Lipolytic, Hormonal, and Muscle Quality Differences of Female Endurance Athletes with Higher vs Lower Body Fat

Tristan J. Ragland
LIPOLYTIC, HORMONAL, AND MUSCLE QUALITY DIFFERENCES OF FEMALE ENDURANCE ATHLETES WITH HIGHER VS LOWER BODY FAT

By

TRISTAN JOSEPH RAGLAND

A Dissertation submitted to the Department of Nutrition and Integrative Physiology in partial fulfillment of the requirements for the degree of Doctor of Philosophy

2021
Tristan Ragland defended this dissertation on June 7, 2021.
The members of the supervisory committee were:

Michael J. Ormsbee
Professor Directing Dissertation

Diana Williams
University Representative

Robert C. Hickner
Doctoral Committee Member

Jeong-Su Kim
Doctoral Committee Member

The Graduate School has verified and approved the above-named committee members and certifies that the dissertation has been approved in accordance with university requirements.
ACKNOWLEDGMENTS

“It takes a village to raise a child” and for the last five years my education, this project, and my growth as a researcher and academic, have been my baby. So many people have play minor and major roles in assisting and lifting me to this major accomplishment and none of this would have been possible without them.

First, Ashley, and my girls, Paidyn and Evarie, thank you for being my anchor in the storm and my light in the darkness. I would not have been able to push through the growing pains of this experience without your constant love and support. Also, thank you Mom, Dad, LaDuska, and Nathan who have always been there to support me and encourage me in all my educational experiences, especially those early years when it was not always the easiest or the most fun.

Next, Dr. Michael J. Ormsbee I give you my deepest gratitude for all the time, effort, and council you have given me. We have shared quite a ride through IRB approvals and withdrawals, mobile metabolic cart trouble shooting, and finally a pandemic! Thank you for your patience, advice, and “fire lighting” to help me accomplish this goal.

To my committee, Dr. Robert C. Hickner thank you for all your help and support in helping me learn the microdialysis technique. I could not have accomplished this without your willingness to share your experience and resources. Dr. Jeong-Su Kim thank you for the use of your lab, and always having an open door if I had any questions in regard to research or teaching. I have thoroughly enjoyed learning from you on a regular basis. Dr. Diana Williams thank you for helping me grow as a researcher by asking questions outside my normal wheelhouse. Your input and inquiry have helped me see obesity and its related issues on a much grander scale.

And last by not least, the Institute of Sports Science and Medicine team thank you for all the help and support during my education and especially my project. Dr. Brandon D. Willingham for being a friend, a sounding board, and for keeping me just sane enough to accomplish this goal. Shiloah Fuller for always making the lab a fun and lighthearted place. Haylee Colannino for all your help in my ever-changing project. Casey Greenwalt and Lillie Renteria for always being willing to help, even at the last minute! You are all amazing! Thank you again for helping me grow as a person.
TABLE OF CONTENTS

LIST OF FIGURES ........................................................................................................... vii

LIST OF TABLES ............................................................................................................. viii

ABSTRACT ........................................................................................................................ ix

CHAPTER 1 INTRODUCTION ....................................................................................... 1

  Specific Aims and Hypotheses ..................................................................................... 4

CHAPTER 2 REVIEW OF LITERATURE ........................................................................ 6

  Introduction .................................................................................................................. 6

  Adipose Physiology ...................................................................................................... 8

    Lipolysis .................................................................................................................... 8

    Catecholamines ....................................................................................................... 8

    Insulin .................................................................................................................... 11

    Growth Hormone ................................................................................................. 13

  Microdialysis ............................................................................................................. 14

  Muscle Quality ......................................................................................................... 17

  Ultrasound .............................................................................................................. 20

  Impact of Acute Exercise ......................................................................................... 21

  Impact of Endurance Training ................................................................................... 22

  Conclusion ................................................................................................................. 24

CHAPTER 3 METHODOLOGY ....................................................................................... 26

  Experimental Design and Methodology ................................................................. 26

    Participants ........................................................................................................... 26

    Inclusion Criteria ................................................................................................. 26

    Exclusion Criteria ............................................................................................... 27

    Sample Size and Attrition .................................................................................. 27
<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>COVID-19 Safety Procedures</td>
<td>28</td>
</tr>
<tr>
<td>Participant Recruitment, Retention, and Compliance</td>
<td>29</td>
</tr>
<tr>
<td>Data Collection</td>
<td>30</td>
</tr>
<tr>
<td>Testing Day 1</td>
<td>30</td>
</tr>
<tr>
<td>Anthropometrics</td>
<td>30</td>
</tr>
<tr>
<td>Fitness Testing</td>
<td>31</td>
</tr>
<tr>
<td>Functional Muscle Quality Testing</td>
<td>31</td>
</tr>
<tr>
<td>Compositional Muscle Quality Testing</td>
<td>32</td>
</tr>
<tr>
<td>7-Day Lifestyle Measurements</td>
<td>33</td>
</tr>
<tr>
<td>Testing Day 2</td>
<td>33</td>
</tr>
<tr>
<td>Lipolytic and Metabolic Testing</td>
<td>33</td>
</tr>
<tr>
<td>Microdialysis Ethanol Outflow/Inflow Ratio</td>
<td>35</td>
</tr>
<tr>
<td>Circulating Hormone Response to Exercise</td>
<td>35</td>
</tr>
<tr>
<td>Statistical Analyses and Interpretation of Results</td>
<td>36</td>
</tr>
<tr>
<td><strong>CHAPTER 4 RESULTS</strong></td>
<td>38</td>
</tr>
<tr>
<td>Participant Characteristics</td>
<td>38</td>
</tr>
<tr>
<td>Lipolytic Response to Exercise</td>
<td>39</td>
</tr>
<tr>
<td>Interstitial Glycerol</td>
<td>39</td>
</tr>
<tr>
<td>Ethanol Concentrations</td>
<td>40</td>
</tr>
<tr>
<td>Hormone Concentrations</td>
<td>41</td>
</tr>
<tr>
<td>Insulin</td>
<td>41</td>
</tr>
<tr>
<td>Growth Hormone</td>
<td>41</td>
</tr>
<tr>
<td>Muscle Quality</td>
<td>42</td>
</tr>
<tr>
<td>Compositional Muscle Quality</td>
<td>42</td>
</tr>
<tr>
<td>Functional Muscle Quality</td>
<td>42</td>
</tr>
</tbody>
</table>
Health and Lifestyle Markers.................................................................................................................. 43
Blood Chemistry ......................................................................................................................................... 43
Exercise Logs ............................................................................................................................................ 43
Activity and Sleep Data.............................................................................................................................. 44
Food Logs................................................................................................................................................ 45

CHAPTER 5 DISCUSSION.......................................................................................................................... 46
Lipolysis .................................................................................................................................................... 46
Hormone Concentrations .............................................................................................................................. 48
Muscle Quality ........................................................................................................................................... 49
Health and Lifestyle Parameters ................................................................................................................ 50
Limitations................................................................................................................................................ 52
Future Directions...................................................................................................................................... 53
Conclusion and Practical Application ......................................................................................................... 54

APPENDIX A IRB APPROVAL .................................................................................................................. 55
APPENDIX B INFORMED CONSENT ....................................................................................................... 57
APPENDIX C EXERCISE LOG ...................................................................................................................... 63
APPENDIX D FOOD LOGS .......................................................................................................................... 64
APPENDIX E RPE SCALE ........................................................................................................................... 65
REFERENCES ............................................................................................................................................... 66
BIOGRAPHICAL SKETCH ........................................................................................................................... 84
LIST OF FIGURES

Figure 1: Lipolytic Cascade .......................................................... 9
Figure 2: Insulin Cascade and Lipolysis ................................... 11
Figure 3: Trained vs Sedentary Lipolytic Response to Exercise ....... 15
Figure 4: Glycemic Effect on Lipolysis during Exercise ................. 16
Figure 5: Lean vs Obese Lipolytic Response to Resistance Training .... 17
Figure 6: Strength to Mass Ratio .................................................. 19
Figure 7: Study Design ................................................................. 26
Figure 8: Testing Day 1 ................................................................. 30
Figure 9: Microdialysis and Metabolic Testing Day Timeline ............. 34
Figure 10: Lipolytic Response to Exercise ........................................ 39
Figure 11: Ethanol Outflow:Inflow Ratio ........................................ 40
Figure 12: Insulin Concentrations ................................................. 41
Figure 13: Growth Hormone ......................................................... 41
Figure 14: Functional Muscle Quality .......................................... 42
Figure 15: Compositional Muscle Quality ...................................... 42
LIST OF TABLES

Table 1: Participant Descriptive ................................................................. 38
Table 2: Blood Chemistry ...................................................................... 43
Table 3: 7-Day Exercise Data ................................................................. 44
Table 4: Activity and Sleep Data ............................................................. 44
Table 5: 7-Day Food Log Data ............................................................... 45
ABSTRACT

Introduction
The popularity of endurance events has grown recently with an increased number of participants who have an elevated body fat percentage. The influence of chronic endurance training on adipose tissue physiology in these recreational athletes is unknown. The purpose of this investigation was to determine the effect of chronic endurance training on lipolytic rate and measurements of muscle quality (MQ) between two groups of female endurance athletes with two different levels of body fatness.

Methods
Female endurance athletes (N=14) with >8 yrs of training experience were divided based on body fat % into lower body fat (LBF; N=5, age=32.1±8.2yrs, BF%=19.4±4.7%, BMI=21.8±1.7kg/m2) or higher body fat (HBF; N=9, age=36.1±8.3 yrs, BF%=31.1±3.1%, BMI=23.9±3.2 kg/m2) groups based upon a BF cut-off of the 30th percentile. Two visits were completed: 1) anthropometrics, BF%, fitness, strength, and MQ testing. US histogram analysis and isokinetic force production of the rectus femoris were used to quantify MQ (IMF% and N-m/cm2), and 2) SCAAT lipolysis was measured 1 hr before, during cycling at ~70% VO2peak for 45 min, and after exercise for 2h.

Results
SCAAT lipolysis was not significantly different pre-exercise or not post-exercise (p > 0.05). However, there was a significant difference during exercise F(1,12) =15.01, p=.002, η2=.556 (LBF=3090.6±1394.1μM, HBF=1003.2±654.3μM) between groups. There were no significant differences between groups in insulin or growth hormone (p > 0.05). There were no significant differences between groups in functional or compositional muscle quality (p > 0.05).

Conclusion
Chronic endurance exercise training of recreational female athletes appears to alleviate the expected decrements in muscle quality associated with excess adiposity among individuals in the EBF group; however, here remains a lower resting and exercise-induced lipolysis in the HBF than LBF group. Further investigations should consider possible mechanisms or lifestyle factors contributing to these observed differences.
CHAPTER 1
INTRODUCTION

Obesity is a major health concern in the United States. An estimated 33% of the adult population is currently classified as obese, and this number is estimated to rise to 50% of adults by the year 2030\(^1\). Obesity is associated with comorbidities such as the metabolic syndrome, prediabetes, and type 2 diabetes mellitus\(^2\). It is reported that obesity and its related comorbidities cost the United States approximately $150 billion each year\(^3\).

Over-fatness is more than just an excessive accumulation of weight, but rather a complex condition characterized by an accumulation of adiposity resulting in physiological differences compared to individuals with normal/healthy body composition. Excess adiposity is certainly associated with individuals who are classified as overweight and obese, but also includes an increasing number of normal-weight individuals as well\(^4,5\). Arner et. al.\(^6\) have demonstrated perturbations to lipolysis (high basal/resting and low stimulated/exercise-induced rates) in adipocytes is linked to future weight gain. Indeed, the low stimulated lipolytic response to catecholamines during exercise has been demonstrated in obese populations in a number of research studies\(^7-12\). Likewise, obesity is associated with decrements in relative aerobic capacity, fuel utilization\(^13\), and muscle quality\(^14\). Multiple studies have also documented that individuals with elevated adiposity have a higher prevalence of insulin resistance\(^4,5,15-19\). Healthy normal weight individuals with an elevated body fat percentage, also termed normal weight obese, have normal fasted blood glucose concentrations, accompanied by elevated fasted insulin levels when compared to healthy weight individuals with a low body fat percent\(^5,11,20\).

Insulin resistance is linked to many physiological changes that alter the body’s ability to respond to common external stimuli, such as exercise. Insulin resistance is strongly linked to increased lipid deposition in skeletal muscle\(^21\). Ectopic fat storage, in the form of intramuscular fat accumulation, decreases the overall quality of the muscle making it less efficient and less metabolically flexible\(^22\) and is prevalent even in young obese populations\(^23-26\). However, this physiological response does not always hold true. Endurance trained athletes store lipids intramuscularly without the presence of
insulin resistance\textsuperscript{23,27–30}. Elite runners have been shown to maintain a normal insulin response to a glucose load despite having 50% more intramuscular triglycerides than a sedentary obese group\textsuperscript{29}.

Likewise, some epidemiological studies\textsuperscript{31} have reported the existence of individuals with apparently healthy physiology despite excess adiposity. The lack of additional physiological disturbances, such as markers of metabolic syndrome and cardiometabolic disturbances, in some obese individuals has led to the inference of a phenotype termed metabolically healthy obesity\textsuperscript{32–36}. Yet, as it is well documented, excess adiposity can lead to disturbances in physiology that are not readily measured for the diagnosis of metabolic syndrome or type 2 diabetes. The physiological disturbances in adipocyte lipolysis\textsuperscript{11} and altered substrate utilization\textsuperscript{6–12} at rest and during exercise, alterations in circulating insulin and growth hormone concentrations as well as decrements in muscle quality\textsuperscript{23–26}, lead many to believe that metabolically healthy obesity is mostly a transitory state\textsuperscript{2,37}. Particularly, in individual living a sedentary lifestyle\textsuperscript{38}. Recently, it has been shown that only 26% of men and 19% of women are meeting the federal recommendations for physical activity\textsuperscript{39}. Similarly, even healthy weight individuals, based on body mass index, can display excess adiposity as well as the cardiometabolic abnormalities usually associated with obese individuals\textsuperscript{40}. This condition is frequently referred to as normal weight obesity\textsuperscript{34,40–43}.

Physical activity appears to attenuate some of the detrimental effects of excess adiposity. It has been demonstrated that having a cardiorespiratory fitness level $>$28 ml/kg/min reduces the risk of all-cause and cardiovascular disease mortality despite excess adiposity\textsuperscript{34,44,45}. Exercise has been shown to be an effective intervention to improve health in a variety of populations, including those with obesity\textsuperscript{2,46–49}. As such, the American College of Sports Medicine recommends everyone engage in 150 minutes of moderate or 75 minutes of high intensity physical activity on a weekly basis\textsuperscript{50}.

Despite the low prevalence of those engaging in the recommended amount of regular physical activity, the popularity of endurance events (i.e. marathons, etc.) has increased approximately 20 fold from the 1970s to present with the anthropometrics of participants changing over this timeframe as well\textsuperscript{51,52}. Accordingly, many endurance races now include divisions for heavier weight male and female runners\textsuperscript{52}. It was
reported that overweight (according to BMI) runners comprised approximately 15% and 31% and obese runners 31% and 33% of the female and male participants, respectively. Hence, many recreational endurance athletes are overweight despite their presumed high level of activity. On one hand, these data are positive as regular exercise reduces the risk of cardiovascular disease and all-cause mortality in overweight and obese populations\textsuperscript{53,54}. On the other hand, it remains to be determined if this level of training eliminates health disparities and alters physiological mechanisms, such as differences in lipolysis, in this population.

Twelve weeks of endurance training has been shown to improve body composition in obese and overweight individuals\textsuperscript{46}. De Glisezinski et. al.\textsuperscript{55} also demonstrated endurance training helps balance the anti- and pro-lipolytic mechanisms within subcutaneous fat. Similarly, Richterova et. al.\textsuperscript{48} has also shown 12 weeks of endurance training increases exercise-induced lipolysis in obese women. Endurance training has also been shown to effectively improve metabolic health and improve cardiovascular fitness\textsuperscript{2}. Interestingly, van Loon et. al. has shown that endurance trained athletes have as high or higher intramuscular triglyceride storage as type 2 diabetic and sedentary overweight individuals without any sign of insulin resistance that usually accompanies these conditions\textsuperscript{20}. These data highlight the metabolic modifying properties of chronic training in muscle tissue. However, it is unclear if these modifications also exist in a population of chronically trained normal weight obese females regularly competing in endurance events.

A lot of work has been done to determine the differences between lean and obese sedentary populations\textsuperscript{55–68}. Likewise, the beneficial effects of endurance training has been shown in a variety of obese cohorts\textsuperscript{46,56,69–78}. \textit{However, it is unknown if females with higher body fat who exercise regularly have disrupted fat metabolism, circulating insulin and growth hormone levels, as well as muscle quality compared to their female counterparts with lower body fat of a similar training history.} This investigation determined if differences in these physiological conditions are possibly contributing to continued retention and/or accumulation of excess adiposity in these recreational athletes. Adding to the breadth of research in lifestyle and therapeutic interventions but aimed directly at those meeting or exceeding the physical activity
guidelines while maintaining a potentially detrimental amount of adiposity despite their physical activity. It is imperative that an investigation designed to determine the differences in fat metabolism and muscle quality in recreationally active endurance trained females be undertaken to understand the potential mechanisms leading to differences in body composition and health markers in those exceeding the physical activity guidelines for Americans. Therefore, the purpose of this investigation was to determine the differences between female endurance athletes of higher and lower body fat, with similar years of training experience and frequency, on markers of: lipolytic response to exercise, insulin and growth hormone concentrations around an acute bout of exercise, as well as functional and compositional muscle quality. Our central hypothesis was that female endurance athletes with a higher body fat percentage would have significant differences in markers of fat metabolism, hormone levels, and muscle quality compared to female endurance athletes with lower body fat despite an equivalent training history. Thus, being regularly active may increase heart health, but may not produce some of the other expected adaptations to exercise.

Specific Aims and Hypotheses

We tested our hypothesis with the following specific aims:

Aim 1: To determine if resting and exercise stimulated lipolysis in the subcutaneous abdominal adipose tissue was different between female endurance athletes (18 – 45 years) with a higher verse a lower body fat percentage.

**Hypothesis:** Female endurance athletes with higher body fat have an increased resting lipolytic rate and a decreased physical activity stimulated lipolytic rate compared to the female endurance athletes with lower body fat.

To accomplish Aim 1, lipolysis was measured in subcutaneous abdominal adipose tissue via microdialysis. Two probes were perfused with a saline/ethanol solution to measure resting and exercise stimulated lipolysis. Measurements were performed on a single day for 60 minutes before, ~45 minutes during, and for 120 minutes after exercise. The exercise stimulus was a 45-minute ride on a
cycle ergometer at approximately ~2.5 watts/kg of lean body mass (~70% of estimated VO\textsubscript{2peak}). Dialysate was collected before exercise, every 15 minutes for 60 minutes, immediately pre- and post-exercise, and every 15 minutes for 120 minutes post-exercise.

**Aim 2:** To determine if circulating insulin and growth hormone levels, at rest and around an exercise bout, were different between female endurance athletes with higher body fat verse female endurance athletes with lower body fat.

*Hypothesis:* Female endurance athletes with higher body fat have higher resting insulin levels and lower post-exercise growth hormone levels compared to the female endurance athletes with lower body fat.

To accomplish Aim 2, a total of three blood draws were collected, during the microdialysis testing day. Collections were collected pre-exercise, immediately post-exercise, and at 120 minutes post-exercise for the analysis of insulin and growth hormone concentrations. Serum hormones were compared between groups.

**Aim 3:** To determine if compositional and functional muscle quality of the upper leg was different between female endurance athletes with higher body fat verse female endurance athletes with lower body fat percentage.

*Hypothesis:* Female endurance athletes with higher body fat have increased intramuscular fat percentage and a lower functional muscle quality compared to female endurance athletes with lower body fat.

To accomplish Aim 3, intramuscular fat percentage of the rectus femoris was assessed via analysis of ultrasonography echo intensity and related calculations\textsuperscript{79}. Ultrasonography cross-sectional measurement of the rectus femoris was compared to the extension force collected form the isokinetic strength test (FMQ = N-m/cm\textsuperscript{2}).
CHAPTER 2
REVIEW OF LITERATURE

Introduction

Obesity affects ~33% of the United States adult population and is estimated to increase to 50% by the year 2030\(^1\). Some experts maintain this number is severely underestimated as BMI does not consider body composition. Gujral et. al. demonstrated individuals with excess fat and normal body weight prevalence is as high as 40% in some populations\(^80\). The most common form of diagnosis for obesity is the BMI scale (18.5 to 24.5 normal/healthy, 25 – 29.9 overweight, and \(\geq\) 30 obese\(^2,81\), but as stated previously has the potential to miss individuals who may be at a heightened risk of health complications. Obesity has a multifactorial etiology often simplified to an imbalance of energy intake and expenditure. However, it has also been reported that when compared to healthy weight individuals, obese populations have physiological anomalies such as reduced metabolic rate, fat oxidation, exercise induced lipolytic rate, and metabolic flexibility\(^82,83\). Insulin resistance is a prevalent condition associated with obesity and is marked by abnormally elevated insulin concentrations to maintain proper metabolic homeostasis\(^84\).

Absolute measurements of resting metabolic rate in obese and non-obese people are not different from each other\(^16\). It is generally concluded that if a difference in RMR is evident between obese and non-obese individuals, it is not large enough to correlate or predict an increase in weight, even throughout a period of four years\(^16\). However, when RMR is adjusted for differences in fat-free mass, fat mass, and sex, obese individuals experience a significantly lower relative resting metabolic rate, which adds yet another risk factor for continued weight gain\(^85,86\).

Additionally, obese individuals have an impaired ability to mobilize fat in response to exercise and oxidize fat at rest and during exercise\(^78,87\). On a systemic level, respiratory exchange ratio (RER) can be used to estimate the type of macronutrient oxidized for energy transfer during rest or activity. RER is calculated by dividing the amount of ventilated carbon dioxide by the amount of ventilated oxygen (VCO\(_2/\)VO\(_2\)) during metabolic processes. The range of this scale runs from \(~0.7\) to \(~1.0\) with the low end of the continuum representing greater fat oxidation and the higher end
representing greater carbohydrate oxidation. At rest, the RER of obese people tends to be higher than normal weight individuals indicating a reduction in fat oxidation\textsuperscript{16}. This elevated RER could be caused, at least in part, by several key physiological changes evident in obese individuals.

Notably, obese individuals have a marked change in muscle fiber type distribution\textsuperscript{88}. Obese individuals tend to have a higher prevalence of type II muscle fibers possibly leading to the decreased oxidative capacity\textsuperscript{16}. Similar physiological changes associated with obesity have been observed in normal weight individuals participating in bed rest studies\textsuperscript{89}. These changes include decreased insulin sensitivity, increased low-grade systemic inflammation, and decreased lipolysis in response to circulating catecholamines during exercise\textsuperscript{6,11,90}. In addition, bed rest patients develop decreased muscle quality as demonstrated by reduced force production in skeletal muscle (per cross-sectional area) and increased intramuscular fat accumulation. Thus demonstrating decreased physical activity appears to be a driving force behind some of the abnormal physiological conditions in obese populations, and these dysfunctions seem to begin in healthy weight individuals when a sedentary lifestyle is adopted\textsuperscript{89}. Importantly, women tend to exercise less than recommended\textsuperscript{39,49,91,92}, potentially increasing the risk of obesity and poor muscle quality\textsuperscript{2,93}.

However, the popularity of endurance events (i.e., half and full marathons, etc.) has increased 20 fold, from the 1970s to now\textsuperscript{94}. Interestingly, the demographics of participants has also changed\textsuperscript{51}. From 1980 to 2002 the average race time to complete the marathon lengthened from \~3.5 hours to \~4.5 hours\textsuperscript{52}. Likewise, many endurance races include “Clydesdale” and “Athena” divisions for heavier weight male and female runners, respectively\textsuperscript{52}. As such, there has been an increase of overweight and obese participants in these races. For example, Vadeboncoeur et. al. showed that out of 250 runners, according to BMI, approximately 15\% of female and 31\% of male participants were classified as overweight, with an additional 31\% of female and 33\% of male runners classified as obese\textsuperscript{52}. Therefore, many recreational endurance athletes have higher body fat despite their high level of activity. On one hand, these data are positive as regular exercise reduces cardiovascular disease and all-cause mortality in overweight and obese populations\textsuperscript{34,45,53,54}. Yet, it is well documented in sedentary
obese individuals with excess adiposity have disturbances in adipocyte lipolysis\textsuperscript{11} and altered substrate utilization\textsuperscript{6–12} at rest and during exercise, and decreased muscle quality\textsuperscript{23–26}. However, it is unknown if individuals with higher body fat, who exercise regularly, have disrupted fat metabolism, circulating hormones, or muscle quality. No study has directly determined if differences exist in these physiological markers between recreational female athletes with a higher body fat and female athletes with a lower body fat when training status is equivalent.

**Adipose Physiology**

Adipose tissue is the primary storage site for lipids. One major function of adipose tissue is the release of free fatty acids and glycerol into circulation to provide these substrates for oxidation in working skeletal muscle\textsuperscript{95}. In populations with a healthy body composition, adipose tissue typically makes up $\leq 20\%$ of body mass in men and $\leq 30\%$ in women\textsuperscript{96}. However, in obese populations, adipose tissue can constitute greater than 30$\%$ body mass and in extreme cases has been shown to be more than 50$\%$ body mass\textsuperscript{96}. Further, adipose tissue is composed of many types of cells. Predominantly, adipocytes with smaller amounts within the stoma vascular fraction including preadipocytes, stem cells, and immune cells (macrophages, lymphocytes, pericytes, endothelial cells and fibroblasts). Catecholamines and insulin are the primary hormonal regulators of adipose tissue lipolysis, with growth hormone playing an important role in conjunction with exercise of sufficient intensity and duration\textsuperscript{11,56,96–101}.

**Lipolysis**

**Catecholamines**

Exercise has many physiological effects, one of the main effects on adipose tissue is the initiation of lipolysis. Lipolysis is the release of glycerol and free fatty acids into circulation for active muscles and other tissues\textsuperscript{95,102} to utilize as a substrate for the increased demand of ATP production. Exercise-induced lipolysis is driven mainly by the catecholamines (epinephrine and norepinephrine) which are released from the adrenal medulla in response to stress\textsuperscript{103}. Catecholamines bind to the $\beta$-adrenergic receptor in
white adipose tissue to stimulate the intracellular lipolytic cascade. When catecholamines bind to the G-protein coupled receptors they activate the $G_{\alpha s}$ subunit$^{96}$. The $G_{\alpha s}$ subunit activates adenylyl cyclase and intracellular levels of cAMP increase. cAMP phosphorylates, and thereby activates, of protein kinase A. Protein kinase A phosphorylates perilipin and hormone sensitive lipase increasing the lipases’ action at the lipid droplet resulting in increased lipolysis (Figure 1)$^{96}$. Many of the data and information gained in adipose physiology has come from both animal and human studies.

However, a direct comparison between species is not always appropriate as rodent and human fat cells have significantly different lipolytic rates when extracted and cultured$^{102}$. Human cells have a higher basal lipolytic response than rodents, however, when stimulated by pro-lipolytic agents the animal cells respond much more robustly than human cells$^{102}$. This is most likely due to the differences between the adrenergic receptors in human and rodent adipose tissue. Three types of beta-adrenergic receptors exist ($\beta$-ARs: $\beta_1$, $\beta_2$, and $\beta_3$). The $\beta$-receptors have differing amino acid composition and not all adipocytes express every type. Human adipose tissue is mostly composed of white adipocytes and contain $\beta_1$-AR and $\beta_2$-AR. The $\beta_3$-AR is expressed

**Figure 1: Lipolytic Cascade**
Diagram of the adipocyte intracellular pro-lipolytic cascade. With permission Morigny et. al., 2016
in humans but only in brown adipocytes. The main function of brown adipocytes, and the β3-receptor, is to activate non-shivering thermogenesis, which is thought to play a non-significant role in energy expenditure in humans. Rodent adipocytes express all three β-ARs in both white and brown adipocytes thus explaining, to some degree, the larger lipolytic response to catecholamines\textsuperscript{104}. Likewise, human adipocytes also contain α-2 adrenergic receptors (α-AR) which have a higher affinity for catecholamines but are anti-lipolytic on human adipocytes. α-ARs are not present in rodent adipocytes\textsuperscript{95}.

The dominant lipolytic pathway activated by exercise is the adrenergic signaling cascade\textsuperscript{105}. In obese populations, the lipolytic response to exercise in subcutaneous abdominal adipose tissue (SCAAT) is blunted due to a preferential inhibition of lipolysis\textsuperscript{105}. This inhibition is thought to be due to a greater amount or activation of the α-AR's or a downregulation of the β-AR\textsuperscript{12}. Using norepinephrine and isoprenaline (β-AR agonist) to stimulate adipocytes, Langin et. al.\textsuperscript{10} demonstrated a 40% lower lipolytic rate in cultured human adipocytes biopsied from obese subjects compared to non-obese subjects.

Similar results were observed by Ormsbee et. al.\textsuperscript{11} when obese and lean participants were exposed to an acute resistance-exercise bout. Microdialysis was used to quantify lipolysis via glycerol release. Three microdialysis probes were utilized in the study; one was perfused with a control solution consisting of 10mM saline/ethanol mixture, one with an α-AR antagonist (control solution + phentolamine), and one with a β-AR antagonist (control solution + propranolol). Resistance exercise was used to stimulate catecholamines release and the control probe used to measure the adipose response to this stimulus. The obese group showed no significant difference (p>0.05) to all three conditions at any time point, whereas the lean group showed resistance exercise to stimulate the expected elevation of glycerol in the control probe, an increased release of glycerol compared to the control in the phentolamine probe, and a decreased release of glycerol in the propranolol probe. These data demonstrate a blunting of lipolysis still prevalent in the obese cohort even when inhibiting the α-AR by phentolamine\textsuperscript{11}. 
Insulin

Insulin is the primary circulating lipolytic inhibitor\textsuperscript{96,99,106}. As insulin binds to its receptor two pathways of lipolysis inhibition are activated (Figure 2). The first is the phosphorylation of intracellular tyrosine on the insulin receptor. Tyrosine phosphorylation activates insulin receptor substrate-1 (IRS-1) which directly inhibits PKA phosphorylation, this action inhibits PKA's activation of HSL at the lipid droplet. The second action is the activation of phosphoinositol-3-kinase (PiP3) pathway. As PiP3 concentration increases in the cell, protein kinase B is activated and leading to the activation of phosphodiesterase 3B (PDE3B) which converts cAMP to 5'-AMP resulting in a decreased activation of HSL, thus blunting lipolysis\textsuperscript{96,107}. This is especially true in studies investigating the lipolytic effect of an acute bout of exercise in obese and insulin resistance populations\textsuperscript{10,108}, as these populations have higher levels of circulating insulin. Even at basal levels, studies have shown obese and type 2 diabetic people to have higher levels of circulating insulin. Likewise, it has been shown that a compensatory mechanism exists in overweight and obese individuals. Participants with excess adiposity with normal fasted blood glucose levels have significantly higher fasted insulin levels\textsuperscript{11,20}. This finding points to an altered mechanism in nutrient partitioning that would not be detected by a normal

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{insulin_diagram.png}
\caption{Insulin Cascade and Lipolysis}
\end{figure}

Diagram of insulin’s role in mediating the lipolytic cascade. IRS activates PDE, PDE degrades cAMP to 5'-AMP decreasing cAMP action and all the other downstream targets. IRS also has a direct inhibitory effect on PKA.

Nishino et. al. 2007
blood glucose or a glycosylated hemoglobin test. Both of which are prominently used for diagnosing issues with metabolism and glycemic control. High levels of circulating insulin might help explain the blunting of lipolysis present among obese populations during and after physical activity\textsuperscript{11}.

Interestingly, SCAAT lipolysis in the basal/resting state has been shown to be increased in the obese phenotype\textsuperscript{6,102,108,109} despite the elevated insulin levels. The increased basal rate of lipolysis is thought to be produced by hypertrophied adipocytes and has been shown to be linked to future weight gain in women\textsuperscript{6}. Insulin resistance has been shown to be associated with increased basal lipolysis independent of BMI or age\textsuperscript{110}. Following weight loss, obese people with the largest decrease in basal lipolysis had the greatest increase in insulin sensitivity\textsuperscript{110}. Potentially, high basal lipolysis in conjunction with low levels of fat oxidation leads to increased circulating fatty acid concentrations. These conditions lead to a melee of physiological complications where fat accumulation in the liver and muscle\textsuperscript{111} increase, further reducing insulin sensitivity in these metabolically important tissues. Insulin sensitivity has also been seen to transiently shift toward resistance in an acute flux of infused non-esterified fatty acids (NEFA)\textsuperscript{112}. The insulin resistant state becomes further complicated as higher levels of circulating NEFA and glycerol are used by the liver for gluconeogenesis exacerbating hyperglycemia\textsuperscript{113}. Low-grade systemic inflammation has also been shown to be a contributor to increased basal lipolysis and insulin resistance\textsuperscript{114}.

However, several studies have demonstrated exercise as a plausible mechanism to improve insulin sensitivity and decrease low-grade systemic inflammation which may alter basal lipolytic rates. Beneficial effects on insulin sensitivity has been seen in endurance training in normal and overweight sedentary\textsuperscript{84}, overweight and obese sedentary\textsuperscript{115}, and young healthy males\textsuperscript{116}. Resistance training has also shown improvements in insulin sensitivity in type 2 diabetics\textsuperscript{117–119}, pre-diabetics\textsuperscript{120}, and obese men\textsuperscript{105}. Likewise, high intensity interval training has demonstrated similar results in overweight and obese populations\textsuperscript{46,72,121}. 
Growth Hormone

Growth hormone is released from the anterior pituitary and can be produced in over 100 isoforms\textsuperscript{122–124}. Growth hormone has many metabolic effects, several of which are mediated by the increased synthesis of insulin-like growth factors from the liver\textsuperscript{123} and produce the growth effects generally attributed to the hormone. However, the focus of this review will be on the direct effects of growth hormone on adipose tissue and how this effect changes with increased stored fat within the adipose tissue and the body.

Growth hormone is well established as a prolipolytic hormone under fasted conditions. Under such conditions GH assists in switching the primary substrate of metabolism from carbohydrates to fat in an attempt to facilitate sparing of proteins\textsuperscript{125}. Interestingly, the same amount of growth hormone does not always have the same amplitude of lipolysis across different fat depots\textsuperscript{126}. Bredella et. al.\textsuperscript{127,128} have demonstrated that the administration of growth hormone, in young metabolically healthy obese men and women, decreased SCAAT, while having no effect on gluteal subcutaneous adipose tissue. Growth hormone’s effect on adipose tissue is through regulating fat metabolism and storage in the adipocyte. Some research has shown growth hormone to decrease the activity of lipoprotein lipase (LPL)\textsuperscript{129}, and increase the activity of HSL\textsuperscript{130,131}. Other research has demonstrated that growth hormone’s effects on lipolysis may not have a direct effect but are ascertained via enhancing the lipolytic actions of catecholamines in cultured adipocytes\textsuperscript{132,133}. Likewise, it is hard to delineate the effect growth hormone may have on an obese population in response to exercise training. Ormsbee et. al.\textsuperscript{11} demonstrated a reduction in serum growth hormone concentration in sedentary obese men, compared to sedentary lean men, in response to a single bout of resistance exercise. Interestingly, exogenous growth hormone administration has been shown to decrease SCAAT size\textsuperscript{127,128}. These data potentially demonstrate a beneficial effect of growth hormone on adipocyte lipolysis and a possible mechanism as to why obese individuals have a difficult time losing and maintaining weight. Endurance training\textsuperscript{115}, resistance training\textsuperscript{120,134}, and high intensity interval training\textsuperscript{135} have all demonstrated the ability to improve insulin sensitivity which has been shown to normalize growth hormone secretion\textsuperscript{136}. Considering these data, it is important to take into account serum growth hormone concentrations as some of the
variability in lipolysis and to try to elucidate the effect of chronic exercise on serum growth hormone levels in overweight/obese populations.

Microdialysis

Microdialysis is effective for the in situ study of adipose tissue and adipocyte function as it allows for a constant sampling of metabolites from the interstitial space. It may also be used to deliver pharmacological agents to the tissue to directly activate certain receptors on the adipocyte in vivo. Microdialysis is a tool used to determine the metabolism and blood flow of a local region of tissue (<1 cm³). Most commonly, microdialysis has been used in adipose or muscle tissue, however researchers also utilize this technique in tendons, skin, bone and even the brain in human studies. The typical microdialysis probe is a double cannula design developed by Urban Ungerstedt in the early 1980s. The probe is inserted into the tissue using a catheter type device consisting of a needle surrounded by a plastic guide. The needle and guide are inserted into the tissue and the needle is removed. The probe is inserted through the plastic guide and into the tissue. Once the probe is in place, the guide is removed, and the probe is secured to the insertion site. After securing the probe, the inlet tube is attached to a perfusion pump providing a continuous flow of perfusate into the tissue. The perfusate passing through the probe creates a perfusion system creating a gradient for the diffusion of metabolites across the probe membrane and carried out of the probe to a collection tube for analysis.

Microdialysis has been used in a multitude of studies to test lipolysis in SCAAT. Although not statistically significant, De Glisezinski et. al. showed healthy trained individuals' lipolytic response appears to be increased above healthy sedentary individuals by a decrease in the activation of the α-AR. Seven trained and fifteen sedentary men cycled at 50% VO₂max for 60 minutes. Two microdialysis probes, one with an α-AR antagonist (phentolamine) and one with a ringer solution (control) were used to determine the rate of lipolysis unencumbered by the influence of the inhibitory effects of the α-AR and under normal physiological conditions, respectively. Under normal physiological stimuli from exercise the untrained population tended to have a lower extracellular glycerol concentration (497 ± 60 vs. 605 ± 68 μmol/l; p>0.05)
compared to the trained population. The difference in lipolysis between the groups was normalized by the phentolamine probe (Figure 3).

However, the untrained participants had a significantly increased calculated AUC in extracellular glycerol concentration compared to the control probe (phentolamine: 15,638 ± 2,371 vs control: 11,025 ± 1,612; p<0.05), where the trained individuals had no difference between phentolamine and control. These data indicate endurance training likely modulates adipocyte responsiveness to catecholamines. The effect is seen in the reduction of the anti-lipolytic effects of the α-ARs. The study also demonstrates that healthy non-active persons have a lower response to exercise stimulated lipolysis than those regularly engaging in exercise.55

Recently, microdialysis was used to investigate the effects of differing glycemic index drinks (low, high, and non-glycemic control) on lipolysis in SCAAT in elite runners. It was determined that the glycemic index of the drinks had no effect on SCAAT lipolysis during exercise (Figure 4). In a study, comparing the response of lean and obese sedentary men to a bout of resistance exercise, Ormsbee et al. used microdialysis to determine lipolytic rate in response to exercise induced CATs as well as pharmaceutical perfusion into SCAAT.

Figure 3: Trained vs Sedentary Lipolytic Response to Exercise
The lipolytic response to an acute bout of cycling in trained and untrained men. Extracellular glycerol collected via microdialysis. Open circles phentolamine probe, closed circles control probe. *significant compared to pre- and post- resting levels.

With permission De Glisezinski et. al. 2001
To determine the actions of each of the adrenergic receptors’ roles in response to the exercise, three-microdialysis probes were inserted into the SCAAT and two probes contained pharmaceuticals to test activation of adrenergic receptors. One of the probes was perfused with phentolamine (α-AR antagonist), one with propranolol (β-AR antagonist), and one with ringer solution as a control probe. The researchers also demonstrated that resistance exercise was a potent stimulator (p<0.001) of catecholamine release in both cohorts and of lipolysis (p<0.05) in the lean cohort. Interestingly the obese cohort was unable to produce the same level of glycerol via lipolysis as the lean participants in response to the acute bout of resistance exercise. Each participant had three microdialysis probes inserted into the SCAAT.

The data show no change in glycerol levels between the three-microdialysis probes in the obese participants demonstrating a blunted response to exercise even when phentolamine (α-AR antagonist) was perfused into the SCAAT (Figure 5). Phentolamine is expected to maximally increase lipolysis, above the normal catecholamine stimulus created by exercise, as it inhibits the anti-lipolytic effect of the α-AR mechanism. These results support that obese individuals have a blunted lipolytic response to an exercise stimulus11.

![Figure 4: Glycemic Effect on Lipolysis during Exercise](image)

Interstitial glycerol concentration before, during, and after a moderate to high intensity run on a treadmill in elite male runners. PL=Placebo non-glycemic, G=Gatorade high-glycemic, UCAN low-glycemic.

With permission Baur et. al. 2017
Similarly, Allman et. al.\textsuperscript{142} used microdialysis to determine the effect of an acute bout of resistance exercise on lipolysis in resistance-trained women. They determined resistance exercise to be a potent stimulator of lipolysis (pre: 596.7 ± 82.8 μmol, mid: 832.2 ± 106.8 μmol, post: 961.4 ± 116.3 μmol; p<0.05) and the increase in lipolysis resulted in a 3-fold increase in fat oxidation rate (pre: 13.73 ± 3.87 g/h, post: 31.12 ± 5.24 g/h; p=0.006) compared to baseline measurements\textsuperscript{142}. As important as acute studies are in understanding exercise’s immediate effects on physiology, it is increasingly important to determine the effects of longitudinal training and the chronic effects of repeated acute stimuli on physiology and the potential adaptations these stimuli create.

**Muscle Quality**

The musculoskeletal system is a large contributor to many physiological mechanisms including glucose disposal and mechanical movement. On average, skeletal muscle makes up about 40% of body mass and 30% of RMR in healthy normal weight
adults. Skeletal muscle has the largest absolute capacity for glycogen storage, due to the sheer volume of skeletal muscle in the body, and accounts for ~80% of glucose disposal under insulin stimulated conditions. The ability to respond to insulin-stimulated glucose uptake has a direct correlation with muscle mass and is inversely correlated with fat mass.

Type 2 diabetes and insulin resistance is commonly known to impair skeletal muscle’s ability to transport glucose intracellularly in response to insulin’s signal. Under these conditions skeletal muscles start to display physiological dysfunction such as changes in composition seen as an increase in intramuscular fat accumulation (IMFA), and a decrease in size, capillarity, metabolic capacity, and force production. Specifically, Sucharita et. al. demonstrated IMFA to be positively and significantly correlated (r = 0.76, p<0.01) with HbA1c levels in normal and overweight individuals with prediabetes. Importantly, both an acute bout of resistance exercise and endurance exercise have been shown to improve insulin sensitivity for up 24 hours and ~48 hours following activity, respectively. Endurance and resistance training programs have also shown beneficial effects on insulin sensitivity. The understanding of muscle’s role in systemic homeostasis continues to develop. As such, it is imperative to determine the overall quality of skeletal muscle and its potential to alter glucose regulation in response to different training modalities.

Muscle quality is a term used to describe a muscle’s functional capacity and tissue composition when considering such variables as: metabolic efficiency, force production, and IMFA. Although muscle quality is multifactorial, it is generally considered to be the ratio between muscle strength and power per kilogram of muscle mass (N-m/kg). muscle quality is known to decrease in elderly populations and can lead to sarcopenia. Sarcopenia is defined as an age-related decrease in muscle mass and strength, resulting in decreased overall metabolic function. However, recent studies demonstrate that a decrease in strength occurs before a decrease in muscle mass. This suggest that a decrease in muscle strength is leading to decreased physical activity and is driving the decreased muscle size, overall health, and quality of life in older adults. For these reasons, it is important to try to determine exercise training’s role in maintaining and improving muscle quality.
Even with similar BMI’s, obese individuals with higher body fat percentage showed an accelerated decline in muscle quality compared to normal weight individuals \((p=0.021)\)\(^{152}\). The rate of decline in muscle quality is significantly faster \((p<0.001)\) in women compared to men with an average rate of decline per year being \(-0.025 \pm 0.003\) and \(-0.010 \pm 0.003\) Nm/cm\(^2\) respectively\(^{152}\). Indeed, despite the trend for obese people to have a greater absolute strength than their healthy weight counterparts, force production relative to body mass is impaired in this population\(^{157-159}\). In fact, differences in force production per kilogram of body mass differ by as much as 34.5% \((p<0.001)\) in obese compared to lean individuals at three different isokinetic speeds \((Figure 6)\)\(^{158}\).

Furthermore, this relationship remains even when controlling for body composition and comparing force production to lean mass where the obese participants had an average decrease of 6% compared to the lean group \((r=0.29-0.49, p<0.001)\)\(^{159}\). The disparity in functional muscle quality has been linked to several physiological differences in muscle tissue between obese and lean people including increased intramuscular fat accumulation, insulin resistance, pro-inflammatory cytokines, and decreased anti-inflammatory cytokines and myokines\(^{152,154}\). Therefore, it is imperative to determine if

**Figure 6: Strength to Mass Ratio**

Torque-velocity normalized to body mass. Open bars lean cohort, Filled bars obese cohort. The x-axis are different measures of isokinetic speed *** significantly different from lean \(p<0.001\).

With permission Maffiuletti et. al., 2007
muscle quality can be maintained and improved in obese women to help improve relative strength and potentially improve some of the underlying physiological impairments associated with low muscle quality.

**Ultrasound**

The most common ways to measure muscle quality include magnetic resonance imaging (MRI), computed tomography (CT), and ultrasound\(^{151,160}\). While CT and MRI scans are ideal for estimating muscle quality, however, CT and MRI are expensive and can be invasive. Thus, ultrasound is a technique that is not cost prohibitive and has been demonstrated to be a valid and reliable method for assessing muscle quality\(^{79,161,162}\). Additionally, ultrasound can be housed in more clinics and research facilities for a reduced cost and without the potential hazards of MRI and CT\(^{162}\). Ultrasound measurements of intramuscular fat accumulation via echo intensity are highly correlated to functional measures of muscle quality\(^{163}\). Echo intensity is independent and negatively correlated (\(r = -0.4, p<0.01\)) to muscle quality\(^{163}\) this correlation is increased when combined with BMI (\(r = 0.79, p<0.001\)) compared to BMI alone (\(r = 0.66, p<0.001\)) on measurements of muscle quality\(^{155}\), providing an excellent detection tool for muscle quality.

Ultrasound has also been shown to be a valid form of detecting and quantifying intramuscular fat accumulation. Young et. al.\(^{79}\) found a moderate to strong correlations between ultrasound and MRI in measurements of the rectus femoris and the correlation was made even stronger when controlling for subcutaneous fat thickness (uncorrected: \(r = 0.79\), corrected: \(r = 0.91\))\(^{79}\). Burton and Stock\(^{161}\), demonstrated the ultrasound equations produced by Young and colleagues\(^{79}\) have a high reliability (ICC: uncorrected \(r = 0.95\), corrected \(r = 0.98\), A.U.) for testing intramuscular fat accumulation in a healthy young population. Ultrasound may help to understand the functional outcome of obesity related changes in lipolysis, fat oxidation, and insulin sensitivity as a response to exercise. Recently, three weeks of HIIT showed no significant difference in echo intensity compared to pre-training measures\(^{164}\) and measurements of muscle quality via echo intensity have not been investigated around an endurance training intervention. The majority of work in this area has been performed with resistance exercise as the
main intervention, demonstrating resistance training has a favorable effect on muscle quality and has the ability to modify fat stores in the muscle\textsuperscript{14}. The next logical step is to determine if these results can translate to adipose tissue physiology during long term interventions.

**Impact of Acute Exercise**

Physical activity is the largest modifiable component of daily energy expenditure\textsuperscript{39}. Yet, participation in daily physical activity has decreased in both adults and children in recent years. Ng & Popkin\textsuperscript{165} has demonstrated a decline in MET hours/week of 235 in 1965 to 160 MET hours/week in 2009, and is estimated to continue to decrease to 126 MET hours/week by 2030. Inactivity plays a major role in the body’s ability to mobilize fat from adipocytes\textsuperscript{89}. Exercise has been shown to be an important driver to stimulate an increase in lipolysis and fat oxidation\textsuperscript{166}.

However, De Glisezinski et. al.\textsuperscript{55} demonstrated even healthy weight, but sedentary, individuals have a hindered catecholamine stimulated lipolytic response to endurance exercise compared to active healthy counterparts. Stich et. al.\textsuperscript{12} demonstrated obese populations to have a greater blunting of lipolysis due to a greater activation of the $\alpha$-AR and decreased activity of the $\beta$-AR activity. Moreover, Ormsbee et. al.\textsuperscript{11} also demonstrated obese sedentary men experience an even greater decrease in lipolysis to resistance training than healthy weight, sedentary, counterparts. These data indicate the importance for everyone, but especially obese individuals, to engage in physical activity regardless of weight. However, the specifics of the physical activity are still yet to be determined.

Exercise is viewed as a vital part of helping people improve their health and is recommended as part of a healthy lifestyle\textsuperscript{50}. The majority of research has been performed using endurance training and its effects on metabolic risk factors associated with obesity\textsuperscript{2,134,167–174}. However, there is a growing body of evidence that resistance training may have favorable effects on obesity and comorbidities\textsuperscript{2,169,172,175}. Most research supports moderate to high intensity exercise is needed for beneficial changes in health markers, as a dose-dependent response is apparent with exercise\textsuperscript{2}. Yet, the dosage of exercise is still a topic of much debate as some researchers disagree with
regular recommendations and state they are still too low for anyone trying to change and maintain a different weight phenotype\textsuperscript{176}.

**Impact of Endurance Training**

Endurance training has been shown to modulate SCAAT's intracellular mechanisms that control metabolism after 10 weeks of training\textsuperscript{116}. Riis et. al.\textsuperscript{116} showed, in healthy untrained men that after training adipose insulin sensitivity was improved (Adipo-IR: Pre 16.7 ± 12.8 to Post 11.4 ± 6.8; \( p=0.03 \)). In adipose tissue insulin sensitivity using the Adipo-insulin resistance index which is calculated by multiplying plasma insulin (pmol/L) concentration by plasma NEFA (mmol/L) concentration. 19 healthy sedentary young men were recruited and exercised three times per week for 40 minutes at an average intensity of \(~65\%\) maximum power output for 10 weeks\textsuperscript{116}. The focus of the study was to determine the effect of training on protein expression and mRNA abundance associated with lipid storage and breakdown via adipose tissue biopsy. The intervention demonstrated that training increased expression of the insulin receptor (+54\%, \( p=0.03 \)), GLUT4 (+17\%, \( p>0.05 \)), and hexokinase-II (+76\%, \( p=0.006 \))\textsuperscript{116}. Kreb’s cycle enzymes, such as succinate dehydrogenase (SDH), were also increased indicated by an increased expression of \textit{SDHA} gene (+70\% \( p=0.04 \)). These data demonstrate that endurance training improves adipose tissue glucose handling. However, the expressions of lipid droplet associated proteins, and G-coupled receptor proteins, normally involved in lipolysis, had no significant change in response to training\textsuperscript{116}. The lack of change in G-coupled receptor proteins and lipid droplet proteins could be explained due to participants being healthy young men. Therefore, these proteins were already at the physiological level to facilitate the changes in oxidative enzymes and other proteins shown to increase in this study. The study also reported the 10-week intervention to have a significant effect on increasing \( VO_{2\text{max}} \) (+20\%, +5.4 ± 11.2 ml/kg/min, \( p<0.001 \)) and decreasing whole body fat percentage (-1.93\%, \( p=0.01 \)) compared to the control group.

Henriksson et. al. demonstrated two months of submaximal (150 – 225 W) endurance training (ET) shifts muscle toward being more efficient in using fat as a fuel substrate in young (22 – 24 yrs.) healthy weight (61 – 76 kg) males\textsuperscript{177}. Investigators
determined a significant rise in SDH activity, via muscle biopsy, between the untrained and trained legs (3.8 vs. 5.2 μmol/g, p<0.05) of each participant. They also observed a shift toward greater fat oxidation during exercise with training (RQ: trained 0.91 vs. untrained 0.96, p<0.05) The authors concluded the shift toward fat oxidation trended to be an increased oxidative capacity with a correlation between fat utilization and SDH from training (r = 0.79, 0.05 < p < 0.1)177. Likewise, lipid depots within the muscle have been shown to decrease with endurance training167.

Duncan et. al.84 demonstrated that 6 months of walking significantly improved insulin sensitivity (pre: 2.54 ± 2.74, post: 4.41 ±3.30 μU/ml/min, p<0.005). Insulin sensitivity in this study was measured using an intravenous glucose tolerance test where a dextrose-saline solution (0.5 g dextrose/kg) was infused for 3 minutes, and 14 blood samples were taken over the next 3 hours. This test was performed pre- and post the 6-month walking intervention. Notably, this improved insulin sensitivity did not coincide with any change in BMI or weight loss84. These data indicate that exercise can modulate insulin signaling independent of body weight reduction, further supporting the use of endurance training for improving metabolic health in obese populations.

Similar results were observed by Houmard et. al.115 in a 6-month endurance exercise study. Insulin sensitivity was measured from the results of an intravenous glucose tolerance test115. The study cohort consisted of 154 sedentary overweight or obese individuals. The participants were randomly assigned to either a control group or one of three exercise groups: 1) low-volume moderate intensity (LVMI) (~12 miles/wk at 40 – 55% VO2max), 2) low-volume high intensity (LVHI) (~12 miles/wk at 65-80% VO2), or 3) high-volume high intensity (HVHI) (~20 miles/wk at 65 – 85% VO2). The average amount of time for each group to finish the exercise prescription was ~115 min/wk for the low-volume high intensity group, and ~170 min/wk for the other two groups115.

Fasting insulin concentrations significantly increased (pre: 7.6 ± 0.6, post: 8.7 ± 0.7 μU/ml; p<0.001) in the control group and decreased in all of the exercise groups (LVMI – pre: 11.3 ± 1.4, post: 8.1 ± 1.0 μU/ml; HVHI – pre: 8.9 ± 0.9, post: 8.0 ± 0.7 μU/ml, p<0.05). Likewise, insulin sensitivity significantly (p<0.001) improved in all of the training groups and significantly (p<0.05) declined in the control group. Interestingly, when comparing the magnitude of change between the exercise groups to the control
group, all had a significant improvement (p<0.001). However, the exercise programs that required ~170 min/wk resulted in an even greater improvement (LVMI = 88 ± 18.7%; LVHI = 37.6 ± 8.9%; HVHI = 82.7 ± 15.3%), and were significantly higher than the LVHI group (p<0.05)\textsuperscript{115}. These data indicate the potential relevance of a time/volume threshold that must be met to see such marked improvements in insulin sensitivity.

As discussed in the Microdialysis section previously, endurance training has also been shown to increase activity of the adipose tissue lipolytic cascade by increasing in the activation of β-AR and concurrently decreasing the anti-lipolytic activity in the adipocyte\textsuperscript{55}. This increase in lipolysis has been demonstrated by a number of studies\textsuperscript{8,47,48,73,166,178}. Particularly, Richterova et. al.\textsuperscript{48} showed ET increased lipolysis by decreasing the anti-lipolytic effect of the α-AR during 40 minutes of exercise in obese women (n=11, 39.2 ± 1.9 yrs., 86.4 ± 2.1 kg, 31.3 ± 0.6 BMI). The training consisted of 12 weeks, 5 days/wk of ET at 50% VO\textsubscript{2}peak for 40 minutes. Although, non-significant (p = 0.07) during exercise (AUC for 40 min of exercise), the AUC of extracellular glycerol for the entire testing day (40 minutes exercise and 50 minutes rest) was significantly higher compared to pre-training values. They also found that peak glycerol levels during exercise in the control probe before training were 61 ± 14 μmol/l and following training increased to 95 ± 14 μmol/l.

Martins et. al.\textsuperscript{46} demonstrated isocaloric high intensity interval training compared to moderate endurance training had similar effects on body composition with significant changes (p<0.05) between pre and post training. However, there was no significant difference between interventions on fitness measurements in an obese population after 12 weeks of training\textsuperscript{46}.

Conclusion

The condition of maintaining excess adiposity is multifactorial with some of the main complications arising from dysregulated lipolysis, low insulin sensitivity, and low muscle quality. It is crucial to gain a greater understanding of how this condition responds to chronic exercise training in female athletes. Endurance exercise is highly recommended as the best exercise modality for weight loss and improved health.
However, there has been no work investigating the effects of chronic endurance training in recreational female athletes with a higher body fat percentage compared to recreational female athletes with a lower body fat percentage. There is a lack of information on how lipolysis, circulating hormones, and muscle quality, compare between these two cohorts. With the increased popularity of endurance events in recent years, it is important to investigate if these physiological processes are different in this specific population. A greater understanding of these parameters could lead to additional insight into potential mechanisms that could be targeted by interventions to improve health and wellbeing. The purpose of this investigation was to determine the effect of chronic endurance training on lipolytic rate, hormone concentrations, and measurements of muscle quality of female endurance athletes with differing levels of body fat. This investigation increases the understanding of potential mechanisms associated with adverse health conditions linked to excess adiposity in those exceeding the physical activity guidelines for Americans.
CHAPTER 3
METHODOLOGY

Experimental Design and Methodology

The study consisted of two experimental days (Figure 7), one at the Institute of Sports Science and Medicine (ISSM) and one at the Sandels building at Florida State University. Participants arrived at the laboratory in a fasted state for laboratory testing days. All participants filled out a health history questionnaire\textsuperscript{179}, physical activity questionnaire\textsuperscript{180}, and signed the informed consent before any testing was performed.

Participants

Inclusion Criteria

Two groups of female participants were recruited for the study: 1) those with lower percent body fat (< 30% BF) and 2) those with higher percent body fat (>30% BF). Participants were between the ages of 18 and 45 years old and weight stable (± 2 kg) for the three months prior to enrollment\textsuperscript{48} Participants self-reported a history of regular endurance exercise on most days of the week for at least one hour and competed in endurance races for about two years. No exclusion was made for race or socioeconomic status. Participants were eumenorrheic and premenopausal.

<table>
<thead>
<tr>
<th>Informed consent and Initial testing</th>
<th>1 week Physical activity, food, and sleep tracking</th>
<th>Microdialysis and Exercise testing</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Day 1</strong></td>
<td></td>
<td><strong>Days 8 - 10</strong></td>
</tr>
<tr>
<td>Informed consent</td>
<td></td>
<td>Microdialysis</td>
</tr>
<tr>
<td>Bodex</td>
<td></td>
<td>Blood markers</td>
</tr>
<tr>
<td>Inclusion testing</td>
<td></td>
<td>RMR</td>
</tr>
<tr>
<td><em>(BMI, Body comp, Est. VO_{2peak})</em></td>
<td></td>
<td>Rest &amp; Post-Ex Fat Ox</td>
</tr>
<tr>
<td>Blood pressure</td>
<td></td>
<td>45 min @ 70% Est. VO_{2peak}</td>
</tr>
<tr>
<td>Ultrasound</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Study Packet</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Figure 7: Study Design**

Body Mass Index (BMI), Est. VO_{2peak} (Estimated highest oxygen consumption during test), RMR (Resting Metabolic Rate), Post-Ex Fat Ox (Post-exercise fat oxidation)
Exclusion Criteria

Participants who were not engaged in regular physical activity and endurance training for less than the last two years were excluded from the study. Participants with 1) resting blood pressure above 160 mmHg systolic or 100 mmHg diastolic; 2) type 1 or type 2 diabetes; 3) other medical problems in which exercise is contraindicated, such as chronic infections, were excluded. Diagnoses, signs, or symptoms of cardiovascular, respiratory, or musculoskeletal disease or injury that would interfere with completing this study were excluded. Participants did not have any musculoskeletal injuries in the six months prior to enrollment that would have prevented them from engaging in endurance training. Participants who were pregnant or lactating and had an irregular menstrual cycle. Smokers and those with diagnosed eating disorders were excluded.

Sample Size and Attrition

To complete the subcutaneous abdominal adipose tissue (SCAAT) interstitial glycerol measurements for aim 1, a power analysis of a study comparing the resting lipolytic rate between obese and lean women\textsuperscript{181} showed a sample size of ten (five per group) achieved 90% power to detect a difference of 54.1 μmol/L in dialysate glycerol with an pooled standard deviation between groups of 22.59 μmol/L, with significance set at $\alpha = 0.05$ and an estimated effect size of $d = 2.39$. Another study\textsuperscript{182} compared lean and obese women and showed a sample size of four (two per group) achieves 90% power to detect a difference of 24.7 μmol/L in dialysate glycerol with an average standard deviation 3.1 μmol/L with significance set at $\alpha = 0.05$ and an estimated effect size of $d = 6.99$. Therefore, completing a total sample size of eight participants (four per group) was set to achieve a power of 95% an average detectable difference of 41.37 μmol/L, an average standard deviation of 12.5 μmol/L, significance set at $\alpha = 0.05$, and an estimated effect size of $d = 3.31$, for the microdialysis portion of this investigation.

To complete aim 2: Heptulla et. al.\textsuperscript{183} investigated the difference in fasted insulin between obese and lean adolescent females and showed a sample size of six (three per group) achieved a 90% power to detect a difference of 38.9 μU/ml and an average standard deviation of 8.6 μU/ml with significance set at $\alpha = 0.05$ and an estimated effect size of $d = 3.48$. Likewise, van der Merwe et al.\textsuperscript{182}, compared fasted insulin levels
between lean and obese women and showed a sample of six (three per group) achieved 90% power to detect a difference of 7.09 μU/ml with an average standard deviation 1.04 μU/ml with significance set at \( \alpha = 0.05 \) and an estimated effect size of \( d = 3.4 \). Thus, six participants (three per group) were required to achieve >95% power for the insulin portion of the investigation with an average detectable difference of 22.97 μU/ml, and average standard deviation of 4.8 μU/ml, and significance set at \( \alpha = 0.05 \), and an estimated effect size of \( d = 4.9 \). Kanaley et. al.\textsuperscript{184} investigated the difference in circulating growth hormone between lean and obese women and showed a sample size of six (three per group) achieved 90% power to detect a difference 28.6 ng/L and an average standard deviation 6.75 ng/L with significance set at \( \alpha = 0.05 \) and an estimated effect size of \( d = 3.6 \).

To complete aim 3, a study comparing younger and older adults', including both men and women, echo intensity analysis via photoshop Hioki et al\textsuperscript{185} showed a sample size of 13 (seven per group) achieved 90% power to detect a difference of 18.5 A.U. with an average standard deviation between groups of 9.35 A.U. with significance set at \( \alpha = 0.05 \) and an estimated effect size of \( d = 1.98 \) for measurements of compositional muscle quality. In a resistance training study of men and women with diabetes, Brookes et. al.\textsuperscript{14} showed a sample size of four (two per training group) achieves 99% to detect a difference of 52 Nm/kg with an average standard deviation between groups of 5 Nm/kg with significance set at \( \alpha = 0.05 \) and an estimated effect size of \( d = 10.2 \) for measurements of functional muscle quality.

Based on the power analyses, fourteen participants (seven per group) were recruited for all variables to be statistically powered to observe a difference if one were to exist.

**COVID-19 Safety Procedures**

During each data collection day, the research team followed COVID-19 protocols to increase the safety of the participants and research team, as well as reduce the risk of the potential spread of COVID-19. All participants were required to wear a face covering while in the lab. Testing days were scheduled with at least two hours between participants to allow adequate time for cleaning and disinfecting of equipment. During
all procedures that require close proximity to the participants, less than six feet, the research team wore required gloves, face covering, and eye protection.

For procedures that were expected to require being within six feet of the participants and possibly touching the participant directly the following additional procedures were also be implemented. Researchers were not touching or within six feet of a participant for longer than approximately two minutes at a time. Only the minimum number of researchers needed to maintain safety for each procedure were used. The following are the procedures that required the additional COVID-19 precautions: microdialysis insertion, biodex strength testing, resting and post-exercise metabolic testing, placement of blood pressure cuff, venous blood draws, body composition testing (BodPod®, Cosmed, Chicago, IL), changing of dialysate collection tubes, and ultrasound measurements. After each test the equipment was thoroughly cleaned and disinfected with approved cleaning solutions and the recommended duration of time for full cleaning to occur, per the solution directions, was allowed before the next use of equipment.

**Participant Recruitment, Retention, and Compliance**

Participants were recruited from the general population of Tallahassee, FL and rural areas within reasonable commuting distances. Recruitment was accomplished via social media and flyers posted. Flyers were posted on campus and at sites of local endurance clubs, and in health clinics and fitness centers.

The overall time commitment was explained in detail at the beginning of the study to confirm their understanding of what was asked of them. A phone call or text message, whichever the participant preferred, was sent within two days of their final day to remind them of their appointment for microdialysis testing. On completion of the study, participants were given their personalized testing information.
Data Collection

During the initial phone screening participants were asked for their height and weight to calculate BMI and training history was assessed. This information was used for early exclusion. The entire study design is depicted in Figure 7. Each testing day was completed in the fasted state (7-9 hours) and after abstaining from caffeine (12 hours) and exercise and alcohol (24 hours). Before any laboratory tests were performed or data collected, written and verbal consent was obtained in accordance with Florida State University’s Human Subject Review Board (Study ID: 00000027, Aug. 5, 2019).

Figure 8: Testing Day 1
Heart Rate (HR), Rate of Perceived Exertion (RPE)

Testing Day 1

For Day 1 of testing (Figure 8), participants arrived in the morning at ISSM for informed consent, anthropometric testing, body composition, resting blood pressure, and fitness testing. Following these measurements participants were instructed to drive to the Sandels building and meet the research staff for ultrasonography of the rectus femoris. All testing was overseen by a certified strength and conditioning specialist.

Anthropometrics

Anthropometric and body composition measurements were assessed on the first day in the lab to ensure inclusion criteria was met. Height and weight were measured with a wall-mounted stadiometer (SECA, Hamburg, Germany) and a digital scale (Detecto®, Webb City, MO, USA) respectively, and used to calculate body mass index (BMI: kg/m²). Fat-free mass, fat mass, and percent body fat was assessed by
BodPod® (Cosmed, Chicago, IL) following the manufactures instructions.

**Fitness Testing**

A submaximal YMCA cycle ergometer (Monarch *Ergomedic 828 E*, Kroons väg, Vansbro, Sverige) test was used to assess current fitness level. In accordance with COVID-19 precautions, participants were asked to complete the test on an ergometer outdoors of the ISSM laboratory and maintaining PPE and social distancing as previously described. The test consisted of three to four, three-minute stages. The first stage of the test started with the participants pedaling against a resistance of 0.5 kg (150 kgm/min) at 50 rpm. Heart rate was recorded at the end of each minute and if the second- and third-minute measurements are within five beats of each other the researcher adjusted the resistance depending on the last heart rate recorded (<80 bpm = 2.5 kg, 80 – 90 bpm = 2.0 kg, 90 – 100 bpm = 1.5 kg, and >100 bpm = 1.0 kg). The participant continued to pedal at 50 rpm through each of the stages and each stage following stage two increased by 0.5 kg. The test concluded when two heart rate readings (between 110 and 150) had been collected in two different stages. The heart rate measurements and intensity for the two stages were used to calculate the slope (slope (a) = (VO\textsubscript{22} – VO\textsubscript{21})/(HR\textsubscript{2} – HR\textsubscript{1})) of a line used to extrapolate maximal oxygen uptake if the test was continued to maximal effort. After determining the slope the participant’s estimated VO\textsubscript{2peak} was calculated using age predicted heart rate max (APHR\textsubscript{max}) and specific heart rate and VO\textsubscript{2} measurements with the following equation: $VO_{2\text{max}} (\text{ml/kg/min}) = a (\text{APHR}_{\text{max}} – HR2) + VO_{2186}$. Before the test, participants were fitted with a chest strap heart rate monitor and watch receiver (Polar, Lake Success, NY) which continuously recorded heart rate for the duration of the test. Participants were also be asked to rate their perceived exertion throughout the test. A Borg scale ranging from 6 to 20, 6 being equivalent to resting and 20 being equivalent to maximal effort, to determine how hard they felt they were working during the fitness test.

**Functional Muscle Quality Testing**

Force production was accessed via isokinetic dynamometry (Biodex Medical Systems Inc, Shirley, NY) using knee extension and flexion at 60°/s for strength testing.
Participants performed 2 sets of 3 reps, the peak power of the sets was used as the measurement of maximal force production (N-m). A 2-minute rest was given between sets. Isokinetic testing is not a usual mode of exercise as it requires equipment generally only found in a lab setting. Likewise, the specific test we will be conducting requires a very short period of time during physical exertion. The test does not increase minute ventilation, as it will take approximately 15 seconds per set for a total of 30 seconds of exercise. Therefore, this test does not increase the risk of COVID-19 spread. However, to reduce any possible risk of spreading COVID-19 participants were required to wear a face covering during this test, as well as social distancing protocols were followed by the investigation team during the test. Cross-sectional measurements of the rectus femoris via ultrasonography (Sandels, described below) with the isokinetic strength test (ISSM) were used to calculate functional muscle quality (FMQ = N-m/cm²).

**Compositional Muscle Quality Testing**

Following a standard 20-minute rest period, including a car ride across campus to the Sandels building, participants were asked to sit on an examination table. Images of the rectus femoris were taken midway between the inguinal crease and the proximal aspect of the patella using an ultrasound machine. Intramuscular fat percentage of the rectus femoris was assessed via analysis of the ultrasound echo intensity and related calculations. All parameters of the sonograph unit (depth, gain, etc.) were kept constant between each participant. The average of the three measurements were used to quantify echo intensity. Echo intensity of the measurements were quantified via Adobe Photoshop histogram analysis (San Jose, CA) and were used to estimate the intramuscular fat percentage using the following equations.

Females: \( y = [(0.062) \cdot (40 \cdot z + x)] + 7.901 \)

Males: \( y = [(0.114) \cdot (40 \cdot z + x) + 1.126] \)

* \( x \) = raw echo intensity, \( y \) = intermuscular fat percent, \( z \) = subcutaneous adipose thickness
7-Day Lifestyle Measurements

At the end of the testing on this day the participants were given a packet with food and exercise logs, an accelerometer (Actigraph WGT3X-BT, Pensacola, FL), to use over the following seven days. The information packet also contained contact information for the research team in case a problem should arise with any of the equipment.

During the next 7 days, participants kept track of their daily training routines by recording their distance, time to completion, and the rate of perceived exertion for training sessions. They wore the accelerometer (Actigraph WGT3X-BT, Pensacola, FL) to record their non-structured physical activity, and to track sleep duration and quality. The data provided by the accelerometers are given as an average of percentages for time spent standing, laying, or sitting during the waking hours of the day. The sleep data provided by the monitors are sleep time (min), number of waking events, and sleep efficiency (%).

Participants also filled out food logs for all seven days for quantifying of their normal diet. Information from the food logs were analyzed with ASA24®, an online food catalog from the National Institute of Health (https://asa24.nci.nih.gov), for caloric and macronutrient (carbohydrate, protein, and fat) content. Training logs, accelerometer data, and food logs were used to explore potential differences between the two groups.

Testing Day 2

After the 7-day period of at home data collection, participant reported back to the lab for the second day of testing (occurring on 7±3 days after day 1). Participants arrived in the morning at the ISSM for microdialysis testing, metabolic measurements, exercise, and blood draws (Figure 9).

Lipolytic and Metabolic Testing

Two microdialysis probes were inserted percutaneously 5 – 10 cm lateral to the umbilicus for collection of interstitial glycerol for quantification of lipolysis using sterile procedures. The microdialysis probes were perfused with a solution of 0.9% sodium chloride and ~10 mM ethanol (for blood flow quantification) at 2.0 µL/min11,140.
Interstitial glycerol concentration were calculated via the following equation similar to previous studies\textsuperscript{187,188}:

\[
\text{Gly} \text{ \textit{in vivo}} = \left( \frac{\text{Gly dialysate} (1 - \text{EtOH dialysate/perfusate})}{\text{in vitro EtOH relative recovery}} \right) \div \left( \frac{\text{in vitro Gly relative recovery}}{\text{in vitro}} \right)
\]

After a 60-minute microdialysis probe equilibration period, resting dialysate was collected for a total of 60 minutes (collection vials changed every 15 minutes). During the last 30 minutes of the pre-exercise session resting metabolic rate was collected. Participants were asked to lie in a supine position for 30 minutes to measure resting energy expenditure and respiratory exchange ratio, for quantification of whole-body fat oxidation at rest\textsuperscript{142}. Next, participants completed a 45-minute cycle ergometer (Monarch \textit{Ergomedic 828 E}, Kroons väg, Vansbro, Sverige) ride. The resistance was set to between 2 – 3 watts per kilogram of lean body mass to elicit approximately 70\% of their estimated VO\textsubscript{2peak}. In accordance with COVID-19 precautions, the cycling was performed outside the lab in the open air and while maintaining a safe and recommended social distance (at least 6 feet). During the ride, participants could drink ad libitum. One dialysate collection occurred immediately before and after the completion of the ride. Immediately after the cycling bout the participant were escorted back into the lab and were asked to lie down in a supine position for 30 minutes for continuous gas collection to measurement post-exercise energy expenditure, respiratory exchange ratio, and fat oxidation.
Dialysate was again collected every 15 minutes for 120 minutes following the exercise session. Dialysate was stored at 4°C for analysis of ethanol within 24 hours and subsequently stored at -80°C until batch analysis of all dialysate glycerol samples (CMA600, Solna, Sweden) could be completed. Participants could sit up and read quietly in the lab for all post-physical activity data collection except during metabolic testing. Fat oxidation was measured again in the same supine position for the last 30 minutes of the 120-minute post-exercise period.

**Microdialysis Ethanol Outflow/Inflow Ratio**

Ethanol (~10 mM) was included with the perfusion medium to monitor adipose tissue blood flow in the area of the microdialysis probe\(^11\). Ethanol diffuses over the dialysis membrane and is not metabolized in adipose tissue to any significant extent. The Ethanol is transported away from the local area by the microcirculatory blood flow in the immediate vicinity of the probe membrane. An enzymatic fluorometric method\(^{188}\) is used to measure the ethanol in the perfusate and dialysate. Blood flow is expressed as a ratio of the ethanol concentration in the dialysate (outflow) to the ethanol concentration in the perfusate (inflow):

\[
\text{Ethanol outflow/inflow ratio} = \frac{\text{ethanol dialysate}}{\text{ethanol perfusate}}
\]

The ethanol outflow/inflow ratio is inversely related to the local adipose tissue blood flow. Indirect calorimetry using a Parvo metabolic cart (TrueOne 2400; Parvomedics; Salt Lake City, UT) was used to measure resting energy expenditure and determine whole body fat oxidation at pre-(rest), immediately post-, and at 120 minutes post-exercise.

**Circulating Hormone Response to Exercise**

Venous blood samples, via antecubital vein were collected to measure concentrations of insulin, human growth hormone, blood lipids, and glucose. A total of three blood draws were collected, during the microdialysis testing day immediately before and after the exercise bout, as well as 120 minutes post-exercise. Plasma
insulin and growth hormone were compared between groups and used as a covariate for lipolytic action if significant differences exist (Figure 9).

All blood was collected in red top serum vacutainers and were centrifuged for 15 min at 3,500 rpm at 4°C and aliquots serum was stored at -80°C for later batch analysis to limit day-to-day assay variability. HbA1c was measured with a DCA Vantage analyzer (Siemens, Tarrytown, NY), respectively. Serum insulin (BioVendor, RIS006R, Czech Republic) and human growth hormone (BioVendor, RCD017R, Czech Republic) were assessed via enzyme-linked immunosorbent assay according to manufacturer’s instructions. Blood was drawn immediately following metabolic testing for resting measurements, immediately after the cycling bout, and at 120 minutes’ post exercise.

**Statistical Analyses and Interpretation of Results**

Descriptive statistics were calculated for all variables and are included as means and standard deviations for normally distributed continuous variables and medians, minima, and maxima for non-normally distributed continuous variables. Frequency and percentages were calculated for categorical variables. Distributions of outcome variables were examined graphically for symmetry and for outliers. Extreme outliers were investigated for technical or clerical errors. If the size of the measurement could not be attributed to such an error, it was included in the analysis.

To test the hypothesis that **female endurance athletes with higher body fat have a significantly decreased lipolytic rate in response to a 45-minute cycle ergometer ride at 70% of estimated VO$_{2peak}$ compared to female endurance athletes with lower body fat**, a two-way repeated measures ANOVA was performed. The analysis was used to determine interactions (group X time) in interstitial glycerol concentrations at rest, immediately after, and for 120 minutes post-exercise. Significance was located using a Tukey post-hoc analysis.

To test the hypothesis that **female endurance athletes with higher body fat have a significantly higher level of circulating insulin and significantly lower level of post-exercise human growth hormone compared female endurance athletes with lower body fat**, a two-way repeated measures ANOVA was performed. The analysis was used to determine interactions (group X time) in circulating hormone
concentrations at rest, immediately after, and for 120 minutes post-exercise. Significance was located using a Tukey post-hoc analysis.

To test the hypothesis that female endurance athletes with higher body fat have a significant decrease in compositional and functional muscle quality of the upper leg compared to female endurance athletes with lower body fat, an Independent Student t-test was performed. The analysis was used to assess any significant group effects in compositional and functional muscle quality between groups.

An Independent Student t-test was used for descriptive statistics and secondary analysis of health and lifestyle markers between groups. Age and fat mass were analyzed as covariates and were reported if interactions were determined as significant. Significance was set a p ≤ 0.05, data are reported as mean ± SD, and all statistical analyses were performed using SPSS (IBM, Armonk, NY version 27) with post hoc analysis performed on Prism (GraphPad®, San Diego, CA version 9).
CHAPTER 4
RESULTS

Participant Characteristics

A total of twenty five female endurance athletes (runners, cyclists, and triathletes) were pre-screened, via phone or email, for inclusion in the study. Three were unable to participate due to having a BMI >30 kg/m². Two lived outside of a reasonable commute to the lab. Three didn’t respond back to the initial contact for more information and four didn’t respond/contact the investigation team when at the start of menses, and one was excluded from analysis as an outlier with a VO₂peak greater than three standard deviations above the mean. Ultimately, fourteen female endurance athletes volunteered for this study and were stratified into two groups based on body fat percentage, lower body fat (LBF, <30%, N=8) and higher body fat (HBF, ≥30%, N=6). Descriptive statistics for each group are in Table 1. The HBF group had a significantly higher body fat percentage (t = -5.22 (10.3) p < 0.000, d = 2.55), lower VO₂peak (t = 3.96 (12), p = 0.002, d = 2.14), and higher body fat mass (kg) (t = -3.16 (12), p = 0.008, d = 2.94).

Table 1: Participant Descriptive

<table>
<thead>
<tr>
<th>Variables</th>
<th>Lower Body Fat (n = 8)</th>
<th>Higher Body Fat (n = 6)</th>
<th>Sig.</th>
<th>Eff.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yrs.)</td>
<td>Mean (SD)</td>
<td>Mean (SD)</td>
<td>p</td>
<td>d</td>
</tr>
<tr>
<td></td>
<td>32.3 (8.8)</td>
<td>38.7 (6.7)</td>
<td>.161</td>
<td>.81</td>
</tr>
<tr>
<td>Height (m)</td>
<td>1.65 (0.06)</td>
<td>1.66 (0.05)</td>
<td>.714</td>
<td>.20</td>
</tr>
<tr>
<td>Weight (Kg)</td>
<td>61.7 (7.6)</td>
<td>65.6 (7)</td>
<td>.352</td>
<td>.49</td>
</tr>
<tr>
<td>Body composition (%)</td>
<td>23.4 (4.5)</td>
<td>32.8 (2.1)*</td>
<td>&lt;.000</td>
<td>2.55</td>
</tr>
<tr>
<td>Body Mass Index (Kg/m²)</td>
<td>22.7 (2.5)</td>
<td>24 (3.2)</td>
<td>.428</td>
<td>.44</td>
</tr>
<tr>
<td>Estimated VO₂peak (ml/kg/min)</td>
<td>40.7 (3.5)</td>
<td>33.5 (3.2)*</td>
<td>.002</td>
<td>2.14</td>
</tr>
<tr>
<td>Estimated VO₂peak (ml/FFM(kg)/min)</td>
<td>53.2 (3.7)</td>
<td>49.9 (4.9)</td>
<td>.474</td>
<td>.78</td>
</tr>
<tr>
<td>Training Experience (yrs.)</td>
<td>6.9 (2.8)</td>
<td>8.3 (3.8)</td>
<td>.473</td>
<td>.38</td>
</tr>
<tr>
<td>Training Frequency (d/wk.)</td>
<td>4.9 (1.1)</td>
<td>4.7 (1.4)</td>
<td>.681</td>
<td>.23</td>
</tr>
<tr>
<td>Resting Metabolic Rate (kcals/day)</td>
<td>1516.7 (207.8)</td>
<td>1551.3 (125.7)</td>
<td>.725</td>
<td>.19</td>
</tr>
<tr>
<td>Fat Mass (Kg)</td>
<td>14.7 (4.4)</td>
<td>21.6 (3.5)*</td>
<td>.008</td>
<td>2.94</td>
</tr>
<tr>
<td>Fat Free Mass (Kg)</td>
<td>47 (3.9)</td>
<td>44 (3.8)</td>
<td>.171</td>
<td>.33</td>
</tr>
<tr>
<td>Fat Free Mass Index (FFM(kg)/m²)</td>
<td>17.3 (1.4)</td>
<td>16.1 (1.8)</td>
<td>.158</td>
<td>.81</td>
</tr>
</tbody>
</table>

*Denotes significantly different between groups.

Eff. = effect size; d = 0.2 small effect; 0.5 medium effect; 0.8 large effect
Lipolytic Response to Exercise

Interstitial Glycerol

Glycerol was measured at 13 different time points throughout the second lab visit before, during and after an acute bout of exercise. Figure 10a shows the averages of the pre-exercise, exercise, and post-exercise time points, while Figure 10b shows the group responses at all 13 time points. Pre- and post-exercise glycerol showed a non-significant difference between groups of 65.3 µM (LBF = 275.1±90.7, HBF = 209.8±65 µM, F (1,12) = 2.2 p = 0.161, η² = 0.157) and 155 µM (LBF = 383.9±212.4 µM, HBF = 228.9±64.5 µM F (1, 8.6) = 3.8 p = 0.84, η² = 0.197), respectively. However, the glycerol response during exercise showed a significant difference of 1504.6 µM (LBF = 2393.3±1537.7 µM, HBF = 889.3±423.2 µM, F (1, 12) = 5.34 p = 0.04, η² = 0.308) between groups.

Figure 10: Lipolytic Response to Exercise
Measurements of SCAAT interstitial glycerol concentration
Figures displayed as M(SEM)
*Denotes significantly different between groups.
**Ethanol Concentrations**

Ethanol was measured in the perfusate (inflow) and dialysate (outflow) before, during and after an acute bout of exercise. There were no significant differences between groups in the ethanol outflow/inflow ratio at any of the 13 collection time points ($p = 0.418$), or averaged pre-exercise, during exercise, or for 120 minutes post-exercise ($p = 0.305$), respectively.

![Ethanol Outflow:Inflow Ratio](image)

*Figure 11 Ethanol Outflow:Inflow Ratio*
Measurements of interstitial glycerol concentration
Figures displayed as M(SEM)
*Denotes significantly different between groups.*
Hormone Concentrations

**Insulin**

Pre-exercise, immediately post-exercise, and 120 minutes post-exercise insulin concentrations were not different between groups (Pre-exercise: LBF = 5.95±2.7 µU/ml, HBF = 7.79±4.2 µU/ml, F = 0.77 (1, 9) p = 0.403 η² = 0.08) (Post-exercise: LBF = 18.69±8 µU/ml, HBF = 17.67±8.3 µU/ml, F = 0.05 (1, 11) p = 0.83 η² = 0.01) (120 minutes post-exercise: LBF = 5.34±2.3 µU/ml, HBF = 3.26±1.1 µU/ml, F = 2.01 (1, 4) p = 0.23 η² = 0.34, CV = 6.9%). There was a significant time effect within groups with the post-exercise insulin concentration significantly higher than the pre- and 120-minute post-exercise (p = 0.002)

**Growth Hormone**

Pre-exercise, immediately post-exercise, and 120 minutes post-exercise insulin concentrations were not different between groups (Pre-exercise: LBF = 0.82±0.6 ng/ml, HBF = 0.5±0.05 ng/ml, F (1, 6.1) = 2.16 p = 0.191 η² = 0.14) (Post-exercise LBF = 5.92±4.7 ng/ml, HBF = 5.6±4.2 ng/ml, F (1, 11) = 0.02 p = 0.899 η² = 0.002) (120 minutes post-exercise: LBF = 0.62±0.2 ng/ml, HBF = 0.61±0.2 ng/ml, *significantly different from pre- and 120 post-exercise*
F (1, 9) = 0.01 \; p = 0.92 \; \eta^2 = 0.001, \; CV = 7.9\%). There was a significant time effect within groups with the post-exercise growth hormone concentration significantly higher than the pre- and 120-minute post-exercise (p = 0.005)

**Muscle Quality**

**Compositional Muscle Quality**

Intramuscular fat percentage (Figure 15) was a non-significant difference of 0.1% (LBF = 13.5±0.93%, HBF = 13.6±0.92%, F (1, 12) = 0.03 \; p = 0.859, \; \eta^2 = 0.003, \; \text{RawEl} \; CV = 5.3\%) between groups.

**Functional Muscle Quality**

Functional muscle quality (Figure 14) was significantly higher in the LBF group with a difference of 0.5 Nm/cm² (LBF = 5.2±0.5 Nm/cm², HBF = 4.7±0.5 Nm/cm², F (1, 12) = 5.04 \; p = 0.04, \; \eta^2 = 0.296) between groups. However, the parameter of AGE was a significant covariate (F (1, 12) = 13.2 \; p = 0.006, \; \eta^2 = 0.59) and eliminated the significant
difference in functional muscle quality ($F (1, 12) = 2.5, p = 0.15, \eta^2 = 0.22$) between groups.

### Health and Lifestyle Markers

#### Blood Chemistry

There were no significant differences in any of the following health markers: HbA1c, total cholesterol, HDL, triglycerides, ALT, AST, fasted glucose, nHDLc, TC:HDL ratio, LDL, and VLDL (Table 2).

#### Table 2: Blood Chemistry

<table>
<thead>
<tr>
<th>Variables</th>
<th>Lower Body Fat (n = 8)</th>
<th>Higher Body Fat (n = 6)</th>
<th>Sig.</th>
<th>Eff.</th>
</tr>
</thead>
<tbody>
<tr>
<td>HbA1c (%)</td>
<td>Mean (SD)</td>
<td>Mean (SD)</td>
<td>$p$</td>
<td>$d$</td>
</tr>
<tr>
<td></td>
<td>5.2 (.1)</td>
<td>5 (.2)</td>
<td>.081</td>
<td>1.03</td>
</tr>
<tr>
<td>Cholesterol (mg/dL)</td>
<td>177.9 (40.6)</td>
<td>160.8 (19.5)</td>
<td>.370</td>
<td>.520</td>
</tr>
<tr>
<td>HDL (mg/dL)</td>
<td>69.6 (15.9)</td>
<td>65.7 (13)</td>
<td>.642</td>
<td>.266</td>
</tr>
<tr>
<td>Triglycerides (mg/dL)</td>
<td>70.1 (18.1)</td>
<td>77.7 (29.6)</td>
<td>.585</td>
<td>.313</td>
</tr>
<tr>
<td>ALT (U/L)</td>
<td>26 (9.1)</td>
<td>23.8 (4)</td>
<td>.600</td>
<td>.300</td>
</tr>
<tr>
<td>AST (U/L)</td>
<td>27.4 (9.9)</td>
<td>22.3 (7.1)</td>
<td>.317</td>
<td>.583</td>
</tr>
<tr>
<td>Glucose (mg/dL)</td>
<td>94 (7)</td>
<td>91.5 (3)</td>
<td>.413</td>
<td>.453</td>
</tr>
<tr>
<td>Insulin (µU/ml)</td>
<td>6 (2.7)</td>
<td>7.8 (4.2)</td>
<td>.403</td>
<td>.532</td>
</tr>
<tr>
<td>nHDLc (mg/dL)</td>
<td>108.1 (29.3)</td>
<td>95.7 (20)</td>
<td>.398</td>
<td>.489</td>
</tr>
<tr>
<td>TC/HDL (%)</td>
<td>2.6 (.3)</td>
<td>2.5 (.5)</td>
<td>.757</td>
<td>.177</td>
</tr>
<tr>
<td>LDL (mg/dL)</td>
<td>94.1 (27.2)</td>
<td>79.8 (17.3)</td>
<td>.291</td>
<td>.617</td>
</tr>
<tr>
<td>VLDL (mg/dL)</td>
<td>14 (3.5)</td>
<td>15.5 (5.9)</td>
<td>.580</td>
<td>.318</td>
</tr>
</tbody>
</table>

*Denotes significantly different between groups.

Eff. = effect size; $d = 0.2$ small effect; 0.5 medium effect; 0.8 large effect

### Exercise Logs

There was no significant difference in the seven-day exercise data in years of minutes of exercise per week, training frequency, miles run per week, training heart rate, percent of age predicted heart rate, or average rate of perceived exertion for training sessions, number of training day during the week (Table 3).
Table 3: 7-Day Exercise Data

<table>
<thead>
<tr>
<th>Variables</th>
<th>Lower Body Fat (n = 8)</th>
<th>Higher Body Fat (n = 6)</th>
<th>Sig.</th>
<th>Eff.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Time Training (min/wk.)</td>
<td>Mean (SD)</td>
<td>Mean (SD)</td>
<td>p</td>
<td>d</td>
</tr>
<tr>
<td></td>
<td>272 (168.1)</td>
<td>412.4 (247.4)</td>
<td>.229</td>
<td>.685</td>
</tr>
<tr>
<td>Distance (miles/wk.)</td>
<td>27.9 (19.7)</td>
<td>52.8 (55.7)</td>
<td>.260</td>
<td>.639</td>
</tr>
<tr>
<td>Training Heart Rate (HR) (bpm)</td>
<td>147 (20)</td>
<td>144 (16)</td>
<td>.788</td>
<td>.167</td>
</tr>
<tr>
<td>Percent Age Predicted HR (%)</td>
<td>78.2 (7)</td>
<td>79 (10)</td>
<td>.886</td>
<td>.089</td>
</tr>
<tr>
<td>Training RPE</td>
<td>12.7 (2.6)</td>
<td>13.3 (1.3)</td>
<td>.602</td>
<td>.289</td>
</tr>
<tr>
<td>Number of Training Days</td>
<td>5.3 (1.8)</td>
<td>4.8 (1.8)</td>
<td>.681</td>
<td>.277</td>
</tr>
</tbody>
</table>

*Denotes significantly different between groups.

**Eff. = effect size; d = 0.2 small effect; 0.5 medium effect; 0.8 large effect**

**Activity and Sleep Data**

There was no significant difference in percent time spent standing (LBF = 28.4±8%, HBF = 30±3% p = 0.65, d = -0.26) between groups. The HBF group spent significantly more time laying down with a difference of 6.39% (LBF = 18.8±5%, HBF = 25.2±5%, t (12) = -2.38, p = 0.035, d = -1.29) between groups. The HBF group also spent significantly more time sitting with a difference of 3.83% (LBF = 14.5±2%, HBF = 18.3±2%, t (12) = -2.95, p = 0.012, d = -1.6) between groups. There was no significant difference in sleep time, number of waking events, and sleep efficiency (Table 4).

Table 4: Activity and Sleep Data

<table>
<thead>
<tr>
<th>Variables</th>
<th>Lower Body Fat (n = 8)</th>
<th>Higher Body Fat (n = 6)</th>
<th>Sig.</th>
<th>Eff.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Time Standing (%)</td>
<td>Mean (SD)</td>
<td>Mean (SD)</td>
<td>p</td>
<td>d</td>
</tr>
<tr>
<td></td>
<td>28.4 (8)</td>
<td>30 (3)</td>
<td>.606</td>
<td>.255</td>
</tr>
<tr>
<td>Time Laying (%)</td>
<td>18.8 (5.1)</td>
<td>25.1 (4.9)*</td>
<td>.035</td>
<td>1.287</td>
</tr>
<tr>
<td>Time Sitting (%)</td>
<td>14.5 (2.4)</td>
<td>18.3 (2.3)*</td>
<td>.012</td>
<td>1.595</td>
</tr>
<tr>
<td>Time Sleeping (min)</td>
<td>371.5 (148.4)</td>
<td>427.4 (18.4)</td>
<td>.382</td>
<td>.507</td>
</tr>
<tr>
<td>Wake Episodes (#)</td>
<td>15.4 (4.1)</td>
<td>16.8 (6.2)</td>
<td>.624</td>
<td>.280</td>
</tr>
<tr>
<td>Sleep Efficiency (%)</td>
<td>87.7 (5.9)</td>
<td>87.2 (5.3)</td>
<td>.865</td>
<td>.097</td>
</tr>
</tbody>
</table>

*Denotes significantly different between groups.

**Eff. = effect size; d = 0.2 small effect; 0.5 medium effect; 0.8 large effect**
Food Logs

There were no significant differences between groups for calories, carbohydrate, protein, or fat intake. However, the HBF groups tended to have a higher absolute and relative caloric intake with differences of 185 kcals/day and 1.6 kcals/kg, respectively, between groups (p > 0.28). This increased caloric intake is most likely due to a slightly higher carbohydrate intake (35.2 g/day and 0.9 g/kg) and fat intake (26.8 g/day), respectively (p > 0.13) (Table 5).

Table 5: 7-Day Food Log Data

<table>
<thead>
<tr>
<th>Variables</th>
<th>Lower Body Fat (n = 8)</th>
<th>Higher Body Fat (n = 6)</th>
<th>Sig.</th>
<th>Eff.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calories (kcals/day)</td>
<td>1751.5 (277.3)</td>
<td>1936 (179.5)</td>
<td>.277</td>
<td>.752</td>
</tr>
<tr>
<td>Carbohydrate (g/day)</td>
<td>167.8 (59.2)</td>
<td>203 (85.2)</td>
<td>.459</td>
<td>.502</td>
</tr>
<tr>
<td>Protein (g/day)</td>
<td>77.8 (17.2)</td>
<td>78.5 (17.7)</td>
<td>.954</td>
<td>.038</td>
</tr>
<tr>
<td>Fat (g/day)</td>
<td>83.2 (12.9)</td>
<td>110 (61.4)</td>
<td>.317</td>
<td>.689</td>
</tr>
<tr>
<td>Relative Calories (kcals/kg/day)</td>
<td>28.7 (3.8)</td>
<td>30.3 (2.3)</td>
<td>.464</td>
<td>.496</td>
</tr>
<tr>
<td>Relative Carbohydrates (g/kg/day)</td>
<td>2.8 (0.7)</td>
<td>3.7 (0.8)</td>
<td>.130</td>
<td>1.04</td>
</tr>
<tr>
<td>Relative Protein (g/kg/day)</td>
<td>1.3 (0.3)</td>
<td>1.2 (0.2)</td>
<td>.753</td>
<td>.210</td>
</tr>
<tr>
<td>Relative Fat (g/kg/day)</td>
<td>1.3 (0.2)</td>
<td>1.3 (0.3)</td>
<td>.966</td>
<td>.028</td>
</tr>
</tbody>
</table>

*Denotes significantly different between groups.  

Eff = effect size; d = 0.2 small effect; 0.5 medium effect; 0.8 large effect.
CHAPTER 5
DISCUSSION

Controversy remains as to whether an individual can be considered healthy with higher levels of body fat\textsuperscript{32–37,45,189–191}. While some argue cardiorespiratory fitness negates the effects of elevated adiposity on cardiovascular disease and all-cause mortality\textsuperscript{34,36,44,192}, others argue there are still health consequences in maintaining a higher body fat\textsuperscript{32,37,193}. The purpose of this study was to determine the physiological differences that would exist between female endurance athletes of higher and lower body fat percentages.

The primary findings from this study were: 1) there was a blunted lipolytic rate, often associated with elevated adiposity in the HBF group compared to the LBF group, 2) circulating insulin and growth hormone were not significantly different between the LBF and HBF groups, and 3) compositional muscle quality was not significantly different between groups, nor was functional muscle quality when considering age as a covariate. Furthermore, our exploratory parameters of blood chemistry were not significantly different between groups and interestingly, the activity tracking data showed that the HBF female endurance athletes spend a more significant percentage of time engaged in sedentary behavior than the LBF group.

Lipolysis

Our first aim was to determine if the lipolytic response to an acute bout of endurance type exercise would be different between the two groups. Previous research by Ormsbee et. al.\textsuperscript{11} demonstrated that sedentary obese men have a blunted response to an acute bout of resistance training. However, Richterova et. al.\textsuperscript{48}, De Glisezinski et. al.\textsuperscript{47,55} and others\textsuperscript{73} have demonstrated that a 12-week endurance training protocols can increase the lipolytic rate of SCAAT in previously sedentary obese individuals in response to perfused pharmaceuticals as well as to an exercise bout. These data suggest that adipocyte lipolysis does adapt to training.

Yet, Stallknecht et. al.\textsuperscript{143} demonstrated no difference in lipolytic rate stimulated by epinephrine infusion in highly trained vs. sedentary healthy men. However, there are a couple of differences compared to the current study. For instance, adipose tissue
blood flow was determined using 133-Xenon clearance and blood flow was elevated in the trained men during epinephrine infusion, whereas the current study showed no difference in blood flow between groups. Also, although epinephrine is a prominent stimulator of lipolysis, it has been recently demonstrated that atrial natriuretic peptide (ANP) can contribute to as much as 50% of the lipolytic action of adipocytes during exercise\textsuperscript{145,194}. By perfusing only epinephrine into the SCAAT depot the investigators potentially missed the lipolytic response of other cellular mechanisms, including ANP.

Stinkens et. al.\textsuperscript{195} also showed a 12-week bout of concurrent exercise training did not increase lipolytic rate in obese men compared to baseline measurements. Some potential limitations to the study include that the dose of exercise was likely suboptimal as it only consisted of two endurance exercise session/week and one resistance training session/week. The endurance training session consisted of a 30-minute bout of cycling at about 70% $\text{VO}_{2}\text{max}$, missing the American College of Sports Medicine (ACSM)\textsuperscript{50} recommendation of 150 minutes of moderate or 75 minutes of vigorous aerobic activity per week. Likewise, the resistance training session falls just shy of the two to three times per week of the ACSM guidelines. It also consisted of performing eight different exercises using three sets of ten repetitions at 60% one-rep max (1RM), the National Strength and Conditioning Association (NSCA) recommends performing $>15$ repetitions at 60% 1RM or prescribing 75% of 1RM for a set of ten repetitions for an optimal dose of resistance training\textsuperscript{196}.

It remains unknown if chronic training (>1 yr.) can normalize lipolytic response to a bout of exercise. The present study demonstrates, among female endurance athletes, those who have HBF have lower interstitial glycerol in the SCAAT as compared to LBF female endurance athletes, despite similar training experience, frequency, and intensity. It stands to reason, as sedentary individuals start to exercise and adapt to the new stimulus, an individual with lower body fat would maintain the elevated lipolytic rate to a bout exercise compared to a person with higher body fat. This concept would explain the difference seen in our data between the two groups.
Hormone Concentrations

The second aim of the study was to determine if differences exist in the insulin and growth hormones response to an acute bout of exercise between endurance trained females with HBF or LBF. From our data, the low lipolytic response observed among our participants cannot be attributed to elevated insulin levels or decreased post-exercise growth hormone, as no significant difference was measured in either of these lipolytic modulating hormones. Our data contrast those of Ormsbee et. al.\textsuperscript{11} reported a blunting of lipolysis that was attributed to high concentrations of basal insulin and lower concentrations of post-exercise growth hormone release. Interestingly, the insulin response to exercise in the present study, although not significant, may point to a physiological pattern between the groups. Pre-exercise the HBF group had a slightly elevated insulin concentration, similar to Ormsbee et. al.\textsuperscript{11} and others\textsuperscript{67,89,111,187,197–199}, but did not have as robust an increase in response to fasted exercise, and seems to have a lower post-exercise insulin concentrations compared to the LBF. In the Ormsbee et. al.\textsuperscript{11} cohort, the increased insulin in response to RT is potentially a sign of metabolic inflexibility in their obese participants. The lack of increased insulin in the HBF group could be pointing to a greater amount of fat oxidation, however we were not able to measure fuel utilization due to COVID-19 restrictions. It would be interesting to determine if this pattern persists with exercise in the post-prandial and post-absorptive states in cohorts of healthy individuals or those with obesity or diabetes.

Curiously, the growth hormone response in our study was different than was anticipated with lower concentrations of post-exercise growth hormone release than has been observed in other studies. First, we expected to see higher concentrations in growth hormone secretion to the exercise bout, based on work by Kraemer et. al.\textsuperscript{200} and others\textsuperscript{201–203}, who demonstrated that women have a more robust growth hormone release following a bout of exercise training of a similar intensity as this study. The difference could be the type of training completed, as these studies used weightlifting as the mode of exercise. However, our growth hormone concentrations following exercise were similar to the resistance trained women of Allman et. al.\textsuperscript{142}, who had lower circulating concentrations after an acute bout of resistance training than the male participants of Ormsbee et. al.\textsuperscript{11}. Second, we expected to see a drastically lower growth
hormone secretion in the HBF individuals following exercise as it is well documented that obese individuals have the blunted growth hormone response to exercise\textsuperscript{11,12,26,183,184,204–208}.

The blunted growth hormone secretion observed in the present study following exercise may be due to the exercise bout being performed on a cycle ergometer. Optimal growth hormone release may require the exercise stimulus to incorporate eccentric muscle contractions as part of the training session\textsuperscript{200}. Likewise, although 70\% VO\textsubscript{2peak} should be sufficient intensity to induce growth hormone secretion\textsuperscript{123,209}, due to COVID-19 restrictions, we were unable to collect expired air during the exercise bout. Thus, we cannot fully determine if the participants were exercising at 70\% their maximal capacity.

**Muscle Quality**

The third aim of the study was to determine if muscle quality was different between endurance trained female athletes of HBF vs LBF. Compositional muscle quality was not significantly different between the groups in the present study. These data refute multiple studies that have shown individuals with higher body fat to have a higher level of intramuscular triglyceride accumulation\textsuperscript{29,155,210}. However, they support the notion that chronic endurance training increases the storage of lipids within the muscle as an adaptation to such training\textsuperscript{29,210}. This is further demonstrated as our LBF groups had a higher raw echo intensity (~50 AU) than a group of sedentary women (~40 AU) in a study by Ismail et. al.\textsuperscript{155}. When considering raw EI for compositional muscle quality, both groups within the present study were closer to the sedentary elevated BMI group in the Ismail et. al. study\textsuperscript{155}. Yet when using the IMF\% estimation equations produced by Young et. al.\textsuperscript{79} and tested against MRI for reliability by Burton et. al.\textsuperscript{161}, the non-significant difference between the groups of the present study is decreased even more drastically to less than 1\% difference between the HBF and LBF groups. The likely cause of this difference in IMFA measured by raw EI and the equations is the control for different variables within and around the measurement site such as subcutaneous fat thickness and corrected EI\textsuperscript{79}. 
Maffiuletti et al.\textsuperscript{158} demonstrated that obese individuals, compared to lower weight individuals, have a decreased functional muscle quality but difference in force production between their groups was reduced to non-significance when relative force to mineral free lean mass of the leg was used in the ratio instead of weight. These findings are similar to the present study in which the HBF group initially had a significantly lower FMQ than the LBF group. However, when we compared force to cross-sectional area our statistical significance decreased but was not eliminated ($p = 0.04$). Age was a significant covariate. When considered in the statistical model it eliminated the significant difference in FMQ between HBF and LBF groups, indicating age to be a major contributor to loss in force production, substantiating many other studies previous findings\textsuperscript{93,153,155,211,212}. This is an important point, as all of the participants were considered extremely active by physical activity standards, decrements in force production have a stronger association with age. This could also be attributed to the fact that during the 7-day inter-testing week only two of the participants noted participating in any form of resistance type training. Further investigations and interventions should be used to determine the extent to which RT could help prevent or slow this decline, especially in physically active adults who have a desire to continue an active lifestyle as they age.

Beginning in the 4\textsuperscript{th} decade, functional muscle quality decreases as we age\textsuperscript{93,153,155}, it is an important measure to consider in preventing increased morbidity and mortality rates. The importance this topic becomes even more prevalent when excess adiposity is added to already under used musculature\textsuperscript{24,154,211,212}. This is substantiated in the present study as the significant decrease in FMQ between the two groups was reduced to non-significant with the addition of age as a covariate to this parameter. These data demonstrate that the presence of force decline with age and draws into the light the importance of interventions to prevent such a decline.

**Health and Lifestyle Parameters**

In line with the purpose of this investigation, to determine differences between endurance trained females of differing body fatness, the investigators included additional exploratory measurements to the study. Body composition and body fat
percentage were significantly different between the groups as a strategic methodological choice. The groups were stratified by body fat percentage as to determine the effect of adiposity on the aims previously discussed. Interestingly, only three of the participants could be considered overweight according to BMI, two in the HBF and one in the LBF, respectively. Thus, adding to the growing portion of scientific literature demonstrating altered physiological indices attributed to excess fat more than excess weight. The additional parameters, that were collected and measured, have been shown to influence weight gain and physiological abnormalities that are associated with metabolic dysfunction often accompanying the obese phenotype. These additional parameters were body composition, blood chemistry, exercise training characteristics, non-structured physical activity, sleep quality, and dietary characteristics.

The etiology of the normal weight obese individual is not new per se, but it is becoming a more recognized issue with people of normal weight. These individuals present metabolic disfunction and it is increasingly important to determine more effective diagnosis techniques as it is no longer sufficient to assume normal weight translates to healthy. However, the other health markers often reported as abnormal with elevated body fat (i.e., BP, glucose, TC, HDL, LDL etc.) appeared to be normal and not different between the HBF and LBF groups in the present study. These data support the notion being physically active and having a moderate level of fitness decreases indices and incidences of CVD and all-cause mortality and strengthens the recommendations that all people should be exercising regularly.

Intriguingly, there were some significant differences found in the non-structured physical activity data. Specifically, the data supports pervious research that individuals with HBF tend to be more sedentary compared to LBF counterparts despite having similar training volume and frequency. The increased amount of sedentary time could be contributing to the alterations in physiology similar to the physiological disturbances seen in detraining and bedrest studies. These studies have demonstrated significant decline in insulin sensitivity in healthy young individuals after 7 days of bed rest similar to those with clinical levels of insulin resistance. Bergouignan et. al. demonstrated a resistance to an exercise bout in healthy young individuals following a
time of detraining\textsuperscript{176}. These data demonstrate, again, the importance of regular and consistent exercise for everyone.

**Limitations**

There were several limitations to the present study. The most prevalent of which is COVID-19. Due to the pandemic the investigators were unable to perform any maximal testing in the lab. Thus, a submaximal cycle ergometer test was used for the estimation of aerobic capacity creating a scenario in which we were unable to say with all certainty the participants were exercising at the proper intensity during the exercise day. However, heart monitors were worn continuously during the exercise session. Heart rate has been shown to be a valid measure of exercise intensity. The exercise intensity was also dependent on the participant’s ability to maintain a constant pedal cadence of \(~90\) rpms. The cycle used for the exercise session was a Monarch with a turn-style resistance band, thus is the cadenced decreased or increased it shifted the number of watts being generated by the participant. This situation also made it impossible to measure expired gasses during exercise. Thus, our ability to determine fuel utilization differences between groups was severely limited. COVID-19 also presented a unique training environment for the participants. We tried to determine the amount of physical activity/training for each participant with Actigraphs and training logs during the week between experimental visits. The average time spent training during this week was still greater than the physical activity guidelines\textsuperscript{50}, however, we are unable to determine if the pandemic reduced the amount of training these women engage in under normal circumstances.

While we investigated the exercise-induced lipolytic system, we understand that fat metabolism is a multi-factorial process. There are many secretagogues that can affect lipolysis and fat oxidation. We measured insulin and growth hormone as regulators of lipolysis to understand their potential mechanisms. Due to monetary constraints, we were unable to perfuse adrenergic agonist and antagonists into the SCAAT to directly measure adrenergic control of lipolysis. It is well known that $\alpha$-AR and $\beta$-AR ratio regulates lipolysis and changes with endurance training\textsuperscript{12,55,105}. Had pharmaceuticals been used in the present study a deeper understanding of specific
adrenergic regulation in this population would have been achieved. Also, atrial natriuretic peptide (ANP) and inflammatory markers are known to effect lipolysis\textsuperscript{27,145,194,217–219}. We did not collect or analyze these markers and as such will not be able to fully elucidate if differences in lipolysis, hormone levels, and muscle quality are due to differences in low-grade systemic inflammation. However, this does leave the opportunity of additional work on inflammatory markers for future research.

While we gained a greater understanding of how lipolytic rate, circulating hormones, and muscle quality in female endurance athletes with HBF compares to female endurance athletes with LBF, we do not know how lipolysis is influenced specifically from different modes of exercise. There were no direct measurements of muscle tissue or cellular components, such as mitochondrial density or protein content.

Finally, the study lacked a sedentary cohort to compare the group values too. If a LBF sedentary cohort had been included, it would have allowed for the determination of how close to homeostatic physiology the HBF population is getting through their training. If an obese sedentary cohort had been able to be recruited, then the data would have allowed for the determination as to the extent exercise training was improving the active HBF physiology.

**Future Directions**

Obesity is becoming more of a health concern with each passing year. It is imperative to determine the optimal combination and dose of lifestyle interventions to help individuals mitigate or eliminate the deleterious effects, often associated with excess fat. This investigation has demonstrated that HBF leads to perturbations in adipocyte function. This course of inquiry can be pursued by determining if the amount of time being inactive is more important than the amount of time exercising as some have already indicated\textsuperscript{34,39,216,220}. Exercise interventions could be prescribed in multiple doses per day instead of just one long dose. Exercise and meal timing may also play a role in the optimal prescription for improving health. Investigations aimed to develop better screening and diagnostic tools to improve point of care\textsuperscript{221} for individuals with HBF whether overweight or not. The investigation of other systemic health mediators also needs to be investigated. Determining how secretagogues from adipose and muscle
tissue affect the other tissue as well as every other system in the body is needed and then the determination of what kind of dietary and exercise prescription can alter and optimize the levels of those adipokines and myokines for improved health.

**Conclusion and Practical Application**

Blunted lipolysis appears to persist in HBF female endurance athletes despite a similar chronic training history to LBF female endurance athletes. Circulating insulin and growth hormone concentrations where not significantly different between groups suggesting a benefit to chronic training in HBF female endurance athletes. However, further investigations are warranted to determine the full effect of training on changes in hormone concentrations in these populations. Interestingly, there was a significant difference between groups in sedentary behavior with the HBF group spending more time sitting and laying than the LBF group. Further investigation is warranted to determine the extent sedentary time during the day influences the maintenance of elevated adiposity in this and other populations. These data could lead to a greater understanding of how exercise recommendations and prescriptions could be optimized by breaking up sedentary time with structured exercise. Furthermore, Intramuscular fat percentage in female endurance athletes were similar but age appears to be the driving force behind decrements in functional MQ in the HBF group. Therefore, exercise training appears protective to muscle quality despite significant differences in body composition.
APPENDIX A
IRB APPROVAL

FLORIDA STATE UNIVERSITY
OFFICE of the VICE PRESIDENT for RESEARCH

APPROVAL

August 5, 2019

Tristan Ragland

Dear Tristan Ragland:

On 7/24/2019, the IRB reviewed the following submission:

<table>
<thead>
<tr>
<th>Type of Review</th>
<th>Expedited</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(2)(b) Blood samples from others;</td>
</tr>
<tr>
<td></td>
<td>(4) Noninvasive procedures</td>
</tr>
<tr>
<td>Title</td>
<td>Endurance and resistance training’s effects on adipocyte and muscle quality rehabilitation in an obese population</td>
</tr>
<tr>
<td>Investigator</td>
<td>Tristan Ragland</td>
</tr>
<tr>
<td>Submission ID</td>
<td>STUDY00000027</td>
</tr>
<tr>
<td>Study ID</td>
<td>STUDY00000027</td>
</tr>
<tr>
<td>Funding</td>
<td>None</td>
</tr>
<tr>
<td>IND, IDE, or HDE</td>
<td>None</td>
</tr>
<tr>
<td>Documents Reviewed</td>
<td>• DXA Use Table - 6-21-2019.docx, Category: Other;</td>
</tr>
<tr>
<td></td>
<td>• Food Log.docx, Category: Other;</td>
</tr>
<tr>
<td></td>
<td>• Informed Consent Form, Category: Consent Form;</td>
</tr>
<tr>
<td></td>
<td>• IRB Application, Category: IRB Protocol;</td>
</tr>
<tr>
<td></td>
<td>• Recruitment Flyer, Category: Recruitment Materials;</td>
</tr>
</tbody>
</table>

The IRB approved the protocol, effective from 7/24/2019 to 7/23/2020 inclusive. Research approved using the expedited procedure include the approval categories. Before 7/23/2020 or within 30 days of study close, whichever is earlier, you are to submit a completed continuing review and required attachments to request continuing approval or closure. You can submit a continuing review by navigating to the active study and clicking Create Modification / CR.

If continuing review approval is not granted before the expiration date of 7/23/2020, approval of this study expires on that date.

Page 1 of 2
You are advised that any modification(s) to the protocol for this project must be reviewed and approved by the IRB prior to implementation of the proposed modification(s).

Federal regulations require that the Principal Investigator promptly report any new information related to this protocol (see Investigator Manual (HKP-103)).

You are required to submit a Continuing Review at least 60 days before the protocol expiration date of 7/23/2020 to request continuing approval or closure. If the continuing review approval is not granted before the expiration date, approval of this protocol expires on that date.

In conducting this protocol, you are required to follow the requirements listed in the Investigator Manual (HKP-103), which can be found by navigating to the IRB Library within the IRB system.

Sincerely,

Human Subjects Research Office
humansubjects@fsu.edu
APPENDIX B
INFORMED CONSENT

Permission to Take Part in a Human Research Study

Title of research study: The effect of regular exercise experience on lipolysis, circulating hormones, and muscle quality between overweight and lean recreational endurance athletes.

Investigator: Tristan Ragland and Dr. Michael Ormsbee

Key Information: The following is a short summary of this study to help you decide whether or not to be a part of this study. More detailed information is listed later on in this form.

Why am I being invited to take part in a research study?
We invite you to take part in a research study designed to compare the differences between recreational athletes of different body sizes on fat cell release of fatty acids. The study will also compare circulating levels of blood hormones, as well as muscle fat content and relative strength of the dominant leg.

What should I know about a research study?
- Someone will explain this research study to you.
- Whether or not you take part is up to you.
- You can choose not to take part.
- You can agree to take part and later change your mind.
- Your decision will not be held against you.
- You can ask all the questions you want before you decide.

Why is this research being done?
The purpose of the research is to compare the difference between recreational athletes, of different body sizes or fitness, who have participated in endurance activities over the last few years on the body’s ability to release fatty acids from fat cells in the storage compartment under the skin, specifically in the stomach region. The study is also comparing the difference between body size and hormone levels in the blood at rest and after exercise (three blood draws will be used to collect blood hormones around an exercise session), as well as fat content of the muscle in the upper leg. We will also test the strength of the muscle of the upper leg to compare it to its relative size.

How long will the research last and what will I need to do?
We expect that you will be in this research study for about 2 weeks. Testing will be held over the course of 2 days. Day 1 consists of height, weight, waist circumference, body composition testing, and the performance of a submaximal fitness test on a stationary bike, ultrasound measurements of the mid-thigh and maximal muscle contraction of the leg on the Biodex machine. Day 2 consists of microdialysis testing of the under-skin fat in the stomach region, resting metabolic testing, and blood sampling. Each of which will happen before, during, and after a moderate-intensity exercise session.
Permission to Take Part in a Human Research Study

Days 1 will take place at the beginning of the 2-week period. At the end of the day of testing you will be given an instruction packet for the next 7 days. During this week you will be asked to record everything you eat for the 7 days. You will also be asked to record all your exercise training sessions. You will also be asked to wear different wrist bands, one will measure how physically activity you are during the week, the second will measure your sleep time and quality. After the 7 days of lifestyle measurements you will be asked to come back to the lab for the final testing day (Day 2).

More detailed information about the study procedures can be found under “What happens if I say yes, I want to be in this research?”

Is there any way being in this study could be bad for me?
Muscle soreness is common from novel exercise or exercise of higher intensity than what is accustomed. However, extreme muscle soreness can lead to hospitalization in some cases. Thus, this research can lead to hospitalization or injury. Infection or bruising is possible during blood sampling.

More detailed information about the risks of this study can be found under “Is there any way being in this study could be bad for me? (Detailed Risks)”

Will being in this study help me in any way?
We cannot promise any benefits to you or others from your taking part in this research. However, possible benefits include knowledge about specific measurements that are known to effect overall health. Further, you will obtain information regarding your estimated fitness level, which may be helpful in designing future training protocols. Additionally, you will receive information regarding your metabolism and fitness.

What happens if I do not want to be in this research?
Participation in research is completely voluntary. You can decide to participate or not to participate. Your alternative to participating in this research study is to not participate.
Permission to Take Part in a Human Research Study

Detailed Information: The following is more detailed information about this study in addition to the information listed above.

Who can I talk to?

If you have questions, concerns, or complaints, or think the research has hurt you, talk to the research team at [Redacted] or Dr. Michael Ormsbee at [Redacted].

This research has been reviewed and approved by an Institutional Review Board ("IRB"). You may talk to them at 850-644-7900 or humansubjects@fsu.edu if:

- Your questions, concerns, or complaints are not being answered by the research team.
- You cannot reach the research team.
- You want to talk to someone besides the research team.
- You have questions about your rights as a research subject.
- You want to get information or provide input about this research.

How many people will be studied?

We expect about 40 people here will be in this research study out of 40 people in the entire study nationally.

What happens if I say “yes” to being in this research?

If you agree and are eligible to participate in this study, we would ask you to do the following: Come to the Human Performance Laboratory in the Institute of Sports Sciences and Medicine (ISSM) at Florida State University to complete an informed consent document, a health history questionnaire, a physical activity readiness questionnaire, a physical activity questionnaire, and eligibility screening. You will also be asked to perform baseline testing for: resting metabolic rate, body composition, and anthropometrics (height and weight), microdialysis testing, submaximal fitness testing (Est. VO2 max), fasted blood-sampling, maximal voluntary contraction of the leg muscles (Biodex), and ultrasound measurements of the thigh.

During your initial/screening visit (Day 1), at the ISSM human research facility your height, weight, resting blood pressure, and body fat percentage (BodPod) will be measured. Then you will perform a seated strength test of your dominant leg on a Biodex consisting of two sets of three reps. The machine will keep your leg at a specific speed during extension and flexion, you will be asked to push and pull against the lever as hard as you can, the machine measures how much force you can produce. Then you will be asked to perform a submaximal cycling test in which you will pedal against a given resistance for 3 – 4 minutes and the resistance will be increased based on your heart rate. You will complete 3 – 4 stages and your fitness level will be estimated from the intensity and heart rate numbers during the test. Then you will be asked to drive to Sandels building on campus (map in participant packet) for ultrasound measurements of the mid-thigh region of the leg on your dominant hand side. This measurement will be taken on the front of the thigh halfway between the kneecap and the bend at the hip. At the end of testing this day you will be given a packet with all of the information for lifestyle measurements for the next week. The packet will include food and exercise logs, a sleep band, and activity band. On this day your next appointment for Day 2 testing will be set. This day of testing will take ~3 hours.

Day 3 will consist of placing a microdialysis probe in the fat just under the skin in the stomach region. Microdialysis is a technique allowing us to collect products of fat breakdown to measure how effective
Permission to Take Part in a Human Research Study

the breakdown of fat is during rest, exercise, and post exercise. Liquid containing different solutions will be sent through the probes by the use of a pump similar to an automated insulin pump. The probe will contain a salt-water solution to collect the normal release of fat breakdown by products. Metabolic rate will be tested during rest, exercise and post-exercise. Resting measures consists of lying in a dark room for about 30 minutes under a hood connected to a chart to measure the amount of oxygen being used. Following the exercise, you will be asked to repeat the resting test for 15 minutes immediately following exercise and last 30 minutes of the next two hours. Blood draws from the front portion of the elbow will also be taken on this day immediately before and after the exercise, and two hours after the exercise. This day of testing will take ~5 hours to complete.

What happens if I say “yes,” but I change my mind later?
You can leave the research at any time it will not be held against you.

Is there any way being in this study could be bad for me? (Detailed Risks)
The study has the following risks: You may experience an increased heart rate, sweat rate, muscle soreness, and fatigue related to exercise testing. Further, you may experience soreness, fatigue, or injury from the exercise testing. All protocols have been previously used in related studies. It should be noted that infection and localized bruising and discomfort may be possible during blood sampling and microdialysis punctures. Qualified research personnel will be present during all experimental trials to ensure that proper procedures are followed. All research personnel will be trained in the techniques and use of the equipment during all testing. Individuals trained in blood draws and microdialysis probe insertion will perform all of these procedures. The risk of injury during the tests will be minimized by careful review of your medical history form.

COVID-19 procedures and precautions: No person who has been exposed to the Coronavirus or who falls into the CDC defined parameters as “high risk” will be allowed to participate in any portion of the study. CDC defined social distancing will occur under all situations that allow for this to happen (i.e. electronically signing of the informed consent, discussion of the study protocol and procedures). Staff and participants will follow the guidelines of proper hand washing and hand sanitizing as well as the use face coverings and PPE when social distancing is not possible such as; blood draws, insertion of the microdialysis probe, changing of microdialysis collections vials, placement of blood pressure cuff, and placement of metabolic chart facemasks for resting and post-exercise testing. Staff will be limited to the absolute minimum number of people to keep the participant safe during testing and collect needed data for each testing day. Participants will be given a copy of the FSU information sheet: Steps we take to help protect you from coronavirus with their informed consent and it will be fully explained to them before the start of their participation in the study.

During each data collection day, the research team will follow COVID-19 protocols to increase the safety of the participants and research team, as well as reduce the risk of the potential spread of COVID-19. All participants will be required to wear a face covering while in the lab. Testing days will be scheduled with at least two hours between participants to allow adequate time for cleaning and disinfecting of equipment. During all procedures that require close proximity to the participants, less than six feet, the research team will be required to wear gloves, face covering, eye protection, and a face shield.

For procedures that are expected to require being within six feet of the participants and possibly touching the participant directly the following additional procedures will also be implemented. Researchers will not be touching or within six feet of a participant for longer than two minutes at a
Permission to Take Part in a Human Research Study

time. Only the minimum number of researchers needed to maintain safety for each procedure will be used. The following are the procedures that will require the additional COVID-19 precautions: microdialysis insertion, biodex strength testing, resting and post-exercise metabolic testing, placement of blood pressure cuff, venous blood draws, body composition testing (BodPod, Cosmed, Chicago, IL), changing of dialysate collection tubes, and ultrasound measurements. After each test the equipment will be thoroughly cleaned and disinfected with approved cleaning solutions and the recommended duration of time for full cleaning to occur, per the solution directions, will be allowed before the next use of equipment.

What happens to the information collected for the research?
Efforts will be made to limit the use and disclosure of your personal information, including research study and medical records, to people who have a need to review this information. We cannot promise complete secrecy. Organizations that may inspect and copy your information include the IRB and other representatives of this organization.

The results of this study may be published but your name or identity will not be revealed. Information obtained during the course of the study will remain confidential to the extent required by law. Your name will not appear on any of the results. No individual responses will be reported in publication, only group responses. Confidentiality will be maintained by the assignment of a code number for each subject in which all data recorded will be based. The only record containing both the participant’s name and code number will be kept by the principal investigator, Dr. Michael Ormsbee, in a locked drawer in his laboratory. All records will be destroyed after a minimum of five years.

Plasma obtained from blood sampling and samples from microdialysis will be stored in a -80° storage freezer in the Institute of Sports Sciences and Medicine. Samples that are not used upon completion of the study will be stored for potential use at a future date (up to 7 years). If identifiers are removed from your identifiable private information or identifiable samples that are collected during this research, that information or those samples could be used for future research studies or distributed to another investigator for future research studies without your additional informed consent.

Can I be removed from the research without my OK?
The person in charge of the research study or the sponsor can remove you from the research study without your approval. Possible reasons for removal include becoming aware of a condition that was described as an exclusion criterion. You may also be removed if you miss any of the testing days as this is the minimum compliance needed to control the proper dose of training.

What else do I need to know?
If you need medical care because of taking part in this research study, contact the investigator and medical care will be made available. Generally, this care will be billed to you, your insurance or other third party. Florida State University has no program to pay for medical care for research-related injury.
Permission to Take Part in a Human Research Study

Signature Block for Capable Adult
Your signature documents your permission to take part in this research.

_________________________  _____________________________
Signature of subject  Date

_________________________
Printed name of subject

_________________________  _____________________________
Signature of person obtaining consent  Date

_________________________
Printed name of person obtaining consent

My signature below documents that the information in the consent document and any other written information was accurately explained to, and apparently understood by, the subject, and that consent was freely given by the subject.

_________________________  _____________________________
Signature of witness to consent process  Date

_________________________
Printed name of person witnessing consent process

7-24-2019
IRB Approval Date
<table>
<thead>
<tr>
<th>Exercise Log of: Week of:</th>
<th>Participant #:</th>
</tr>
</thead>
<tbody>
<tr>
<td>Date &amp; Time</td>
<td>Type of Exercise</td>
</tr>
<tr>
<td>Park of Preferred Exercise</td>
<td>Average HR</td>
</tr>
</tbody>
</table>
### APPENDIX D

## FOOD LOGS

<table>
<thead>
<tr>
<th>Time</th>
<th>Date</th>
<th>Estimated amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>Breakfast –</td>
<td>Date</td>
<td>Estimated amount</td>
</tr>
<tr>
<td>Lunch –</td>
<td>Date</td>
<td>Estimated amount</td>
</tr>
<tr>
<td>Dinner –</td>
<td>Date</td>
<td>Estimated amount</td>
</tr>
<tr>
<td>Additional Food –</td>
<td>Date</td>
<td>Estimated amount</td>
</tr>
</tbody>
</table>
APPENDIX E
RPE SCALE

<table>
<thead>
<tr>
<th>Rating</th>
<th>Perceived Exertion</th>
</tr>
</thead>
<tbody>
<tr>
<td>6</td>
<td>No exertion</td>
</tr>
<tr>
<td>7</td>
<td>Extremely light</td>
</tr>
<tr>
<td>8</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>Very light</td>
</tr>
<tr>
<td>10</td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>Light</td>
</tr>
<tr>
<td>12</td>
<td></td>
</tr>
<tr>
<td>13</td>
<td>Somewhat hard</td>
</tr>
<tr>
<td>14</td>
<td></td>
</tr>
<tr>
<td>15</td>
<td>Hard</td>
</tr>
<tr>
<td>16</td>
<td></td>
</tr>
<tr>
<td>17</td>
<td>Very hard</td>
</tr>
<tr>
<td>18</td>
<td></td>
</tr>
<tr>
<td>19</td>
<td>Extremely hard</td>
</tr>
<tr>
<td>20</td>
<td>Maximal exertion</td>
</tr>
</tbody>
</table>

Table 1. The Borg Rating of Perceived Exertion Scale
REFERENCES


53. Thompson, P. D. *et al.* Exercise and Physical Activity in the Prevention and Treatment of Atherosclerotic Cardiovascular Disease: A Statement From the


64. Ekkekakis, P. & Lind, E. Exercise does not feel the same when you are overweight: the impact of self-selected and imposed intensity on affect and exertion. Int. J. Obes. 30, 652–660 (2006).


90. Reynisdottir, S., Wahrenberg, H., Carlstrom, K., Rossner, S. & Arner, P.


95. Giolo De Carvalho, F. & Sparks, L. Targeting White Adipose Tissue with Exercise or Bariatric Surgery as Therapeutic Strategies in Obesity. *Biology (Basel)*. **8**, 16 (2019).


142. Allman, B. R. *et al.* Fat metabolism and acute resistance exercise in trained


182. van der Merwe, M.-T. et al. Lactate and Glycerol Release from Adipose Tissue in


194. Verboven, K. *et al.* Attenuated atrial natriuretic peptide-mediated lipolysis in


BIOGRAPHICAL SKETCH

TRISTAN JOSEPH RAGLAND

Education

2021      Ph.D., Florida State University, Tallahassee, FL. Major: Exercise Physiology.
          Research Focus: Differences in adipose function, hormonal concentrations, and muscle quality associated with body composition.
          Major Professor: Michael J. Ormsbee, PhD, FACSM

2015      M.S., Marywood University Scranton, PA. Major: Sports Nutrition and Exercise Science
          Major Professor: Angela Hillman, PhD


Professional Experience

2016–2021  Teaching Assistant, Institute of Sports Sciences & Medicine, College of Human Sciences, Florida State University. (APK3110c: Applied Exercise Physiology, PET3322L Anatomy and Physiology 1 Lab, PET3322 Anatomy and Physiology Lecture, PET5367 Nutrition and Exercise Performance, PET6387 Endocrinology in Health and Exercise)

2016–2021  Research Assistant, Institute of Sports Sciences & Medicine, College of Human Sciences, Florida State University.

2015      Adjunct Instructor, Broadview University, Layton, UT

2015      Graduate Assistant, Marywood University, Scranton, PA
Awards and Honors

Outstanding Teaching Assistant Award (2019 – 2020) - Nomination

Kappa Omicron Nu Academic Honor Society (Member 2015)

Manuscripts

Willingham, B.D., Ragland, T.J., Ormsbee, M.J.
Betaine Supplementation May Improve Heat Tolerance: Potential Mechanisms in Humans (2020). *Nutrients*


Adipose Lipolysis Unchanged by Pre-Exercise Carbohydrate Regardless of Glycemic Index (2018). *Medicine and Science in Sports and Exercise*

Conference Presentations

Willingham, B.D., Morrissey, M.C., Kisiolek, J.N., Ragland T.J., Hunt, R.L., Hickner, R.C., Ormsbee, M.J. (Presented 2019). The Effect of Cold Ambient Temperature and Preceding Active Warm-Up on Lactate Kinetics in Female Cyclists and Triathletes. Poster presentation at Regional Meeting, American College of Sports Medicine, Greenville, SC. (Regional)

Daniel A. Baur, Brandon D. Willingham, Kyle M. Smith, Jacob N. Kisiolek, Margaret Morrissey, Patrick G. Saracino, Tristan J. Ragland, and Michael J. Ormsbee (Presented 2017). Glycemic Index Has No Impact on Subcutaneous Abdominal Lipolysis During Exercise Poster presentation at Regional Meeting, American College of Sports Medicine, Chattanooga, TN. (Regional)

Ragland, T.J., Bachman, J.L., Hillman, A. The Effect of Caffeine on EPOC Following HIIT Treadmill Running: 3317 Board #78 May 30, 9 (ACSM Annual Conf. 2015)

Contracts and Grants Awarded

Ragland, T.J. (2021) Graduate School Dissertation Research Grant – Effects of Regular Endurance Training on Lipolysis, Circulating Insulin and Growth Hormones, and Muscle Quality in Recreational Female Athletes of Different Body Fatness ($1,000 Direct costs)

Contracts and Grants Denied


Ormsbee M.J. & Ragland, T.J. (2018) Comparison of Native whey isolate vs concentrate on circulating leucine kinetics – Friesland Campina Grant ($120,000)

Ormsbee M.J. & Ragland, T.J. (2018) The acute and long-term impact of potato protein on muscle, fat, and performance in overweight and obese individuals – LOI to National Potato Foundation ($200,000)

Ragland, T.J. & Ormsbee M.J. (Feb. 2019) *Effects of 12 weeks of endurance and resistance training on adipose lipolysis and muscle quality in an obese population* National Strength and Conditioning Association Doctoral Grant ($15,000)


Ragland, T.J. & Ormsbee M.J. (Feb. 2020) *Effects of resistance vs endurance training on adipose lipolysis, muscle quality, and hormone concentrations in obese women* National Strength and Conditioning Association Doctoral Grant ($15,000)