digesting food can increase human metabolic rate by up to 25%.[114] Diet is an important aspect of athletic training to ensure that athletes consume proper nutrients and enough energy to sustain TEE, body weight, and muscle mass for optimal athletic performance.

In summary, leptin is known as a satiety hormone that may play a greater role in overall energy homeostasis than initially thought. Circulating levels of serum leptin are dependent on body composition due to leptin's sensitivity to adipose tissue. Exercise is a factor that may affect leptin, as exercise is known to impact energy homeostasis and body composition. More research is needed to explore the influence of leptin on exercise as it pertains to maintaining energy homeostasis. The goal of this project was to explore the effect that circulating serum levels of adipose-sensitive leptin have on diet in the form of macronutrients, subjective satiety, and exercise dependence between active and inactive females.

Research Question

Do serum leptin concentrations, dietary intake, subjective satiety, and exercise dependence differ between competitive female runners and inactive females of similar age and BMI?

H_0: There will be no significant difference in serum leptin levels, dietary intake (macronutrient and energy), subjective satiety, and exercise dependence between competitive female runners and inactive females of similar age and BMI.

H_{a1}: Competitive female runners will have significant differences in serum leptin levels across time (fasting, postprandial, and post exercise) compared to inactive females of similar age and BMI.

H_{a2}: Competitive female runners will have significant differences in energy and macronutrient (percentage of overall calories from carbohydrates, protein, fat, and fiber) intake than inactive females of similar age and BMI.

H_{a3}: Competitive female runners will have significant difference in subjective satiety following food consumption (postprandial) compared to inactive females of similar age and BMI.

H_{a4}: Competitive female runners will have significant difference in overall mean exercise dependence compared to inactive females of similar age and BMI.

In addition to the above research question, secondary outcomes will compare energy intake with TEE, as well as to current dietary recommendations for both groups.

CHAPTER III

METHODS

Fifteen competitive female runners (FR) and 15 inactive females (IF) were recruited for this study, 14 FR and 16 IF completed the study. Subjects of this cross-sectional study were of any race or ethnicity between the ages of 18-30 with a BMI of 18.5-24.9 kg/m^2. Runners were defined as actively training with a club or a team at least three months prior to study enrollment and running at least 30 miles per week. Inactive was defined as exercising no more than two times per week, performing a maximum moderate activity no more than 20 minutes in length. The range in BMI was selected because leptin concentrations correlate with total fat mass (FM). The age range was selected because subjects were not likely to present with any contraindications to exercise, as per The American College of Sports Medicine guidelines,[115] which would prevent them from performing the treadmill exercise test that was part of the protocol; but not included in the data for the current project. This was a joint study performed by the Nutrition and Physical Therapy Departments at Texas Woman's University (TWU) at the Houston campus.

Subjects with a normal BMI (18.5-24.9kg/m^2), based on criteria from the Centers for Disease Control,[116] were recruited. Females with a BMI outside this range may have other health complications that could affect the results of this study; therefore, they were excluded. Exclusion criteria included taking any medication known to impact metabolism (e.g. thyroid medication, seizure medication, steroids, herbal stimulants, or caffeine >300

mg/day), smoking, excessive alcohol use (defined for women as four or more drinks per day or eight or more drinks per week),[117] on a weight loss or weight gain regimen, or have had any significant weight changes (≥5% weight change over prior 30 days or ≥7.5% weight change over prior 60 days.) Subjects also could not be pregnant, trying to become pregnant, or lactating. Subjects were asked to consume a bagel during the study so persons with gluten intolerance or Celiac Disease were excluded. Lastly, subjects with a history of spine fracture or surgery were excluded from the study so as not to exacerbate any pre-existing conditions with certain components of the study protocol.

Females were selected for this study due to the difference in hormone concentrations between sexes that could have affected the outcomes of interest for this study. To prevent this, males were excluded from the study.

Local university track and cross-country teams were contacted to invite competitive FR to participate for this study. Flyers (see Appendix C) were posted around TWU at the Houston campus and announcements were made on Blackboard to recruit IF. Interested parties were instructed to contact the nutrition and/or physical therapy graduate students who assisted the principal investigators (PIs). Graduate students scheduled phone or in-person screening sessions that each lasted approximately 20-30 minutes. A screening questionnaire (see Appendix E) determined eligibility. Eligible subjects were invited to participate in the study protocol, which was executed on the TWU-Houston campus. This study implemented rolling recruitment to enroll subjects as they met

qualifications. No more than four subjects were scheduled on any given day and for same day enrollment. Subject appointments were scheduled 30 minutes apart.

Upon enrollment and prior to the start of the study, each subject was assigned a code in order to maintain subject confidentiality. The study protocol lasted approximately four hours and began around 8:00 am on a day that was convenient for the subject, usually Saturdays. Subjects were asked to arrive fasting (other than water), beginning at midnight. Subjects were instructed to arrive in comfortable, lightweight clothing without any metal (e.g. zippers). The nutrition graduate student then reviewed study procedures with the subject, went over potential risks, and then obtained informed consent (see Appendix F). Once consent was obtained, the nutrition graduate student asked the subject to complete the Exercise Dependence Scale-21[27] questionnaire (see Appendix J), a non-exercise test to determine metabolic equivalent (MET) levels (not used for the purpose of this project), and a 24-hour dietary recall (see Appendix H).

For the recall, subjects wrote down all food and beverages (except water) consumed from midnight to midnight the day prior to testing. Dietary intake from this 24-hour recall was later input into the Nutrition Data System for Research (NDSR)[118] by the nutrition graduate student. The NDSR broke data down into macronutrients (specifically carbohydrates, fiber, protein, fat, alcohol, and overall energy intake) for analysis. The Exercise Dependence Scale-21[27] is a questionnaire that asks 21 questions pertaining to exercise dependence. Subjects subjectively answered each question on a scale of one to six; one being never and six indicating always. Questions are formulated to test for

tolerance, withdrawal, intention effect, lack of control, time, reductions in other activities, and continuance. Example questions include: “I exercise to avoid feeling irritable,” “I exercise despite recurring physical problems,” “I spend most of my free time exercising,” “I exercise longer than I plan,” etc.[27] After a subject rates each question, a tally is taken and a subscale mean of each question is scored via SPSS (Statistical Package for the Social Sciences). A score of five or more on a question indicates a subject is at risk for exercise dependence. A subject is classified as exercise dependent by scoring at least three questions above five. The questionnaire differentiates individuals that are at-risk for exercise dependence (5-6), nondependent-symptomatic (3-4), and nondependent-asymptomatic (1-2) based on Diagnostic and Statistical Manual of Mental Disorder-IV (DSM-IV) standards (the most current version available at the time the scale was created).[25]

After questionnaire completion, fasting blood was obtained from subjects by a phlebotomist. Approximately 14 mL of blood was collected into ethylenediaminetetraacetic acid (EDTA) vacutainers three times during the study protocol (fasting, post-prandial, and post-exercise). These blood draws were used to measure serum leptin levels (in pg/mL) and were later analyzed by one of the primary investigators of the study. After the first blood draw, the subject then entered the BOD POD for air displacement measurements by a physical therapy graduate student. Subjects were required to wear tight fitting clothing and a swim cap before entering the BOD POD. The BOD POD is a measure of air displacement plethysmography (ADP; BOD

POD model (Life Measurement, Inc.; COSMED USA Inc.; Concord, CA) and was utilized to obtain REE and TEE via algorithms in the machine's software using FM and FFM values.[119] To estimate TEE, subjects self-reported their daily activity levels (sedentary, low active, active, very active) prior to entering the BOD POD.

Afterwards height, weight, and waist circumference were measured by the nutrition graduate student in triplicate with the average recorded. Subjects were then asked by the nutrition graduate student to consume a Thomas' plain bagel (250 calories, 52gm carbohydrate, 9gm protein, 1gm fat, 2gm fiber) with 16 ounces of water to aid consumption; subjects were not required to finish the water. Immediately following consumption, a ten-minute timer was set and then the subject completed a brief visual analog scale (VAS) questionnaire[120] to assess subjective satiety under the guidance of the nutrition graduate student.

The VAS is a 100mm hedonic questionnaire (Appendix I) that asked subjects to rate each question by drawing a line (from 0-100mm) to measure their satiety of each question. Questions included: "How full do you feel," "Would you like to eat something sweet," "Would you like to eat something savory," etc.[120] Subjective assessments were later measured and recorded by the nutrition graduate student with a mean taken from the eight categories for the overall score.

At the end of the ten-minutes, a post-prandial blood draw was taken from the subject. From here, the subject proceeded to the treadmill test, which was administered by a physical therapy graduate student and not included in this project. The treadmill test

consisted of a ten-minute familiarization period with a mask that was to be worn during the test, followed by a ten-minute warm-up, then the 30-minute exercise test, and lastly a five-minute cool down. The highest degree of intensity reached by subjects during the 30-minute exercise test was perceived exertion of stable VO_2 max.

Ten minutes after the treadmill test, a post-exercise blood draw was taken. This was the third and final blood draw. After the final blood draw, the subject was offered a light snack and beverage before proceeding to the duel-energy x-ray absorptiometry (Horizon W; Hologic Inc.; Marlborough, MA) scan. This whole-body scan was used to measure FM and LM. The DXA scan was determined to be the ideal form of measurement for FM and LM due to the facts that it also accounts for bone density, while the BOD POD does not.[88] Prior to beginning the DXA scan, subjects were required to remove any metal from their body. A physical therapy graduate student conducting the scan positioned the subject's body and placed a foot holder on the subject's feet to maintain proper positioning thus avoiding movement during the scan. For the seven-minute duration of the scan, subjects were asked to remain completely still. Once the DXA scan was finished, a signature of study completion from the subject was obtained, a $10 Target gift card was awarded, parking was validated, and the subject then exited the building.

Subjects were allowed to ask questions at any point during the study protocol. BOP POD results were printed and given to subjects the day of the study. The DXA results were later emailed to subjects who requested a copy.

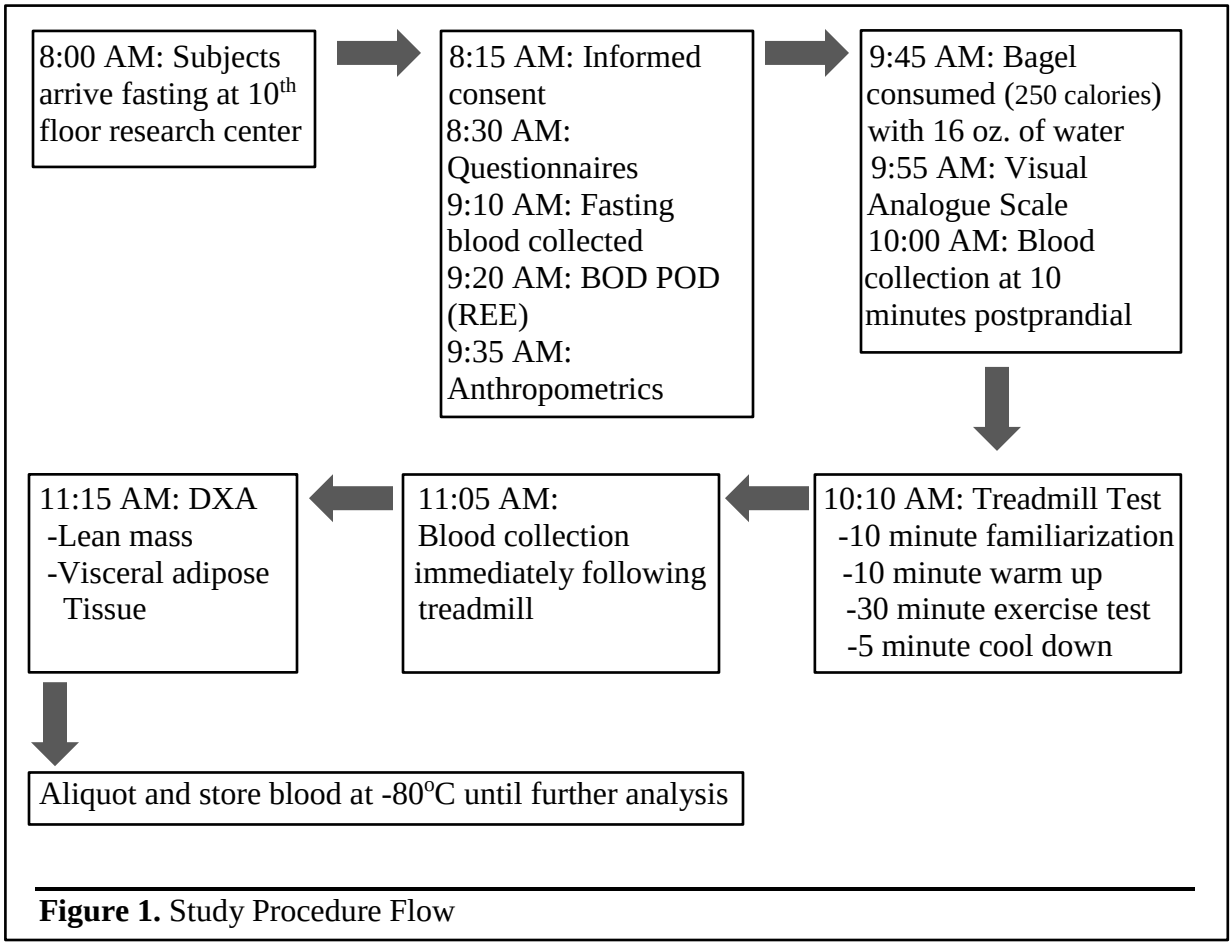

Figure 1. Study Procedure Flow

Statistical Analysis

Descriptive statistics were calculated for all continuous variables, including age, BMI, subjective satiety, dietary intake (overall calories, fiber, alcohol, and percentage of calories from carbohydrate, protein, and fat), and leptin. A preliminary analysis examined the relationships among variables. T-Test and nonparametric analysis compared mean dependent variables (demographics, body composition, diet, satiety) between FR and IF. Analysis of variance (ANOVA) compared mean serum leptin concentrations between groups. Within group concentrations of leptin were analyzed using standard deviations from the mean for each moment in time (fasting, postprandial, and post exercise) in a mixed ANOVA. Data analyses were performed using the Statistical Package for the Social Sciences (SPSS) version 24. The level of significance was set at $P < 0.05$. A *priori* power analysis was conducted using G*Power based on effect sizes derived from Miyazaki et al.[121] Using an analysis of covariance (ANCOVA) model with BMI as the covariate and a power of 80%, alpha level of 0.05 led to a sample size of 19. However, 30 subjects were recruited to allow for attrition and lost data.

CHAPTER IV

RESULTS

Thirty female subjects of the similar BMI and age range participated in this pilot study. Fourteen were FR and 16 were IF. Female runners were recruited primarily through the collegiate track team at a local university, while IF were recruited through TWU and the surrounding area. This study identified differences between the two groups in body composition, macronutrient and overall energy intake, subjective satiety, exercise dependence, and serum leptin levels across time on the day of the study.

Table 1 shows DXA results of body composition along with BOD POD TEE and REE results. In this study, FR had a significantly higher LM (by 10.0%) and TEE (by 38.8%) than IF, along with a lower BMI and FM ($P < 0.000$). Overall body weight was also significantly lower in FR than IF by 11.4% ($P < 0.006$). However, no significant differences were found in REE between FR and IF.

Table 1. The differences in body mass index (BMI), fat mass (FM), lean mass (LM), body weight, resting energy expenditure (REE), and total energy expenditure (TEE) between female runners (FR) and inactive females (IF).

	FR (14)	**IF (16)**	***P* value***
Age (18-30 years)	21.8 ± 3.2	24.7 ± 2.4	0.011*
BMI (18.5 to 24.9 kg/m$^{2)}$	19.9 ± 1.3	23.0 ± 2.8	<0.000*
Percent FM	20.6 ± 2.7	30.8 ± 5.5	<0.000*
Percent LM	75.5 ± 2.5	65.6 ± 5.3	<0.000*
Body Weight (kg)	53.6 ± 5.8	60.5 ± 8.5	0.006*
REE (calories/day)	1172.3 ± 133.7	1197.9 ± 118.1	0.586
TEE (calories/day)	2426.4 ± 276.7	1485.3 ± 146.3	<0.000*

Descriptive variables listed as mean ± standard deviation (SD). REE and TEE obtained from BOD POD; percent FM and LM were obtained through DXA. *P-value less than 0.05 indicates statistical significance.

The FR consumed significantly higher amounts of carbohydrates ($P < 0.004$) and fiber ($P < 0.001$) than IF (see Table 2). No significant differences were found in overall energy intake or any of the other nutrients measured between FR and IF. Subjective satiety using the VAS[120] was assessed immediately following bagel consumption (see Table 3). Differences in post-prandial subjective satiety were not observed between FR and IF in the overall mean score or for each individual question on the VAS.[120]

Table 2. Differences in energy and macronutrient intake between female runners (FR) and inactive females (IF).

	FR (14)	**IF (16)**	***P* value**
Energy (calories/day)	2384 ± 819	1833 ± 667	0.087
Carbohydrates (gm/day)	297.5 ± 97.4	202.7 ± 69.2	0.004*
Average %calorie Carbohydrate	50.5%	43.8%	
Protein (gm/day)	101.7 ± 36.6	80.4 ± 30.5	0.093
Average %calorie Protein	17.4%	17.3%	
Fat (gm/day)	94.2 ± 47.3	78.9 ± 31.4	0.303
Average %calorie Fat	34.6%	37.4%	
Fiber (gm/day)	29.8 ± 10.3	17.9 ± 6.0	0.001*

Descriptive variables listed as mean ± standard deviation (SD). *P-value less than 0.05 indicates statistical significance.

Table 3. Subjective satiety measured by a visual analog scale (VAS)[120] measuring satiety between female runners (FR) and inactive females (IF) following bagel consumption.

	FR (14)	**IF (16)**	***P* value**
Mean Overall Satiety (mm)	43.66 ± 10.5	47.62 ± 9.5	0.287
Hungry (mm)	34.33 ± 22.0	35.41 ± 24.1	0.899
Satisfied (mm)	54.54 ± 20.8	66.49 ± 23.7	0.156
Full (mm)	55.64 ± 32.8	63.36 ± 29.6	0.504
Eat (mm)	48.61 ± 20.6	53.86 ± 24.5	0.534
Pleasant (mm)	48.77 ± 31.6	54.40 ± 32.5	0.635
Sweet (mm)	37.73 ± 35.5	35.23 ± 29.1	0.834
Fatty (mm)	25.76 ± 32.1	26.65 ± 24.1	0.932
Savory (mm)	43.85 ± 31.9	45.52 ± 2.8	0.889

Descriptive variables listed as mean ± standard deviation (SD). One-hundred millimeter (mm) scale for VAS. *P-value less than 0.05 indicates statistical significance.

Exercise dependence was measured via the Exercise Dependence Scale-21.[27] Results of the questionnaire are listed in Table 4. Female runners scored significantly higher than IF in "continuance to exercise despite injury" ($P < 0.008$), "exercising longer than intended" ($P < 0.003$), and "tolerance to exercise," "lack of control in regards to exercise," "reduction in other activities due to exercise," and "the amount of time dedicated to exercise" ($P < 0.000$). The only category FR did not score significantly higher in than IF was "level of exercise performed to avoid withdrawal symptoms of exercise" (see Table 4).

Table 4. Comparison of exercise dependence (ED) scores between female runners (FR) and inactive females (IF).

	FR (14)	**IF (16)**	***P* value**
ED Overall	66.5 ± 13.9	36.53 ± 9.2	<0.000*
ED Withdrawal	10.29 ± 4.6	8.47 ± 3.8	0.254
ED Continue	8.29 ± 4.4	4.6 ± 2.2	0.008*
ED Tolerance	13.57 ± 2.4	8.0 ± 3.6	<0.000*
ED Control	7.57 ± 3.3	3.4 ± 0.8	<0.000*
ED RedACT	3.36 ± 2.3	3.4 ± 0.7	<0.000*
ED Time	12.71 ± 2.9	4.27 ± 1.4	<0.000*
ED Intent	7.71 ± 3.4	4.47 ± 1.7	0.003*

The test includes 21 questions and each question on the scale ranges from 1-6. Results are then input into Statistical Package for the Social Sciences (SPSS) for analysis. Descriptive variables listed as mean ± standard deviation (SD); RedACT refers to reductions in other activities. *P-value less than 0.05 indicates statistical significance.

Serum leptin concentrations were measured at three time points during the study (see Table 5): after an ≥8 hour fast, approximately 10 minutes after consumption of a bagel (postprandial), and ten minutes post exercise (treadmill test). Leptin did not significantly differ within the FR or IF group among fasting, postprandial, and post

exercise blood draws. However, when comparing FR to IF across time, leptin was significantly lower for FR at fasting (67.4%; $P < 0.001$), postprandial (68.3%; $P < 0.000$), and post exercise (70.1%; $P < 0.000$) than IF (see Table 5).

Table 5. Differences in serum leptin between female runners (FR) and inactive females (IF) across time on the day of the study.

	FR (14)	**% Difference FR<IF**	**IF (16)**	***P* value**
Leptin (pg/mL), T0 (fasting)	4.6 ± 3.0	67.4%	14.1 ± 8.8	0.001*
Leptin (pg/mL), T1 (postprandial)	3.8 ± 2.4	68.3%	12.0 ± 7.5	<0.000*
Leptin (pg/mL), T2 (post exercise)	3.5 ± 2.2	70.1%	11.7 ± 6.9	<0.000*

Descriptive variables listed as mean ± standard deviation (SD). *P-value less than 0.05 indicates statistical significance. T0 refers to fasting, T1 refers to postprandial, and T2 refers to post exercise.

Secondary outcomes of this study compared energy intake with TEE, as well as to current dietary recommendations for both groups. Results revealed that while FR had higher TEE than IF ($P < 0.000$); energy intake was not significantly different. Dietary recommendations for carbohydrates, protein, and overall energy intake were different between FR and IF. Female runners consumed more carbohydrates and fiber than IF ($P < 0.004$ and $P < 0.001$ respectively), but there were no significant differences in protein or fat (see Table 6).

Table 6. Differences in total energy expenditure (TEE) compared to energy intake between female runners (FR) and inactive females (IF), as well as dietary recommendations compared to average intakes.

	FR (14)	**IF (16)**	***P* value**
TEE	2426.4 ± 276.7	1485.3 ± 146.3	<0.000*
Energy (calories/day)	2384 ± 819	1833 ± 667	0.087
Energy Recommendation (calories)	2748.5	1800-2000 2018.5**	
Met Energy Recommendation %	7.1%	13% 12.5%*	
Carbohydrates (gm/day)	297.5 ± 97.4	202.7 ± 69.2	0.004*
Carbohydrate Recommendations	6-10g/kg	45-65% of calories	
Met Carbohydrate Recommendation %	29%	44%	
Protein (gm/day)	101.7 ± 36.6	80.4 ± 30.5	0.093
Protein Recommendations	1.2-2.0g/kg	10-35% of calories	
Meet Protein Recommendation %	50%	100%	
Fat (gm/day)	94.2 ± 47.3	78.9 ± 31.4	0.303
Fat Recommendation	20-35% of calories	20-35% of calories	
Met Fat Recommendation %	36%	25%	
Fiber (gm/day)	29.8 ± 10.3	17.9 ± 6.0	0.001*
Fiber Recommendations (gm per 1,000 calories)	14gm per 1,000 calories	14gm per 1,000 calories	
Met Fiber Recommendation %	36%	13%	

Descriptive variables listed as mean ± standard deviation (SD). *P-value less than 0.05 indicates statistical significance. TEE obtained from BOD POD. Carbohydrate recommendations for FR provided by Medicine & Science in Sports & Exercise107 and Nutrition and Athletic Performance.103 Energy intake, carbohydrate, and protein recommendations for IF provided by Dietary Guidelines for Americans 2015-2020.111 Energy intake was also calculated for IF via the **Mifflin-St. Jeor equation122,123 with an activity factor (AF) of 1.5.124 Energy intake for FR was calculated via the Cunningham equation106,123 with an AF of 2.0.124 Percentages of energy recommendations met were based on a 150 calorie range of calculations from predictive equations. Fiber and fat recommendations for both FR and IF provided by Dietary Guidelines for Americans 2015-2020.111 Protein recommendations for FR provided by Rosenbloom and Coleman108 and Nutrition and Athletic Performance.103 Dietary percentage recommendations are for met and does not include exceeded values.

CHAPTER V
DISCUSSION

The primary finding of this study showed leptin concentrations were lower in FR and IF across time on the day of the study, with the FR having lower BMI and FM but higher LM than IF. The FR also had higher TEE than IF despite no difference in REE between groups. The FR also consumed more dietary carbohydrate and fiber than IF without differences in other macronutrients or energy.

Leptin was measured at fasting, postprandial, and post exercise. Results did not differ significantly across time within each group; however, leptin was significantly lower across time in FR than IF. Postprandial responses to leptin within each group may have been greater had subjects not fasted for eight hours prior to the start of the study, had a meal larger than a bagel, and/or had serum blood draws at least four to six hours after postprandial.[29] One study took blood samples from subjects of a normal weight every 30-60 minutes for a 24-hour period to measure differences in serum leptin levels postprandial. Results showed high-carbohydrate meals cause greater postprandial levels of leptin than high-fat meals in women.[29,30] To see significant changes in postprandial serum leptin, it may take ingesting a greater amount of overall energy in addition to increased calories from carbohydrates.

Studies in rodents[3,16-19] and leptin-deficient humans[3,14,15] found leptin to be involved in the modulation of voluntary running and locomotor activity.[3,8] The current study revealed no significant difference in leptin levels for FR or IF before exercise

(postprandial blood draw) or after; however, there were significant differences between the two groups. Differences in adiposity between the two groups may explain the variation as leptin positively correlates with adiposity. Studies have frequently observed low leptin levels in athletes and associate it to an exercise-induced energy deficit[45,68] and/or reduced FM.[68-70] Other studies found that chronic exercise is associated with hypoleptinemia regardless of FM.[68,71] However, a review over research from 1998-2016 found chronic exercise ($\geq$ 2 weeks) is associated with a small decrease in leptin regardless of age and sex.[125] Hypoleptinemia was also observed in subjects performing acute exercise where the duration was not long enough to decrease FM.[68,72-74] In contrast, one study in men found that after an hour of moderate exercise leptin concentrations dropped 18% after a 24-hour period and 40% after 48 hours; but not after a short, intense session.[44,45] It has been suggested that a negative energy balance could explain the decrease in leptin, such as in an ultramarathon or in short bouts of exercise where exertion is extreme.[44] Another study in women reported exercise of $\geq$60 minutes may not show significant variations in leptin until 48 hours post activity.[44,46] Note that the current study measured leptin within 15 minutes post exercise and the 45-minute treadmill session was not intense. Only 30 minutes of the treadmill portion was the exercise test and the highest degree of intensity reached by subjects during that time was perceived exertion of stable VO_2 max; which was not included in the data of this project. A lack of defined intensity could explain why there were not significant changes in serum leptin within each group post exercise.

Body composition was measured due to leptin's positive association with adiposity. Inactive females had a significantly higher FM, body weight, and BMI, even though a concerted effort was made to match BMI between groups. The average BMI of FR was 19.9kg/m^2, while IF had an average BMI of 23.0 kg/m^2. As expected, FR had significantly higher LM than IF. However, no significant differences were found in REE between FR and IF, although IF had a slightly higher mean REE than FR (by 25.6 calories/day). This could be because FR was smaller in terms of overall body weight. The lack of significant results could also be explained by the fact that while REE is dependent on metabolically active tissue, skeletal muscle and adipose tissue use much less energy at rest than the brain and vital organs.[126,127] That statement is supported by evidence presented by the current study where REE was not significantly higher in FR and IF. In contrast, studies report active subjects to have a higher REE than sedentary individuals[128-130] due to a carry-over effect of the increased metabolic rate from exercise.[128] While older studies regard LM as the main influencer of REE,[128,131-133] more current data reveal that body composition only accounts for 20% of REE.[128,133,134] Other factors contributing to REE may include: age, the thermic effect of food due to excessive consumption, disease status, drugs, and emotional stress.[128] Differing hormonal patterns may also impact REE.[128] The current study included age, sex, medical conditions, and drugs or supplements that may affect metabolism in the inclusion and exclusion criteria. Subjects were also asked to fast eight hours prior to participating in the study.

Energy intake is important for metabolic function regardless of activity level; however, as activity increases so should energy intake to compensate for EE. The FR of the current study had an average energy intake that was within 98.3% of their TEE. The average energy intake of IF surpassed their TEE by 23.4%. While FR had a significantly higher TEE than IF, there was no significant difference in overall energy consumption. This could be explained by the overconsumption of energy intake by IF. Energy intake was obtained through 24-hour recalls and analyzed via NDSR,[118] while TEE and REE were obtained through the BOD POD.

Interestingly, while FR met caloric needs within 98.3% of TEE, a majority fell below daily caloric recommendations using the Cunningham equation. Similarly, one study on female college athletes found 91% did not meet estimated energy needs using the Cunningham equation with an AF of 1.8-2.3.[135] Another study also found female athletes consumed significantly less energy intake than recommendations.[136] However, the methodology in that study of 37-41 calorie/kg[136,137] for energy estimates differed from the current study. Garcin et al. found female athletes to have energy intakes closer to Recommended Dietary Allowance (RDA) values than sedentary controls.[138] Similar to the current study, results were based off TEE calculations (TEE = REE x AF)[138,139] with a sport-specific AF and compared to mean energy intake. However, dietary values were compared to French RDAs,[138] which differ from U.S. RDA values; 50-55% carbohydrates, 11%-15% protein, 30%-35% fat[140] versus 45-65% carbohydrates, 10-35% protein, 20-35% fat,[111] respectively.

To compare TEE provided by the BOD POD, energy intake for FR was calculated using the Cunningham equation (22 x FFM (kg) + 500)[106,123] with an AF of 2.0[124] to account for exercise.[103,104] The Cunningham equation is recommended for athletes in a joint position paper by the Academy of Nutrition and Dietetics, Dietitians of Canada, and the American College of Sports Medicine.[103,104] In comparing energy intake to the Cunningham equation, the majority of FR (64.3%) fell below recommendation, 28.6% overconsumed, and 7.1% were within 150 calories of the recommendation.

According to the Dietary Guidelines for Americans 2015-2020,[111] the majority of IF (69%) exceeded recommendations, while 13% met recommendations and 19% fell below recommendations of 1800-2000 calories/day. The problem with a general recommendation of 1800-2000 calories per day is that it is not individualized using factors such as height and weight. The daily caloric recommendations for IF were also calculated via the Mifflin-St. Jeor female specific equation (10 x weight(kg) + 6.25 x height (cm) - 5 x age (y) – 161)[122,123] with an AF of 1.5 for mildly active.[124] Note that the Mifflin-St.Jeor equation is not recommended for athletes because it underestimates REE due to differences in body composition,[141] e.g. FFM.[123] The Mifflin-St. Jeor equation is recommended for males or females of a young population with a healthy weight.[141] Based on Mifflin-St. Jeor calculations, 76% of IF exceeded recommendations, 12.5% met recommendations, and 12.5% fell below recommendations.

To calculate TEE, multiply REE by an appropriate AF.[114,138,139] Calculations for REE use predictive equations that incorporate specifics about an individual such as

height, weight, LM, etc. into the equation.[106,122] In the current study, the Cunningham equation over predicted REE for FR by an average of 202 calories when compared to the BOD POD. Studies have found the Cunningham equation to vary from indirect calorimetry by a mean underestimate of 39 calories[141] to an overestimate of 122 calories.[122] The Mifflin-St.Jeor equation in the current study also over predicted REE by an average of 148 calories. Similarly, the literature reveals conflicting results with the Mifflin equation varying from indirect calorimetry by a mean overestimate of 136 calories[122] to an underestimate of 220 calories.[142] Comparisons in REE between ADP and predictive equations, such as Cunningham and Mifflin, were not found in current literature. However, when compared to indirect calorimetry, ADP under predicted REE by a mean 351 calories using the Nelson (1992) model.[143] Mean differences in REE between various predictor methods likely vary due to the characteristic differences of subjects and investigator specificity per study.[144]

In regards to macronutrients, only 29% of FR met the daily recommendations of 6-10gm/kg carbohydrates for athletes.[103,107] However, using the recommended 14gm fiber per 1000 calories, 36% met the minimum requirements.[111] Only 44% of IF met general carbohydrate recommendations and 13% met fiber recommendations.[111] In contrast to the current study, previous research suggests female athletes often fail to meet recommended carbohydrate intakes. In one study, 75% fell below recommendations (5gm/kg),[135] and in another subjects consumed <50% of the lower end of the recommendation (6-10gm/kg).[136] In regards to leptin and dietary intake in the current

study, FR having significantly lower levels of leptin could be explained by the fact that FR also consumed significantly more fiber than IF.[29,31-34]

For protein, half of FR met their requirements based off the 1.2-2.0gm/kg recommendation for athletes,[103,108] while 28.6% were above range. Based on the general protein recommendation of 10-35% of calories to come from protein sources,[111] all subjects in the current study were within the recommended range. For protein calories, the current study is again in contrast to prior research. Half of subjects in one study did not meet recommendations,[135] while 55% in another study consumed the minimum recommendation[136] (1.2gm/kg).[135,136] It should be noted that while some studies compared minimum requirements for athletic macronutrient recommendations, the current study compared intake to the recommended range, which may account for some variation.

The recommendation for calories from fat were the same for FR and IF with 20-35% of overall calories to come from fat sources.[107,111] The majority of FR (57%) and IF (75%) consumed more fat calories than recommended, while 36% of FR and 25% of IF were within the recommendation. In regard to fat consumption for athletes, one study revealed similar results with 55% overconsuming fat calories,[136] while 76% in another study consumed $\leq$35% of energy from fat.[135] Regarding alcohol, both FR and IF consumed insignificant amounts.

In assessing exercise dependence (ED), FR had a significantly higher mean than IF in overall mean ED and in six of the seven categories listed on the Exercise

Dependence Scale-21.[27] A higher score indicates a higher level of dependence. The scale defines dependence as an individual presenting with at least three out of seven categories.[27] These results can be rationalized in part by the addictive nature of running.[59,89] Similar to the current study, competitive athletes scored higher than non-competitive subjects on every measure.[145] Men and women from 17 different sports were included and measured using the Exercise Addiction Inventory[146] and the Passion Scale.[145,147] In contrast, another study reported a 28% prevalence rate for exercise dependence specifically in women; seven different questionnaires were used to measure ED.[26] However a more recent review of the literature found various studies to report ED from 3-42%.[60,93-97] Note that results may differ between studies due to the different questionnaires used to measure ED. In the current study, FR had significantly lower levels of leptin and significantly higher levels of ED when compared to IF. This is similar to the study by Fernandes et al.[3] that found that despite a reduction in leptin, physical activity levels were still increased. It is suggested that the "runner's high" associated with reward, could have evolved to promote stamina.[3] Note that other sports, such as cycling during high intensity interval training, report achieving euphoria that is associated with exercise and should be explored as well in relation to leptin.[149]

The present study found no significant differences between FR and IF in overall mean subjective satiety or in any of the eight categories. A study in women found no significant differences in satiety for runners or walkers when compared to at rest; however, satiety differences were not measured between runners and walkers.[148] Previous

research was not found on satiety differences between athletes and sedentary individuals. While appetite is hormonally regulated as a metabolic function, eating can be activated by either metabolic necessity or hedonic drive.[40] Lack of significant results in satiety from the current study could be explained by the age group and start time of the study. Many subjects reported having a hard time consuming the bagel and attributed it to not normally eating that early in the day. Furthermore, the bagel was consumed prior to the treadmill portion of the study protocol. Had subjects ran on the treadmill first, there might have been enough of a negative energy balance created to affect appetite. Under normal conditions, when fat stores are diminished, the decreased leptin levels cause appetite to increase and EE to decrease.[39]

In an attempt to streamline serum leptin concentrations, this study tried to match FR and IF by age group, sex, and BMI range in the inclusion and exclusion criteria. However, significant differences were found in age and BMI between FR and IF. The age difference could be accounted for due to the FR being recruited from a predominately undergraduate university and the IF being recruited from a predominately graduate university. The significant age difference could also in part account for the variation in FM and BMI. The exclusion of pregnant or lactating women, and any medications, supplements, or substances known to impact metabolism to avoid unintended hormonal influences were strengths of this study. Having subjects arrive fasting was a strength in terms of inhibiting the thermic effect of food due to excessive consumption as it pertains

to REE.[128] Screening for sex and significant weight fluctuations were also strengths of this study. Most of these restrictions helped strengthen results of the current study.

However, the current study was not without limitations. It did not screen for birth control or menstrual status in subjects. This is a limitation that could have impacted results as amenorrhea is associated with low leptin levels in women who excessively exercise[126] and birth control is a controlled substance that regulates female menstrual cycles. To see more variation in serum leptin responses, it would have been beneficial to feed subjects a high-carbohydrate meal (60% carbohydrate)[30] as opposed to a bagel after fasting eight hours, and to collect postprandial leptin at least four to six hours after consumption.[30] It would also have been beneficial to increase the intensity and duration of exercise to an hour at a defined moderate pace[44,45] and to collect serum leptin 24 to 48 hours post exercise.[44,46] Another limitation of this study is that a 24-hour recall was performed as opposed to collecting three days' worth of dietary data from subjects. The additional data would have given a more accurate assessment of energy intake. Additionally, the 24-hour recall was performed on a Saturday, therefore recalling data from Friday (i.e. the start of the weekend). Consumption patterns on a weekend tend to differ from that of a typical weekday. Two additional days' worth of data would have also accounted for the difference. Another limitation of 24-hour recalls is that subjects tend to underestimate what they consumed. Having subjects keep a food diary may have helped account for this. Other limitations were not standardizing meal patterns nor collecting activity patterns, and not restricting FR from running the day before the study.

The findings of this study led the author to reject one of the alternative hypotheses, and accept one partially and two in full. The null hypothesis (H0) that there would be no significant differences in serum leptin levels, dietary intake, satiety and exercise dependence between FR and IF is rejected overall.

H0: There will be no significant difference in serum leptin levels, dietary intake (macronutrient and energy), subjective satiety, and exercise dependence between competitive female runners and inactive females of similar age and BMI. Rejected

This study found significant differences in serum leptin between FR and IF across time, therefore the first alternative hypothesis (H1) is accepted.

H_1a: Competitive female runners will have significant differences in serum leptin levels across time (fasting, postprandial, and post exercise) compared to inactive females of similar age and BMI. Accepted

Results showed no significant difference in energy intake between FR and IF. The FR did consume significantly higher amounts of carbohydrates and fiber than IF; however, no significant differences were found between protein, fat, and alcohol consumption. Therefore, the second alternative hypothesis (H2) is partly accepted.

H_2a: Competitive female runners will have significant differences in energy and macronutrient (percentage of overall calories from carbohydrates, protein, fat, and fiber) intake than inactive females of similar age and BMI. Partially Accepted

Due to no significant findings for satiety between FR and IF overall or in any of the eight categories, the third alternative hypothesis (H3) is rejected.

H_3a: Competitive female runners will have significant differences in subjective satiety following food consumption (postprandial) compared to inactive females of similar age and BMI. Rejected

Results from the Exercise Dependence Scale-21[27] revealed FR scored significantly higher than IF overall and in six out of seven categories; therefore, the fourth alternative hypothesis (H4) is accepted.

H_4a: Competitive female runners will have significant differences in overall mean exercise dependence compared to inactive females of similar age and BMI. Accepted

Secondary outcome results from the BOD POD revealed FR had significantly higher TEE than IF. These outcomes also compared energy intake with TEE, as well as to current dietary recommendations for both groups. Female runners did not consume significantly more energy than IF, which could account for why FR had a significantly lower BMI than IF. Concerning dietary recommendations, 29% of FR and 44% of IF met carbohydrate recommendations. For fiber, 36% of FR and 13% of IF met recommendations. Half of FR met protein recommendations and 28.6% exceeded, while all IF met protein recommendations. For fat, 36% of FR met recommendations while 57% exceeded, and 25% of IF met recommendations while 75% exceeded.

Due to the significant differences in age and BMI in the current study, it is recommended to narrow the age criteria and recruit a larger amount of subjects to more narrowly match BMI within 2.0-3.0 kg/m^2. It is also recommended to assess for amenorrhea in recruitment screening, and include a three-day dietary recall along with

meal and activity patterns leading up to the study. Leptin blood draws may also show greater results within groups 24-48 hours post exercise. Due to the different mechanisms that affect serum leptin levels, future studies may want to further explore those mechanisms and measure to what degree each has on leptin.

CHAPTER VI

CONCLUSION

This study sought to find differences in serum leptin concentrations, dietary intake, satiety, and exercise dependence between FR and IF. Female runners had significantly lower leptin than IF across time (e.g. fasting, postprandial, and post exercise). However, within each group there were not any significant differences in leptin across time on the day of the study.

No significant changes were observed in overall energy intake or calories from protein or fat, but FR did consume a significantly higher amount of carbohydrates and fiber than IF. For overall energy intake, the majority of FR fell below recommendations while the majority of IF exceeded recommendations. The majority of FR and IF both met protein recommendations and exceeded fat recommendations. Both groups fell below recommended carbohydrate and fiber recommendations, despite FR consuming significantly more carbohydrates and fiber than IF. This is in part due to different carbohydrate recommendations between FR and IF. Despite differences in activity levels between FR and IF, there were no significant variations in overall satiety or in any of the eight categories on the VAS[120] between groups.

The FR scored significantly higher in mean overall exercise dependence and in six of the seven categories on the Exercise Dependence Scale-21.[27] Withdrawal was the only category not to score significant results. FR reported significantly higher results in

continuance, tolerance, control, reduction in other activities, time spent, and intent of exercise.

Despite FR and IF being recruited from the same BMI range (18.5-24.9 kg/m^2), FR had a significantly lower BMI, FM, and body weight than IF. As expected, FR had significantly higher LM and TEE than IF. However, there were no significant changes in REE between groups.

The implications of this research are that variations in FM for FR and IF of the same BMI range (18.5-24.9 kg/m^2) showed significant differences in serum leptin levels. However, the effect of exercise on leptin was not found within 15 minutes after exercise for either group. Additionally, no differences in serum leptin were detected for either group at fasting or 10 minutes postprandial. Dietary intake of macronutrients was only significantly higher in carbohydrates and fiber for FR than IF, despite FR having higher intake requirements for both carbohydrates and protein. Future research is warranted to further assay the effects of exercise and dietary intake on serum leptin.

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APPENDIX A

IRB Approval Letter

Institutional Review Board
Office of Research
6700 Fannin, Houston, TX 77030
713-794-2480
irb-houston@twu.edu
http://www.twu.edu/irb.html

DATE: September 14, 2016

TO: Dr. Mindy Maziarz
Nutrition & Food Sciences - Houston

FROM: Institutional Review Board (IRB) - Houston

Re: Approval for Impact of leptin and ghrelin on body composition, metabolic rate, and bone density in competitive female runners and inactive females: A pilot study (Protocol #: 19145)

The above referenced study was reviewed at a fully convened meeting of the Houston IRB (operating under FWA00000178). The study was approved on 9/13/2016. This approval is valid for one year and expires on 9/13/2017. The IRB will send an email notification 45 days prior to the expiration date with instructions to extend or close the study. It is your responsibility to request an extension for the study if it is not yet complete, to close the protocol file when the study is complete, and to make certain that the study is not conducted beyond the expiration date.

If applicable, agency approval letters must be submitted to the IRB upon receipt prior to any data collection at that agency. A copy of the approved consent form with the IRB approval stamp is enclosed. Please use the consent form with the most recent approval date stamp when obtaining consent from your participants. A copy of the signed consent forms must be submitted with the request to close the study file at the completion of the study.

Any modifications to this study must be submitted for review to the IRB using the Modification Request Form. Additionally, the IRB must be notified immediately of any adverse events or unanticipated problems. All forms are located on the IRB website. If you have any questions, please contact the TWU IRB.

cc. Ms. Rose Bush, Nutrition & Food Sciences - Houston
Alexis Ortiz

APPENDIX B

IRB Study Extension Approval Letter

TWU
TEXAS WOMAN'S UNIVERSITY
DENTON DALLAS HOUSTON

Institutional Review Board
Office of Research
6700 Fannin, Houston, TX 77030
713-794-2480
irb-houston@twu.edu
http://www.twu.edu/irb.html

DATE: September 5, 2017

TO: Dr. Mindy Maziarz
Nutrition & Food Sciences - Houston

FROM: Institutional Review Board (IRB) - Houston

Re: Extension for Impact of leptin and ghrelin on body composition, metabolic rate, and bone density in competitive female runners and inactive females: A pilot study (Protocol #: 19145)

The request for an extension of your IRB approval for the above referenced study has been reviewed by the TWU IRB (operating under FWA00000178) and appears to meet our requirements for the protection of individuals' rights.

If applicable, agency approval letters must be submitted to the IRB upon receipt prior to any data collection at that agency. If subject recruitment is on-going, a copy of the approved consent form with the IRB approval stamp is enclosed. Please use the consent form with the most recent approval date stamp when obtaining consent from your participants. A copy of the signed consent forms must be submitted with the request to close the study file at the completion of the study.

This extension is valid one year from September 13, 2017. Any modifications to this study must be submitted for review to the IRB using the Modification Request Form. Additionally, the IRB must be notified immediately of any unanticipated incidents. All forms are located on the IRB website. If you have any questions, please contact the TWU IRB.

cc. Ms. Rose Bush, Nutrition & Food Sciences - Houston
Alexis Ortiz

APPENDIX C

Recruitment Flyer

TWU
TEXAS WOMAN'S UNIVERSITY
DENTON DALLAS HOUSTON

Wanted Female Research Volunteers

Purpose – To study the relationship of hormones on body composition, metabolic rate, and bone density in female competitive runners and inactive females.

Benefits – Free blood tests, free body composition analysis and bone density.

Criteria:
1. You must be a female between the ages of 18 and 30 and have a healthy body weight.
2. You must not be pregnant, actively trying to become pregnant, or breastfeeding.
3. You must not be using medications or supplements that impact body weight or metabolism.
4. You must not be on a special diet for weight loss or weight gain *or* had recent changes in body weight.

We are recruiting two groups for this study:

1. **Competitive female runners:** Must be actively training with a club or team at least 3 months prior and logging at least 30 miles per week.
2. **Inactive females:** Must not engage in more than 20 minutes of moderate activity on no more than 2 days each week.

We will ask you to come to Texas Woman's University – Houston one morning. Fasting blood will be collected. Blood will also be collected on 2 occasions after a light breakfast. You will complete two separate body composition analysis tests and a 30-minute treadmill test. The total time you need to spend for the study is 4.5 hours (not counting travel time).

If interested, please email or call for more information:

Dr. Mindy Maziarz **mmaziarz@twu.edu**; 713-794-2375
Dr. Alexis Ortiz **aortiz10@twu.edu**; 713-794-2077
Department of Nutrition & Food Sciences and School of Physical Therapy
Texas Woman's University – Institute of Health Sciences, Houston, TX 77030
There is a potential risk of loss of confidentiality in all email, downloading, and internet transactions.

Connecting Excellence to the Real World

SAC APPROVED 4/27/2016 TWUIRB

APPENDIX D

Recruitment Script

Recruitment Script

Thank you for your interest in the research project "Impact of leptin and ghrelin on body composition, metabolic rate, and bone density in competitive female runners and inactive females: A pilot study."

We are conducting the study to find out the relationship of two hormones, leptin and ghrelin, on body composition, resting metabolic rate, and bone density in female competitive runners and inactive females. This study requires only one visit to the TWU-Houston campus. Each subject in the study will fast overnight and go to the study site on a scheduled morning convenient for you. During the visit, the investigators will take the subject's height, weight and waist and hip measurements and perform two types of body composition analysis. A phlebotomist (person taking blood) will draw one tablespoon of fasting blood. The subject will then consume a bagel with water and have another tablespoon of blood drawn. A 30 minute running treadmill test will occur 30 minutes after the bagel and water are consumed. After the treadmill test, another tablespoon of blood will be collected. The subjects will complete 3 questionnaires over exercise habits, feelings of fullness, and dietary intake. We will analyze the blood samples for hormone (leptin and ghrelin) concentrations.

Why are we doing the study?

Leptin and ghrelin are two hormones that impact food intake, metabolism, and exercise motivation. These hormones may also affect bone density. There is very little research on the relationship of these hormones on body composition and metabolism in females. Our study will investigate these relationships in competitive female runners and inactive females and provide new information in this field.

What are we going to do, and what your participation will be?

Exclusion criteria:

Subjects cannot participate if they are less than 18 years of age or above 30 years of age or if they have a body mass index less than 18.5 or more than 25. They cannot participate if they are currently taking drugs, over the counter mediations, or supplements that impact metabolism (e.g. thyroxine or other thyroid medications, prednisone or other steroids, herbal stimulants such as ephedra or ma huang) or are consuming more than 300 mg caffeine each day. Females who are pregnant, actively trying to become pregnant, or breastfeeding will not be able to participate. Subjects who have lost or gained weight

(≥5% weight change over prior 30 days or ≥7.5% weight change over prior 60 days) or are following a special diet for weight loss or gain cannot participate.

Inclusion criteria:
The competitive female runners must be actively training for at least 3 months prior to entering the study and logging at least 30 miles each week. The inactive females must not engage in more than 20 minutes of moderate physical activity on 2 or more days per week prior to the study.

Confidentiality:
Issues of privacy and loss of confidentiality may cause concern to some subjects. However, confidentiality will be protected to the extent that is allowed by law. Information regarding all subjects will be kept confidential. 1. Several forms used in this study will include identifiable information. All information collected from the subjects will be stored in a locked cabinet. In addition, the subject's name will not appear on any document. Each subject will be assigned a code number that will be used at all times.

Benefits:
Free body composition, resting metabolic rate, oxygen consumption during exercise, bone density, and blood test results if you request.

Thank you. Do you have any questions?

APPENDIX E

Screening Questionnaire

Screening Questionnaire

Impact of leptin and ghrelin on body composition, metabolic rate, and bone density in competitive female runners and inactive females.

ID: _____ Name:_____ DOB and Age: _____
Ethnicity:_____

Email address: _____ Telephone:
_____

Weight (pounds) _____ Height (inches) _____ BMI
_____kg/m^2

Do you smoke? Yes No
How many alcoholic drinks do you consume in One day? _____ One week? _____

Are you pregnant? Yes No
Are you breastfeeding? Yes No
Are you trying to become pregnant? Yes No

Are you on a special diet for weight loss or weight gain? Yes No
Have you lost or gained weight over the past month? Yes No
If yes, how many pounds? _____
Have you lost or gained weight over the past 2 months? Yes No
If yes, how many pounds? _____

Do you have a chronic medical condition? Yes No If yes, specify
_____

What medications do you currently take (amount and frequency)?

What vitamins/minerals/herbs do you currently take (amount and frequency)?

Do you exercise? Yes No
If yes, what is the frequency and duration?

Do you currently run for a competitive team or club? Yes No
If yes, which team/club?
Have you been running over the previous 3 months? Yes No
How many miles do you run each week, on average?

You will be exposed to a bagel containing wheat during the study. Do you have an intolerance or allergy to gluten? Yes No

APPENDIX F

Informed Consent

TEXAS WOMAN'S UNIVERSITY
CONSENT TO PARTICPATE IN RESEACH

Title: Impact of leptin and ghrelin on body composition, metabolic rate, and bone density in competitive female runners and inactive females: A pilot study.

Investigator: Mindy Maziarz, PhD, RDN.....mmaziarz@twu.edu; 713-794-2375
Investigator: Alexis Ortiz, PT, PhD..... aortiz10@twu.edu; 713-794-2077

Explanation and Purpose of the Research

You are being asked to participate in a research study at Texas Woman's University – Houston. The purpose of the study is to explore the relationship of two hormones on body weight, bone health, diet and how full you feel after eating, and exercise habits. We will recruit two groups of females. The first group will be participating in a competitive running program. The second group will not be participating in any type of physical activity.

We will ask the following questions:

1) What is the relationship between the two hormones on body weight and bone health in competitive female runners and inactive females?
2) How do the two hormones impact feelings of fullness after eating and exercise habits in competitive female runners and inactive females?

Research Procedures

For this study we will ask you not eat any food or drink anything (other than water) after midnight and arrive the next morning to the Texas Woman's University (TWU) – Houston campus. The following will explain the study procedure beginning with your arrival on campus. First, the research study will be explained to you in detail and you will have an opportunity to ask questions. You will be asked to fill out questionnaires on your exercise habits and diet. A phlebotomist (the person drawing the blood) will take one tablespoon of blood from a vein in your arm. This amount is less than what is required for an annual blood exam. Then an investigator of your choice (male or female) will measure your height, weight, waist and hip circumference. You will be asked to change into tight-fitting clothing and wear a swimming cap over your hair for body weight measurements. You will then be asked to eat a bagel and drink water. After you eat the food you will answer questions on how full you feel. The phlebotomist will draw another tablespoon of blood. The total amount of blood taken from your arm for the study will be three tablespoons. You will then be asked to exercise on a treadmill for 45 minutes wearing equipment such as a breathing mask. Treadmill time will be divided in 10 minute warm-up, 30-minute running, and 5 minute cool-down. You will be given time to become familiar with wearing the mask before the treadmill run. You will also be able to warm up prior to your run and cool-down after your run. After the treadmill test the phlebotomist will collect the last tablespoon of blood from your vein. You will then have a body scan to determine body weight measurements and bone density. **Remember your participation is completely voluntary and you can ask to stop any study procedure at any time.**

Initials

The time commitment for the study is approximately 4.5 hours, not including your travel to and from TWU. The screening process for this study takes approximately 20-30 minutes. The study procedure will take approximately 4 hours. You will be able to stop any study procedure without any consequences.

Potential Risks

A potential risk to you as a subject is the release of confidential information. Confidentiality will be protected to the extent that is allowed by law. To protect confidentiality, you will be given a numerical code which will be used in all records. Only Dr. Maziarz will know your identity. All records will be stored in a locked filing cabinet in Dr. Maziarz' office. Blood samples will be stored in a secure lab (Biology lab room 10133 at TWU-Houston) where only the investigators have access. The blood samples will be labeled with a code. Records will be shredded within 5 years of completion of the study. The blood samples will be disposed in a biological safety bag at the same time. There is a potential risk of loss of confidentiality in email, downloading, and internet transactions.

Blood will be collected three times during this study. The risks for these procedures are not greater than the risks that may happen when you have blood drawn at your doctor's office. A possible risk involves feeling uncomfortable when blood is taken from your vein. Collecting blood can cause minor pain, bruising, swelling, anxiety, infection, light-headedness, and fainting. You will have access to water to minimize discomfort and pain during the blood collection. A trained phlebotomist will collect your blood which will also minimize risk of discomfort, minor pain, swelling, or anxiety. Another possible risk with blood collection is infection. The phlebotomist will clean your arm with alcohol where the blood will be drawn to minimize risk of infection.

You will be asked to wear tight clothing for the body weight testing which may cause you to become uncomfortable or embarrassed. To minimize this risk, privacy walls have been placed around the body weight testing unit. You may also become embarrassed when your height, weight, waist circumference, and hip circumference are measured. To minimize the risk of embarrassment you will be allowed to choose the investigator, one of which will be female, you would like to perform the testing.

Another possible risk involves radiation exposure from the scan used in body weight and bone health measurements. A whole body scan produces less radiation than one-tenth the dose of a standard chest x-ray and less than one day's exposure to natural radiation. **To the best of your knowledge you attest with certainty you not pregnant.**

Another risk may involve sensitivities or allergies to latex. To minimize this risk, non-latex gloves can be used during the blood draw and non-latex hair caps are available to wear during body weight testing.

Another risk is extreme fatigue from running on the treadmill if you are not accustomed to running. To minimize the risk of extreme fatigue your vital signs and respiration values will be monitored throughout the entire test. You may feel claustrophobic during the BOD POD procedure and as you become familiar with wearing the mask for the treadmill procedure. To minimize the risk of claustrophobia you will be given the option to stop the testing at any time if you wish to do so.

_____

Initials

If you desire to stop the test at any time you will be allowed to do so. Also, the investigators will be assessing your vital signs to determine if it is safe to continue with the testing.

You may become fatigued, light-headed, or feel discomfort from being hungry. You may discontinue testing at any time if this should occur. Light snacks and beverages will be available to you to minimize this risk.

Another risk involves the loss of time. The investigators will attempt to expedite the testing procedures to the best of their abilities. To minimize the risk due to time, all equipment will be set-up in advance and multiple personnel will be available to help so the testing can move as quickly as possible.

The researchers will try to prevent any problem that could happen because of this research. You should let the researchers know at once if there is a problem and they will help you. However, TWU does not provide medical services or financial assistance for injuries that might happen because you are taking part in this research.

Participation and Benefits

Your involvement in this study is completely voluntary and you may withdraw from the study at any time. Following the completion of the study you will receive the following analysis: body weight, bone health, resting metabolic rate, oxygen output during exercise, and blood concentrations of two hormones. If you would like to know the results of this study we will email them to you.*

Questions Regarding the Study

You will be given a copy of this signed and dated consent form to keep. If you have any questions about the research study you should ask the researchers; their phone numbers are at the top of this form. If you have questions about your right as a participant in this research or the way this study has been conducted, you may contact the Texas Woman's University Office of Research at 713-794-2480 or via email at IRB@twu.edu

_____ _____

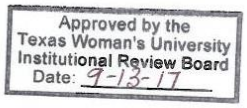
Signature of Participant Date

_____

Printed Name of Participant

*If you would like to know the results of this study please write your email address below:

Email:_____



APPENDIX G

Data Collection Sheet

Data Collection Sheet – Impact of Leptin and Ghrelin on Body Composition, Metabolic Rate, and Bone Density in Competitive Female Runners and Inactive Females

Subject ID _____ **Subject DOB and age: _____**

Date_____ **Ethnicity/Race:_____**

Anthropometrics

Height (in) #1: _____	Height (in) #2: _____	Height (in) #3: _____	Height (in) Mean: _____
Weight (lb) #1: _____	Weight (lb) #2: _____	Weight (lb) #3: _____	Weight (lb) Mean: _____
Waist circ (cm) #1: _____	Waist circ (cm) #2: _____	Waist circ (cm) #3: _____	Waist circ (cm) Mean: _____
Hip circ (cm) #1: _____	Hip circ (cm) #2: _____	Hip circ (cm) #3: _____	Hip circ (cm) Mean: _____

Treadmill Test

Running Time: _____	Distance: _____	Avg. MPH: _____
Avg. VO2: _____	RER: _____	Time: _____

BODPOD

Weight (lb): _____	%Body Fat: _____	%Lean Mass: _____
Weight from Fat (lb): _____	Weight Lean Mass (lb): _____	

Resting Metabolic Rate: _____

APPENDIX H

24-Hour Dietary Recall

24 Hour Dietary Recall

Instructions

A 24-hour recall involves asking subjects to recall and describe all the food and beverages consumed over the previous 24 hours, from waking to sleeping. The recall interview typically takes 20-30 minutes to complete.

The record should include ALL food and beverages consumed including meals, snacks, drinks, "nibbles", sweets, etc.

Main points:

1. Approximate time of eating or drinking
2. Quantity or amount eaten (e.g. 2 tablespoons, 1 cup, 1 slice, etc.)
3. Type of food (e.g. orange juice, dark chocolate, yellow squash)
4. Additional ingredients during preparation and cooking should be included (e.g. oil, sugar)

To ensure accuracy the investigator should probe for additional foods and beverages. For example, asking what types of beverages were consumed at breakfast, between breakfast and lunch, at lunch, etc. Asking "Do you have anything else to eat and/or drink right before bed or once you are in bed?" is also advised.

24 Hour Dietary Recall

Subject ID _____ Date _____ Gender _____

Time	Amount	Description of Food or Beverage

APPENDIX I

Visual Analog Scale (VAS)

Visual Analogue Scale Questionnaire

Subject ID _____ Date _____

Intructions: Draw a line indicating how you feel at the moment regarding hunger and satiety.

How hungry do you feel?

I am not hungry at all _____ I have never been more hungry

How satisfied do you feel?

I am completely empty _____ I cannot eat another bite

How full do you feel?

Not at all full _____ Totally full

How much do you think you can eat?

Nothing at all _____ A lot

How pleasant would you find eating another mouthful of this food?

Very unpleasant _____ Very pleasant

Would you like to eat something sweet?

Yes, very much _____ No, not at all

Would you like to eat something fatty?

Yes, very much _____ No, not at all

Would you like to eat something savoury?

Yes, very much _____ No, not at all

Reference: Flint **2000** *Int J Obes and Metab Dis*

APPENDIX J

Exercise Dependence Scale-21

Exercise Dependence Scale-21

Hausenblas & Symons Downs (2002)

Instructions. Using the scale provided below, please complete the following questions as honestly as possible. The questions refer to current exercise beliefs and behaviors that have occurred in the past 3 months. Please place your answer in the blank space provided after each statement.

1	2	3	4	5	6
Never					Always

1. I exercise to avoid feeling irritable._____
2. I exercise despite recurring physical problems._____
3. I continually increase my exercise intensity to achieve the desired effects/benefits._____
4. I am unable to reduce how long I exercise._____
5. I would rather exercise than spend time with family/friends._____
6. I spend a lot of time exercising._____
7. I exercise longer than I intend._____
8. I exercise to avoid feeling anxious._____
9. I exercise when injured._____
10. I continually increase my exercise frequency to achieve the desired effects/benefits._____
11. I am unable to reduce how often I exercise._____
12. I think about exercise when I should be concentrating on school/work._____
13. I spend most of my free time exercising._____
14. I exercise longer than I expect._____
15. I exercise to avoid feeling tense._____
16. I exercise despite persistent physical problems._____
17. I continually increase my exercise duration to achieve the desired effects/benefits._____
18. I am unable to reduce how intense I exercise._____
19. I choose to exercise so that I can get out of spending time with family/friends._____
20. A great deal of my time is spent exercising._____
21. I exercise longer than I plan._____

APPENDIX K

Blood Collection, Separation, and Storage Procedures

Blood Collection, Separation, and Storage Procedures

Impact of leptin and ghrelin on body composition, metabolic rate, and bone density in competitive female runners and inactive females: A pilot study

A. Perform venipuncture

1. Place subject in seated position and perform venipuncture using 21G or 23G needle. Follow standard safety precaution procedures. Place used needles in sharps container.
2. Fill one pre-labeled BD P800 tube with 7mL of blood, then fill one pre-labeled EDTA vacutainer with 7mL of blood. The tubes should have subject ID, date, and time of blood collection labeled on the side.
3. Mix both tubes gently. Place on ice for 2 hours.

B. Separate whole blood from serum

4. Turn centrifuge on (back of machine).
5. Place EDTA and P800 tubes filled with blood in centrifuge directly across from each other to balance.
6. Increase speed using arrow to 4 (4000rpm).
7. Increase timer to 15 minutes. Press start.
8. Spin blood. When centrifuge stops take tubes out and place in rack.

C. Aliquot serum

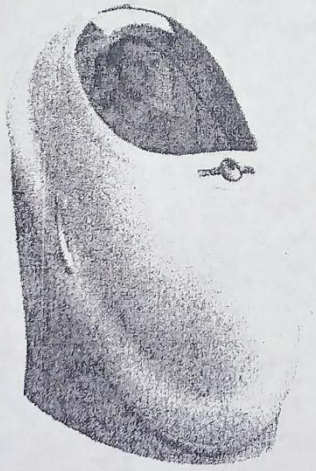
9. Using transfer pipettes (clear, disposable – found in white box on benchtop), take only the serum off the top of the tubes. Add serum to corresponding microcentrifuge tubes that are pre-labeled with subject ID, date, and type of tube (EDTA or P800). Use only one transfer pipette for each blood tube. Discard used transfer pipettes and whole blood tubes in sharps container.
10. When finished filling microcentrifuge tubes, place in white cardboard boxes located in -70 degree C freezer. Make sure EDTA tubes go in EDTA cardboard box; P800 tubes go in P800 cardboard boxes. The serum tubes should remain frozen until analyzed.

APPENDIX L

Attire for BOD POD Examples

BODPOD Clothing

BODPOD Measurement



APPENDIX M

Consent for a Second Horizon W. Duel Energy X-Ray Scan Absorptiometry (DXA) Scan

TWU
TEXAS WOMAN'S UNIVERSITY
DENTON DALLAS HOUSTON

Consent for a Second Horizon W Dual-energy X-ray Absorptiometry (DXA) Scan

Texas Woman's University – Houston

Name of Study Protocol: _____

Primary Investigator: _____

The image quality of your whole body scan is not adequate for reporting your body composition or bone density results. I see that from your scan that (fill in the problem observed) _____. With your permission, I would like to repeat the scan to improve the image quality. The amount of radiation from this scan is about 1 millirem (0.01 mGy/h), or the amount you would receive for 2 hours on a coast-to-coast airplane flight. This scan is voluntary and there is no penalty for refusing.

By signing this document, you have provided consent for me to perform a second scan.

_____ _____

Participant Signature Date

Participant ID _____

_____ _____

Operator Signature Date