LIPID OXIDATION IN FEMALES DURING THE POSTEXERCISE RECOVERY PERIOD: ONE VS. TWO BOUTS OF EXERCISE

A Thesis
Presented
to the Faculty of
California State University, Chico

In Partial Fulfillment
of the Requirements for the Degree
Master of Arts
in
Kinesiology

by
Jennifer L. Barker
Spring 2015
LIPID OXIDATION IN FEMALES DURING THE
POSTEXERCISE RECOVERY PERIOD:
ONE VS. TWO BOUTS OF EXERCISE

A Thesis
by
Jennifer L. Barker
Spring 2015

APPROVED BY THE DEAN OF GRADUATE STUDIES
AND VICE PROVOST FOR RESEARCH:

_______________________________
Eun K. Park, Ph.D.

APPROVED BY THE GRADUATE ADVISORY COMMITTEE:

____________________      __
Kevin G. Patton, Ed.D.
Graduate Coordinator

________________________________
John L. Azevedo, Jr., Ph.D., Chair

_______________________________
George D. Swanson, Ph.D.

________________________________
Michael M. Smith, Ph.D.
DEDICATION

The heart is often analogously described as a hot, smoldering furnace in which the fire of life resides – a fire that keeps blood boiling. The heart, as an organ, as a deliverer of the nutrients and needs of life, and as a keeper and destroyer of love, rules both mind and body. Without the heart comes death. It is the driving force of existence.

I’d like to dedicate this thesis to the inquisitive students, and the current and future educators who have walked along side me on my journey into teaching and research – to those who possess a lot of heart. Your thirst for endless knowledge is my motivation.

I’d also like to dedicate not only this thesis, but also my current place in life, to my parents. Thank you for raising me to constantly pursue goals motivated by happiness. Throughout my childhood, your loving support and positive encouragement to keep my mind open, creative and receptive, has made me into the person I am today, with a soul that is beautifully illustrated. Your influence will mold me into the individual I will become in the future, and for that I will be forever grateful.
ACKNOWLEDGEMENTS

Dr. Jack Azevedo, Jr – Thank you for being a professional and personal support throughout my time here at CSU Chico. You have inspired me to pursue goals I never thought possible, and I will be forever thankful for your motivation. It has been great to be your student. You are, and always will be, my California family.

Dr. Steve Henderson – Thank you for introducing me to the world of physiology. If I hadn’t taken your course, I wouldn’t be in this position – in a place of satisfaction, where my curiosity is constantly being peaked and satisfied. You have helped me find my personal definition of success.

Dr. David Swanson – Thank you for your positive encouragement and personal insight. Your optimism and inspiration has been a constant reminder to stay true to my ideals and pursuit of knowledge.

Dr. Michael Smith – Thank you for all of your help and support.

Martin Friggard – Your assistance with statistics was irreplaceable! Thanks for all of your time!

Dr. Dan Clark – Your courses have helped bridge two areas of academia that will shape my career – physiology and biochemistry. Thank you for your hard work and dedication to students like myself. You have been an inspiring role model.
TABLE OF CONTENTS

PAGE

Dedication ............................................................................................................................... iii

Acknowledgements ................................................................................................................. iv

List of Tables ........................................................................................................................... vii

List of Figures .......................................................................................................................... viii

Abstract .................................................................................................................................... ix

CHAPTER

I. Introduction ......................................................................................................................... 1

   Statement of the Problem ................................................................................................. 3
   Purpose of the Study ......................................................................................................... 4
   Hypothesis of the Study ................................................................................................. 4
   Significance of the Investigation ..................................................................................... 4
   Delimitations of the Study .............................................................................................. 5
   Limitations of the Study ................................................................................................. 6
   Definition of Terms and Acronyms ............................................................................... 6

II. Literature Review ............................................................................................................... 9

   Energy Sources During Exercise .................................................................................... 9
   Responses to Exercise ..................................................................................................... 15
   Substrate Utilization During Exercise ........................................................................... 18
   Substrate Utilization During the Postexercise Recovery Period .................................. 23

III. Methodology .................................................................................................................... 27

   Design of the Investigation ........................................................................................... 27
Population and Sample ................................................................. 27
Location of the Study ................................................................. 29
Length of the Study ................................................................. 29
Treatment ................................................................. 29
Data Analysis ................................................................. 32

IV. Results and Discussion ................................................................. 35

Presentation of the Findings ................................................................. 35
Discussion of the Findings ................................................................. 43

V. Summary, Conclusion and Recommendations ........................................... 48

Summary and Conclusions ................................................................. 48
Research Recommendations ................................................................. 48

References .......................................................................................................................... 50

Appendices

A. Institutional Review Board State of Approval ................................................................. 61
B. Participant Consent Form ................................................................................................. 63
C. Medical and Exercise History Questionnaire ................................................................. 67
D. Recruitment Flier ........................................................................................................... 71
E. Subject Instructional Email ............................................................................................. 73
# LIST OF TABLES

<table>
<thead>
<tr>
<th>TABLE</th>
<th>PAGE</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Characteristics of Study Participants</td>
<td>35</td>
</tr>
</tbody>
</table>

vii
# LIST OF FIGURES

<table>
<thead>
<tr>
<th>FIGURE</th>
<th>PAGE</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. 60-minute Trial Protocol</td>
<td>32</td>
</tr>
<tr>
<td>2. 30-minute Trials Protocol</td>
<td>33</td>
</tr>
<tr>
<td>3. Substrate Oxidation During the Postexercise Recovery Period</td>
<td>36</td>
</tr>
<tr>
<td>4. Substrate Oxidation (Lipid and CHO) vs. Time</td>
<td>37</td>
</tr>
<tr>
<td>5. Substrate Oxidation Between Trials</td>
<td>39</td>
</tr>
<tr>
<td>6. Oxygen Consumption (VO₂) vs. Time</td>
<td>40</td>
</tr>
<tr>
<td>7. RER vs. Time</td>
<td>42</td>
</tr>
</tbody>
</table>
ABSTRACT

LIPID OXIDATION IN FEMALES DURING THE POSTEXERCISE RECOVERY PERIOD: ONE VS. TWO BOUTS OF EXERCISE

by

Jennifer L. Barker

Master of Arts in Kinesiology
California State University, Chico

Spring 2015

Research has shown that there is a difference in postexercise substrate (lipid and carbohydrate) oxidation rates and duration during one bout of exercise, most notably, an increase in lipid oxidation when compared to rest. However, it is unclear what happens to substrate oxidation rates during postexercise recovery during same-day split exercise sessions in females. The purpose of this study was to determine if total postexercise lipid oxidation in recreationally athletic females is affected by frequency of exercise (1 vs. 2 bouts) while keeping total workload the same (60 minutes at 65% VO₂max).

Seven recreationally athletic females were randomly allocated to each treatment and participated as their own control group. During the control trial, resting
VO$_2$, substrate oxidation (lipid and carbohydrate) rates, RER and VO$_{2\text{max}}$ were determined using indirect calorimetry. Subjects were then randomly allocated to each treatment, either 60 minutes of exercise at 65% VO$_{2\text{max}}$ on a cycle ergometer, or two 30-minute bouts at 65% VO$_{2\text{max}}$ on a cycle ergometer, separated by five hours. After each bout, metabolic parameters were collected every 15 minutes for three hours. A significant difference in both lipid oxidation (p < 0.0001) and carbohydrate oxidation (p < 0.005) was found between the 60-min trial postexercise recovery period and the sum of the split 30-min sessions. Significant differences in carbohydrate oxidation were found between exercise and control, and between exercise and all postexercise timepoints (p < 0.008). Although no significant differences in lipid or carbohydrate oxidation were found between the control trial and any of the postexercise timepoints of any trial, trends in increased lipid oxidation and decreased carbohydrate oxidation are seen. These results may indicate that split exercise bouts of identical workload may lead to an overall greater postexercise lipid oxidation when combined, compared to a single exercise session.
CHAPTER I

INTRODUCTION

“If exercise could be packed in a pill, it would be the single most widely prescribed and beneficial medicine in the nation,” stated Robert N. Butler, M.D., and former Director for the National Institute on Aging (Burbank & Riebe, 2002). Exercise is essential to maintain a healthy body composition, promoting physiological homeostasis and decreasing risk for obesity-related disease. The recommended healthy range of body fatness for young adult females is 20-35%, and fitness standards are 16-28% (Powers & Howley, 2012). For both sexes, excess energy consumption leads to increased size and number of adipocytes coupled with unhealthy levels of visceral fat, causing obesity. Obesity can lead to hyperlipidemia and hypertension, increased insulin levels coupled with insulin resistance, metabolic syndrome leading to the onset of Type II diabetes, and the development of cardiovascular disease (Must et al., 1999), the leading cause of death for both men and women in the United States (Murphy et al., 2013). The initial onset of obesity and its associated risks can be prevented by lifestyle changes and the proper incorporation of physical activity. Compoundingly, in 2008, the total healthcare costs in the United States associated with obesity-related conditions was $147 billion (Adult Obesity, 2014).

The benefits of exercise to promote whole-body wellness cannot be overlooked. Current Centers for Disease Control and Prevention (CDC) recommendations state that adults should be engaged in 150 minutes of moderate-
intensity aerobic activity or 75 minutes of vigorous-intensity activity per week, as well as
two days of strength training per week (“How much physical activity,” 2014). The
American Heart Association promotes similar exercise engagement guidelines spread
throughout most days of the week to maintain cardiovascular health and promote a
healthy body composition (“American Heart Association,” 2014). Furthermore, proper
utilization of the FITT principle will dictate physiological adaptations to exercise and
training (“American College of Sports Medicine,” 2013). Choice of exercise will dictate
selection of substrate utilization (Brooks & Mercier, 1994). Many studies have focused
on substrate utilization during exercise with a variety of intensities and types.
Traditionally, male subjects have been selected, even though sex differences have been
noted during rest and exercise. Anne Friedlander of Stanford University has been a
pioneer in investigating sex differences in substrate utilization during exercise and has
opened the door for much of the research that has followed, although many foundational
questions still exist.

Few studies have explored the postexercise recovery period, and sex
differences have been noted during this period of time. It can be suggested that a smaller
amount of research has been conducted involving females due to the complication of
monthly fluctuation in reproductive hormone concentrations and their effect on lipolysis
and lipid oxidation. However, current research is stating otherwise, showing no
significant difference on lipolysis between the follicular and luteal phases of the
menstrual cycle (Casazza et al., 2004).

Recently, it has been discovered that there is a significant increase in
postexercise lipid oxidation in both sexes (Kuo et al., 2004). More specifically, it’s been
noted that males who exercised for 90 minutes at 45% VO$_2$peak or 60 minutes at 65% VO$_2$peak both had increased postexercise lipid oxidation for 21 hours (Henderson et al., 2007). However, postexercise lipid oxidation in females returned to baseline in three hours. Additionally, postexercise lipid oxidation in both females and males is not affected by the power output during exercise.

It could be suggested that a shorter postexercise period of increased lipolysis and lipid oxidation in females assists in the maintenance of a higher essential body fat mass, an attribute inherent to the sex in order to maintain reproductive capability. An evolutionary benefit seems to fit this statement (Wu & O’Sullivan, 2011).

For females pursuing a reduction in CVD risk or exercise-induced fat loss, a question remains. It is currently unknown how multiple bouts of aerobic exercise completed in the same day (split sessions) will affect total postexercise lipid oxidation. If there is an effect, and total lipid oxidation increases, multiple shorter bouts could be a potential strategy for increased reduction in risk for CVD, increased fat loss and potentially a favorable change in body composition.

Statement of the Problem

Until recently, few studies have focused on postexercise substrate utilization, and even further, during recovery in females. The research question proposed was whether or not total postexercise lipid oxidation would be affected if exercise is divided into multiple (split) bouts conducted within the same day. If so, split exercise sessions may prove to beneficially alter resting metabolic rate specifically in females, promote
positive changes in body composition, and potentially decreasing risk for obesity-related diseases.

Purpose of the Study

The purpose of the study was to determine if total postexercise lipid oxidation in recreationally athletic females is effected by frequency of exercise (1 vs. 2 bouts) while keeping total workload the same (60 minutes at 65% VO₂max).

Hypothesis of the Study

The null hypothesis stated that there would be no difference in total lipid oxidation between one and two bouts of exercise. However, it was proposed that there would be a difference in total postexercise lipid oxidation in females when comparing one and two bouts of exercise.

Significance of the Investigation

Many training studies for performance analysis have been conducted, many with males, but less with females. Additionally, many studies have been conducted utilizing only one bout of exercise as treatment for metabolic and substrate utilization analysis, and primarily done during the exercise period, not during recovery. Yeo et al. (2008) analyzed skeletal muscle adaptation after one bout of endurance training followed by two bouts every second day. Male endurance trained triathletes and cyclists were used. Again, this study analyzed impact on performance, but did not delve deeper into the differences between sex or female performance. Investigations regarding EPOC have
looked at sex-specific characteristics (Børsheim & Bahr, 2003; Imamura et al., 2004; Phelain et al., 1997).

An increase in research focusing on female adaptations to exercise has been conducted primarily within the last two decades, but many questions remain unanswered. Further research will provide a greater understanding of sex differences and lead to a more sex-specific prescription of exercise to promote health, fitness and training, and potential body composition improvements.

**Delimitations of the Study**

- Population and sample: recreational female athletes, endurance-oriented athletes from Chico, CA. Sample includes females from the Chico Running Club, Chico Masters Cycling Team, CSU Chico students, and others without particular affiliation.
- Age: 19-35 years old
- Preference of activity (in order): cycling, running, other endurance-oriented sport
- Hours of preferred activity per week: 5-10 hours, 4+ exercise sessions/week.
- Subjects must be eumenorrheic, specifically above 12% (essential) body fat.

Body composition will be taken during the first meeting.

- Oral contraceptive use within 6 months of initiation of the study would be a preferred disqualification for participation due to suppression of endogenous ovarian hormone production and increases lipolysis and FFA availability (though no effect on RER during exercise) (Casazza et al., 2004). Due to the difficulty in obtaining subjects that meet this particular inclusion criterion, subjects will need to be tested between days
3-8 of the follicular phase their menstrual cycle to avoid significant fluctuations in hormones that would affect lipolysis. The subjects can either 1). Perform both trials within the 3-8 day window (48-72 hours apart), or 2). Perform each trial one month apart.

- Subjects must refrain from alcohol and caffeine use 24 hours prior to each meeting.
- Subjects must fast 12 hours prior to each meeting.

Limitations of the Study

- Diet and activity between trials will not be controlled, however, between the two 30-minute bouts, dietary intake will be recommended (see Chapter III, Methodology).
- Time constraints may increase the likelihood of participant drop-out. Each subject must meet three times, early in the morning.
- Fasting for 12 hours before each exercise trial and 24-hour refrainment from alcohol and caffeine consumption are required, but cannot be controlled.

Definition of Terms and Acronyms

1. CHO: Carbohydrate, to include glucose, muscle and/or liver glycogen, and lactate (Brooks, 2012)
2. ETC: Electron transport chain
3. FAD/FADH$_2$: Flavin adenine dinucleotide
4. FFA: Free fatty acid
5. FITT Principle: Frequency, Intensity, Time (or duration), Type
6. GAS: General Adaptation Syndrome, the body’s physiological response to training

7. GH: Growth hormone

8. IMCL: Intramyocellular lipid, also known as IMTG; fats stored as droplets within skeletal muscle cells

9. IMTG: Intramuscular triglyceride, also known as IMCL; fats stored as droplets within skeletal muscle cells

10. Lipolysis: Hydrolysis of triglyceride (breakdown into constituent parts), resulting in free fatty acids and glycerol

11. Lipid oxidation: the uptake and oxidation of a free fatty acid during aerobic metabolism, resulting in the production of ATP

12. NAD$^+$/NADH: Nicotinamide adenine dinucleotide (reduced and oxidized, respectively)

13. P$: Inorganic phosphate

14. PCr: Phosphocreatine

15. Postexercise recovery period: Period of time immediately following cessation of exercise

16. $R_a$ and $R_d$: Rate of appearance, and rate of disappearance, respectively

17. Recreational athlete: Definition provided by the AHA: “Individuals participating in a variety of informal recreational sports and circumstances engage in a range of exercise levels from modest to vigorous on either a regular or an inconsistent basis, which do not require systematic training or
the pursuit of excellence and are without the same pressure to excel against others that characterizes competitive sports” (Maron et al., 2004)

18. RER: Respiratory Exchange Ratio

19. SNS: Sympathetic nervous system

20. TCA Cycle: Tricarboxylic acid cycle; the first of two steps in aerobic production of ATP, conducted in the mitochondrial matrix. Also known as the Krebs cycle or citric acid cycle (CAC).
CHAPTER II

REVIEW OF THE LITERATURE

Energy Sources During Exercise

Metabolism is the “sum total of processes occurring in a living organism” (Brooks, Fahey & Baldwin, 2004). In humans, it consists of a system of chemical conversions that utilize various substrates and produce heat. Fuel utilization is conducted in both the cellular cytosol and the oxidative organelle, the mitochondrion. The primary function of the mitochondrion is to synthesize adenosine triphosphate (ATP), a nucleoside that stores chemical energy from the breakdown of three main fuel groups: carbohydrates, fats and proteins. In 1978, Peter Mitchell was awarded the Nobel Prize in Chemistry – he had developed the chemiosmotic theory, providing us with a foundation in understanding mitochondrial bioenergetics and the oxidative synthesis of ATP (Brooks, Fahey & Baldwin, 2004). Several metabolites and substrates are involved in the various processes that incorporate the synthesis and utilization of ATP, explained in detail later in this chapter.

For purposes of this study, metabolic pathways occurring in skeletal muscle fibers will be the primary focus of discussion. Human skeletal muscle has a mitochondrial density of 2-8% (Kayar et al., 1988). In non-oxidative glycolytic fast-twitch skeletal muscle fibers, mitochondrial density has been found to be lower. The mitochondria are more cylindrical in shape with less branching or reticulum interconnection, when compared with more oxidative muscle fibers.
In oxidative skeletal muscle, mitochondrial density is significantly higher compared to glycolytic fast-twitch skeletal muscle (Kayar et al., 1988). Additionally, the researchers found that both classes of mitochondria, subsarcolemmal and intermyofibrillar, are not purely cylindrical in shape, but extend transversely throughout the sarcomere and are quite complex, consisting of reticulum interconnection. This difference allows for an increased oxygen delivery and oxidative capacity within the oxidative skeletal muscle fiber.

Additionally, when comparing metabolite concentrations between oxidative and glycolytic skeletal muscle fiber types, which is determined by the myosin heavy chain ATPase isoform content (Baechle & Earle, 2008), a significant different exists (Kushmerick et al., 1992). At rest, higher concentration of PCr and ATP (in mM) is found in Type IIa fibers compared to Type I and IIx fibers. In contrast, Type I and IIx fibers have a higher concentration of P_i and ADP at rest (in mM and µM, respectively).

**Immediate**

During any metabolic transient, either the onset of exercise and incremental changes in intensity, there is an immediate increased requirement for ATP at the actomyosin ATPase (Hochachka et al., 1991). In response, a quick onset of ATP synthesis is provided by the use of phosphocreatine (PCr). The Lohman reaction is catalyzed by creatine kinase (CK) (Conley et al., 1997). Here, a phosphate and the respective bond dissociation energy is donated to ADP, creating ATP and creatine (Cr) (Powers and Howley, 2012). Creatine and phosphocreatine spatially translocate throughout this circular shuttle between the actomyosin ATPase and the mitochondrion, which makes “creatine the kinetically limiting acceptor that controls respiration” (Meyer,
1988). Restoration of PCr concentrations is only completed during the postexercise recovery period.

The Lohman reaction is a favorable reaction, with a $\Delta G^o = -11\text{ kcal/mol}$ PCr (Brooks, 2012):

\[
\text{PCr + ADP} \xrightarrow{\text{creatine kinase}} \text{Cr + ATP}
\]

Non-oxidative

Glycolysis provides the primary energy source for Type II (glycolytic) muscle fibers. It is the chemical transformation of glucose to pyruvate or lactate, and is the only process that can be conducted without the use of oxygen (Brooks, Fahey & Baldwin, 2004). The process is conducted in the cellular cytosol and produces a net of two pyruvate (aerobic/slow) or two lactate (aerobic/slow and anaerobic/fast), two ATP and two NADH, all from the breakdown of one glucose molecule. During glycogenolysis, the synthesis of pyruvate or lactate from the epinephrine-stimulated breakdown of glycogen, there is a net production of three ATP ($P_i$ is utilized in place of ATP in the phosphorylation of glycogen) (Brooks, Fahey & Baldwin, 2004). Both pyruvate and lactate are utilized in mitochondrial oxidative pathways (TCA cycle), and in glucose production via gluconeogenesis in the liver and kidneys.

Glycolytic sites of regulation exist where one molecule of ATP is utilized for phosphorylation (Raven et al., 2013). The first site of regulation exists where one ATP is utilized to phosphorylate a glucose molecule by hexokinase, creating glucose-6-phosphate (G6P). The phosphorylation is inhibited through negative feedback if the concentration of G6P is too high, and glucose uptake will be inhibited. Additionally, a
second site of regulation, and the rate-limiting step of glycolysis, exists at the phosphorylation of fructose-6-phosphate (F6P) via phosphofructokinase-1 (PFK-1), creating fructose-1,6-bisphosphate. At this site, increased concentrations of ADP, AMP and P_i will up regulate glycolytic flux, where adequate amounts of cytosolic ATP, PCr, and citrate will allosterically down regulate through negative feedback.

**Oxidative**

Oxidative metabolic pathways (the TCA cycle and oxidative phosphorylation/electron transport chain), reside within the mitochondria of Type I and II oxidative muscle fibers. They are utilized during prolonged, low-moderately intense aerobic activity where there is a greater demand for ATP (Brooks, 2012), and additionally during the postexercise recovery period. The higher mitochondrial density found in these fibers allows for the greatest production of ATP through cellular respiration (Kayar et al., 1988).

The TCA cycle is a series of enzyme-catalyzed redox reactions. From glycolysis, pyruvate (or lactate) enters the mitochondrial matrix through monocarboxylate transport proteins (MCT) and is transformed into acetyl-CoA by pyruvate dehydrogenase (PDH), the primary regulatory enzyme of TCA cycle flux when glucose is the initial substrate (Brooks, Fahey & Baldwin, 2004; Powers & Howley, 2012). The TCA cycle has three sites of regulation: citrate synthase, isocitrate dehydrogenase, α-ketoglutarate dehydrogenase, all responsive to fluctuations in ATP/ADP and/or NAD+/NADH. The rate-limiting enzyme of the TCA cycle is isocitrate dehydrogenase.
Non-carbohydrate substrates can also be converted to enter the TCA cycle. Fatty acids (fatty acyl-CoA) can be converted into acetyl-CoA through beta-oxidation in the matrix, and amino acids can be converted into glucose, pyruvate, acetyl-CoA, or several of the TCA cycle intermediates (Powers & Howley, 2012).

For each molecule of acetyl-CoA it receives, the TCA cycle produces a total of two CO$_2$, three NADH (plus one at the PDH complex, linking glycolytic and oxidative metabolism), one FADH$_2$, and one GTP through direct phosphorylation (Powers & Howley, 2012).

The electron transport chain (ETC) is the site of oxidative phosphorylation, and is located in the mitochondrial cristae inner membrane. It begins with the oxidation of NADH and FADH$_2$ at cytochrome Complexes I and II, respectively (Brooks, Fahey & Baldwin, 2005). Hydride ions are removed from the coenzymes, a pair of electrons associated with each ion is removed at the respective cytochromes, and the remaining proton is pumped into the mitochondrial inner membrane space. The electron pair oxidizes the series of cytochrome complexes along the established electrochemical gradient, with oxygen being the terminal electron receptor at Complex IV (therefore being reduced). The hydrogen ions re-enter the matrix through the F$_0$F$_1$ ATP synthase complex, where the proton-motive force generates energy to phosphorylate ADP, creating ATP (Brooks, Fahey & Baldwin, 2005; Powers & Howley, 2012).

Each oxidation of the coenzymes NADH and FADH$_2$ will yield synthesis of 2.5 and 1.5 ATP during oxidative phosphorylation, respectively (Nelson, Lehninger & Cox, 2008). Total ATP yield per mole of glucose or glycogen ranges from 30-32.
**Lipolysis and beta-oxidation**

The utilization of fat as a substrate can be derived from two pools: intramuscular (IMTG) or extramuscular (adipose tissue adipocytes). Lipolysis (hydrolysis of triglyceride) begins slowly, with lipases being activated by rises in catecholamines and glucagon (Powers & Howley, 2012). Plasma FFA and glycerol $R_a$ both increase. Plasma FFA’s are then transported by serum albumin to working skeletal muscle, whereas the glycerol is mainly utilized in gluconeogenic organs, converted to acetyl-CoA and then keytone bodies. Plasma FFA’s enter the cytoplasm via fatty acid transport proteins (FATs), and once in the cytoplasm, are converted to fatty acyl-CoA by way of acyl-CoA synthase. This step utilizes an eqivelant of 2 ATP (ATP $\rightarrow$ AMP + PP$_i$). The CoA is then exchanged for the carrier carnitine by carnitine-acyltransferase-I (CATI), the rate limiting step in fatty acid oxidation (Mole, 1971). The acyl-carnitine can now enter the mitochondrion, where CATII reversely exchanges the carnitine for CoA, therefore allowing the acyl-CoA to enter the beta-oxidation pathway.

The NADH/FADH$_2$ coenzyme and ATP yields from beta-oxidation depend on the length of the fatty acyl-CoA chain entering the process (Raven, et al., 2013). Two carbon fragments are cleaved during the process to subsequently create one acetyl-CoA molecule. In addition, there is a reduction of one NAD$^+$ and one FAD to NADH and FADH$_2$, respectively. The acyl-CoA, less two carbons, continues through the process until complete (i.e. palmitic acid completes the beta-oxidation sequence seven times). The resulting acetyl-CoA molecules enter the TCA cycle, as previously explained.
After a two-ATP activation step, the net result in ATP production utilizing fat as a substrate is much higher, at 106 (if utilizing palmitoyl-CoA), when compared to glucose (30-32 ATP).

Responses to Exercise

**Acute exercise (single bout)**

Kinetic regulation is driven by a rise in ADP. At the onset of exercise, intramuscular concentration of ATP immediately decreases, causing a rise in ADP (Kushmerick et al., 1992). This rise stimulates ATP synthesis at F₀F₁ ATP synthase complex during oxidative phosphorylation. This complex is driven by a proton motive force, and directly leads to a degradation of the H⁺ gradient in the inner membrane space. This gradient degradation stimulates ETC flux, leading to a decrease in NADH, and therefore an increased TCA cycle flux. This ultimately results in greater fuel utilization (Brooks, Fahey & Baldwin, 2005).

**Acute endocrine response.**

The onset of exercise stimulates catecholamine release. Epinepherine stimulates glycogen breakdown after binding to a G protein receptor, signaling a cAMP cascade and eventual amplified activation of phosphorylase (Brooks, Fahey and Baldwin, 2005). Concurrently, the catecholamines stimulate lipolysis in adipose tissue and IMTG (Horowitz & Klein, 2000). Catecholamines also inhibit insulin secretion by the pancreas (Wee et al., 2005). Inhibition of insulin is necessary during exercise for two reasons: it allows for a rise in lipolysis of triglyceride in adipose tissue, and also directs glucose delivery and uptake to working skeletal muscle, preventing storage of substrate.
Adipose tissue also secretes a variety of hormones that effect energy balance. One notable hormone, leptin, has a primary role to stimulate postprandial satiety by signaling the arcuate nucleus of the hypothalamus to reduce production of neuropeptide Y (NPY) (Jéquier, 2002). In addition, leptin enhances insulin sensitivity, promoting FFA oxidation in muscle tissue (Powers & Howley, 2012). According to Duclos et al, (1999), leptin levels decrease during and after an endurance exercise-induced negative energy balance. Simultaneously, an increase in plasma glycerol and FFA, indicative of lipolysis, is negatively correlated with decreased leptin levels.

**Chronic (training)**

Several metabolic factors are affected in response to training, leading to an increased utilization of fat during submaximal performance and overall increased oxidative capacity. Endurance training enhances the ability to oxidize more aerobic sources of fuel by increasing the ratio of oxidative to glycolytic muscle fibers (Baechle & Earle, 2008). In addition, many studies have concluded that training results in an increased dependence on fat as a fuel source at rest, noted by decreases in RMR (Henderson, 2014). On the contrary, fuel shifts to rely more on CHO during prolonged or high-intensity exercise (O’Brien et al., 1993) are shown by increases in glucose turnover rate and hexokinase activity, which lead to glycogen sparing (Donovan & Sumida, 1990).

 Decreased SNS activity and circulating levels of several hormones, including GH and catecholamines, affect substrate utilization (Brooks & Mercier, 1994). Less circulating epinephrine leads to glycogen sparing, and in turn, a decreased lactate response. Additionally, increased lipid and blood glucose utilization in response to training also leads to glycogen sparing. This attenuation is seen through a decrease in
LDH concentrations, and an increase in GLUT-4 and hexokinase in different types of skeletal muscle fibers (Baldwin et al., 1973; Brooks & Mercier, 1994).

An attenuated GH and catecholamine response to training would also decrease lipolysis of adipose tissue (Brooks, Fahey & Baldwin, 2004), and suggest an increase in use of IMTG-derived lipid substrate (Horowitz & Klein, 2000).

Early studies conducted by Holloszy (1967), and by Holloszy, Oscai, Don and Molé (1970), explain mitochondrial enzymatic responses to training, showing the upregulation of several enzymes involved in the ETC and TCA cycle (citrate synthase and isocitrate dehydrogenase), with concentrations doubling. Increases in ETC cytochrome concentration in several fiber types is also an adaptation in response to training (Holloszy & Booth, 1976). An increased use of oxidative pathways, and upregulation of citrate, inevitably downregulates PFK and increases glycogen sparing.

In addition to increases in TCA and ETC enzymes, metabolic adaptations to aerobic training include the upregulation of several beta-oxidation enzymes, including palmityl-CoA synthetase, CPTI and palmityl-CoA dehydrogenase, increasing the capacity for muscle to oxidize lipids 60% (Molé, Oscai & Holloszy, 1971). This, combined with increased PGC-1α activity leading to mitochondrial biogenesis, leads to an overall shift in substrate utilization toward fat (Baar et al., 2002). Increased mitochondrial mass leads to a lower work rate per mitochondrion, allowing for increased lipid utilization and slowing glycogen depletion and the onset of fatigue. Training induces increased fat oxidation, although not from increased lipolysis of adipose tissue, suggesting IMTG may play a greater role than previous research states (Horowitz & Klein, 2000).
After endurance training, absolute CHO utilization indeed decreases as stated above, but relative CHO utilization increases, both due to increase in mitochondrial mass and therefore an ability to achieve a higher relative work rate (Baar et al., 2002; Bergman et al., 1999).

Substrate Utilization During Exercise

Fuel selection

Both the intensity, duration and trained state of the individual will drive fuel selection. Overall carbohydrate use is dictated by metabolic demand set by exercise intensity (Friedlander et al., 1998b; Tarnopolsky, 2007), where lipid utilization is thought to be regulated (Roepstorff et al., 2006a; Tarnopolsky, 2007).

For a typical individual at rest, oxygen consumption is 3.5mL/kg body weight (Powers & Howley, 2012), and the majority of fuel is FFA derived from adipose tissue triglycerides (Horowitz & Klein, 2000). Glucose provides 4.2 kcals/g of chemical energy, whereas fat provides 9.1 kcals/g, making fat an appropriate substrate selection when oxygen consumption is low, and for maximization of energy storage compactness (Brooks, 2012).

As exercise begins and oxygen consumption increases, catabolism of fat is regulated by the rate of lipolysis, determined by level of catecholamines and glucagon stimulating the lipases. Lipolysis of adipose tissue increases, increasing plasma FFA availability for working muscle and providing the majority of lipid substrate at exercise onset (Horowitz & Klein, 2000). But, according to the crossover concept developed by George Brooks and Jacques Mercier (1994), the overall determination of which pool
(intra or extracellular) fat will be derived during an overall bout of exercise is determined by both intensity and duration. In addition, if exercise is conducted in a fasted state, lipolysis is less limited when compared to exercising after the consumption of glucose (Horowitz, Mora-Rodriguez, Byerley & Coyle, 1997). Consuming carbohydrate prior to exercise causes a rise in insulin, which decreases lipolysis and subsequently limits fat oxidation during exercise.

During low-intensity and prolonged exercise, the majority of fat will be derived from plasma FFA (Duclos et al., 1999), with minimal increases noted in glycerol R_s from working muscle, suggesting IMTGs role may be minimal (Brooks, Fahey & Baldwin, 2004). On the other hand, other studies utilizing muscle biopsies and FFA R_d vs. oxidation rates, have conversely suggested that IMTGs provide more than half of total fat oxidized during a prolonged bout of endurance exercise in trained individuals (Horowitz & Klein, 2000). It is agreed upon, however, that as duration increases, adipose-supplied FFA uptake into working muscle increases. As intensity increases, the uptake of plasma FFA decreases, and use of IMTG-derived fatty acids is maximal at roughly 65% VO_2max (Horowitz & Klein, 2000), contributing nearly 50 percent.

Maximal fat oxidation is likely reached at a relative intensity between 60-68% VO_2max for trained individuals (i.e. moderate intensity exercise) (Achten, et al., 2002). As more fast fibers are recruited, the demand for ATP increases exponentially. ATP turnover rates at the actomyosin ATPase are 2-4 times greater in Type IIa than Type I oxidative fibers (Hochachka et al., 1991). At intensities greater than 70-75% VO_2max, fuel selection must crossover from fat to CHO to meet ATP demand and fiber type recruitment resulting from an increased power output and need for non-oxidative energy
sources (Brooks & Mercier, 1994). At higher intensities, “7.7% more energy is liberated per unit oxygen consumed if CHO is the fuel compared to lipid” (Brooks, 2012). Additionally, the increased utilization of CHO also results from an increased catecholamine response proportional to exercise intensity.

CHO oxidation, and primarily an increase in lactate production with high intensity exercise, inhibits lipolysis by affecting pH (Brooks & Mercier, 1994). CPT1 inhibition by increases in malonyl-CoA production, a byproduct of glycolytic pyruvate synthesis, also results in a decrease of fatty acyl-CoA uptake into mitochondria (Nelson, Lehninger & Cox, 2008).

It is important to note that substrate utilization is affected by acute nutritional status. Results have been conflicting, however, exercise conducted under fasted conditions has been shown to result in a lower RER during exercise (Paoli et al., 2011). During the same study, exercise conducted postprandially (breakfast) had a significantly higher RER (0.96 vs. 0.84).

Sex differences in substrate utilization

Women have a greater utilization of lipid during exercise when compared to men (Friedlander et al., 1998a, 1998b and 1999; Tarnopolsky et al., 1990; Tarnoplosky, 2000; Tarnopolsky, 2007; Wu & O’Sullivan, 2011), and have a greater shift toward lipid utilization when performing exercise at the same relative intensity after a period of training (Friedlander et al., 1998b). Several studies have found that females have a lower RER during endurance exercise bouts, denoting an increased lipid utilization (Tarnopolsky et al., 1990; Tarnopolsky, 2000 and 2006). Friedlander et al. (1998b) also
noted the same findings, showing a decrease in RER and an increase in $R_a$ of glycerol at rest and after training.

To follow, females have an attenuated CHO and amino acid use in response to training. It has been suggested that females have a higher ratio of type I skeletal muscle fiber when compared to males, and following suit, a higher ratio of oxidative enzymes (Nygaard, 1981; Roepstorff et al., 2006). In addition, Friedlander, et al, discovered that males have a significantly higher concentration of catecholamines during and postexercise, promoting mobilization of glycogen and overall increased recycling of glucose.

Several studies have noted differences in SNS and adrenal medulla adaptations between sexes, seeing a higher utilization and production of epinephrine in males (Kjaer & Galbo, 1988), which may help to explain several of the substrate utilization adaptations.

Males tend to have a greater CHO oxidation during exercise (Bergman, et al., 1999), in particular glycogen utilization, even though both sexes have an equal amount of relative glycogen stores at rest (Henderson et al., 2007; Tarnopolsky et al., 1990). Zehnder et al., (2005) found that males not only had a greater glycogen utilization, but also had a higher overall IMTG utilization during a prolonged 3-hour exercise bout performed at 60-65% VO$_2$max, and found no difference between sexes in glycogen use and relative contributions of CHO and fat during exercise, which contradicts many studies and adds to the paradigm. It has been generally accepted that females have a greater utilization of IMTG during moderate intensity exercise and is thought to be due to
a higher overall IMTG content in the form of higher lipid droplet abundance (Henderson et al., 2007; Horowitz & Klein, 2000; Roepstorff et al., 2006b; Tarnopolsky, 2007).

Although females utilize more lipid during submaximal exercise compared to men, it is generally more difficult for women to alter body composition, as it is evolutionarily favorable to maintain fat mass above 12-15% to maintain menstruation (Powers & Howley, 2012; Wu & O’Sullivan, 2011). When comparing nine weeks of daily, self-selected exercise bouts, male rats had a lower whole body mass and fat mass compared to female exercised rats, even though the female rats had a higher overall energy deficit (Cortright et al., 1997).

Leptin plays a large role in both the maintenance of fat mass and the production of biologically active estrogen. More adiposity correlates to a greater leptin production, and a decreased leptin production is related to fat loss (Wu & O’Sullivan, 2011). In addition, concentration of circulating estrogen is positively associated with leptin production, although the exact mechanism of action has yet to be discovered.

Endogenous ovarian hormone variations between estradiol and progesterone peaks during a normal menstrual cycle may not affect whole body substrate utilization during or after exercise, perhaps debunking earlier research findings (Casazza et al., 2004). Fluctuations between the follicular phase and luteal phase have no significant impact on lipolysis (measured by glycerol $R_{a}$), glucose or lactate concentrations during a bout of exercise.

Estradiol contributes to the differentiation in substrate selection between sexes. Estradiol decreases FFA oxidation postprandial, promoting an increase in fat mass (Wu & O’Sullivan, 2011). It can also be attributed to an attenuation of glycogen
utilization during exercise (Tarnopolsky, 2007). After the administration of 17-β-estradiol to men, the following observations were noted: a decrease CHO and amino acid utilization during exercise, increase fat utilization, and alter mRNA transcription factors associated with lipid metabolism (but not glucose metabolism) to mimic rates seen in females (Tarnopolsky, 2007).

Substrate Utilization During the Postexercise Recovery Period

Excess Postexercise Oxygen Consumption (EPOC)

After the cessation of exercise, there exists an increase in oxygen uptake relative to RMR, where VO$_2$ remains elevated for some time (Paoli et al, 2011). EPOC is affected primarily by intensity and duration of exercise and demonstrates a curvilinear relationship, where longer and/or more intense bouts of exercise (greater than 50% VO$_2$max) promote a prolonged and more sizable EPOC (Børsheim & Bahr, 2003). It has been found that shorter bouts of exercise (approximately 5-40 minutes) containing a lack of strenuous exercise result in little to no EPOC beyond 40 minutes of exercise cessation.

When overall work is maintained but intensity is changed during a bout of cycling exercise, EPOC rises (Phelain et al.,1997). Additionally, split aerobic exercise sessions (where work is maintained) at a moderately to vigorous intensity may lead to a higher combined EPOC.

Investigations looking specifically at females have followed suit with the conclusions stated above and have shown little difference between sexes, although some studies have noted differences in EPOC during different phases of the menstrual cycle.
(Børsheim & Bahr, 2003). Imamura et al. (2004) investigated EPOC specifically in females, using separate 30 and 60 minute bouts of exercise conducted at 60% VO$_2$max. A significant difference in EPOC was found between the trials and seems to be correlated with the intensity of catecholamine response and related to the duration of exercise and FFA availability and oxidation during and after exercise (promoting EPOC).

**Postexercise recovery**

IMTG is thought to be primarily utilized during prolonged exercise that results in glycogen depletion (Horowitz & Klein, 2000), and therefore must be restored during postexercise recovery. In response to a prior bout of exercise, resting energy expenditure (REE) and lipid oxidation increases for nearly 24-hours and potentially longer into the next day (males), replenishing used stores and triggering a drop in RMR (Friedlander et al., 1998b; Jamurtas et al., 2004). It is also believed that and increased REE is a result of increased protein synthesis and/or thermogenesis.

It is important to note hormonal effects during and after acute bouts of exercise. GH secretion rises during the onset of exercise, and the higher the intensity, the greater the [GH] (Wee et al., 2005). This may be due to several factors attributed to exercise response, such as increased catecholamine and lactate levels. Relevant to this study, GH secretion has a postexercise effect and could stimulate lipolysis in adipose tissue and lipid mobilization 2-3 hours after release (noted predominantly by increased glycerol and FFA in the bloodstream), and a continued increase in lipolysis one hour into recovery. This assumption is supported by the findings Henderson et al. (2007), where postexercise [GH] was significantly higher in males when compared to the resting control group.
The rise in FFA and glycerol could also be attributed to a continued rise in catecholamines, particularly norepinephrine, immediately following exercise cessation in males (Dimsdale et al., 1984; Young et al., 1992). Henderson et al. (2007) also reached the same conclusion, denoting a significant rise in noradrenaline during the postexercise recovery period when compared to control.

Whether or not exercise is conducted postprandially may have an affect on postexercise VO$_2$ and lipid oxidation. Studies using males have noted that exercise conducted in a fasted state will result in a lower RER during exercise when compared to a postprandial state (Paoli et al., 2011). However, during postexercise recovery, VO$_2$ is higher and RER is lower in the postprandial condition, indicating a higher lipid oxidation rate. The study also found this increase lipid oxidation lasted 24 hours, even after resuming a normal diet.

Kuo et al. (2004) compared male and female substrate utilization before, during and after two separate bouts of exercise (89 minutes at 45% and 60 minutes at 65% VO$_2$peak, respectively). Both sexes had a significant increase in lipid oxidation during recovery when compared to a time-matched resting control group, with no significance attributed to the intensity of the exercise bout for both sexes. A study conducted by Imamura et al. (2004) using only females had shown a significant rise in serum FFA immediately following a 60-minute bout of exercise, but not a 30-minute bout of exercise. In addition, significant increases in glycerol were noted after both exercise bouts, but significantly higher after the 60-minute bout.

To further inquire, Henderson et al. (2007) found that males and females significantly differ in the length of time increased postexercise lipid oxidation exists
before returning to baseline. Noted by the $R_a$ of glycerol (to denote lipolysis of extramuscular sources) and the $R_a$ of FFA, when compared to a control group, males have a significantly greater lipid oxidation rate into the next day (Day 2 measurement taken 21 hours after cessation of exercise), before returning to baseline. However, in females, lipid oxidation rates return to baseline in approximately three hours. Utilizing the same protocol as in 2007, Henderson et al. (2010) discovered that females have an increased postexercise plasma triglyceride clearance, with no significant difference found between exercise intensities.

What has not been identified is whether or not shorter, split bouts of exercise will result in similar findings, and whether or not multiple bouts (split sessions) conducted within the same day will have a similar affect.
CHAPTER III

METHODOLOGY

The purpose of the study was to determine if total postexercise lipid oxidation in recreationally athletic females is affected by frequency of exercise (1 vs. 2 bouts) while keeping total workload the same (60 minutes at 65% VO$_2$max). That is, one bout of exercise for 60 minutes at 65% VO$_2$max, or two bouts of exercise for 30 minutes at 65% VO$_2$max, separated by five hours. An exercise intensity of 65% VO$_2$max was chosen because it is moderate intensity, and maximal fat oxidation is found to be between 60-68% for trained individuals (Achten et al. 2002).

Design of the Investigation

The study was set up using a quantitative experimental crossover design, with a time-matched control:

ROX$_1$OX$_2$O

ROX$_2$OX$_1$O

Subjects were randomly allocated to each treatment and participated as their own control group. This design assisted in increasing internal validity of the study.

Population and Sample

Seven healthy female recreational athletes (not sedentary or elite/professional) were recruited from the Chico, California community and campus. Cyclists were preferred, then runners followed by other endurance-type athletes (i.e. swimmers).
Recruitment was conducted by word of mouth, social media, and fliers posted on the CSU Chico campus and at various local bicycle and running shops. A health history questionnaire was administered to ensure subjects met the following criteria:

Inclusion criteria:

- Female
- Age: 19-35 years
- Hours of endurance activity/week: 5-10 hours/week, 4+ exercise sessions/week
- Type of activity: running, cycling
- BMI <28
- Must be eumenorrheic

Exclusion criteria:

- Body fat less than 12%.
- Medications that effect energy metabolism
- Diabetics/pre-diabetics
- Smoking
- Excess alcohol use
- Subjects with orthopedic and/or cardiovascular contraindications to exercise

The subjects were requested to refrain from caffeine and alcohol consumption 24 hours before each trial. Subjects were also asked to fast for 12 hours before each exercise trial, and perform the trials early in the morning.

Subjects were tested between days 3-8 of the follicular phase their menstrual cycle to avoid significant fluctuations in hormones that could potentially affect lipolysis.
The subjects either: 1). Performed the control and both trials within the 3-8 day window (48-72 hours apart), or 2). Perform all three sessions across a one-month period.

Location of the Study

The study was conducted in the Department of Kinesiology Human Performance Laboratory (Yolo, Room 132) at CSU Chico in Chico, CA.

Length of the Study

The study was conducted between May 1, 2014 and August 31, 2014.

Treatment

The subjects met three times. Initial prescreening included body composition analysis via air displacement plethysmography (BodPod, COSMED USA Inc., Concord, CA), BMI, a one-hour resting control period, and determination of VO$_2$max utilizing a cycle ergometer (VO$_2$max will also be scaled per kg body weight, and to kg lean body mass). The subjects were then expected to perform a moderate-intensity cycle exercise on two additional separate occasions during the follicular phase of their menstrual cycle (days 3-8). They conducted the prescreening and trials either: 1.) Within the 6-day window spaced 48-72 hours apart, or 2.) One month apart within the same 6-day follicular phase window. It was imperative that the subjects maintained qualification for participation if waiting one month between trials.

The initial prescreening included a one-hour sedentary control period. The subjects remained seated for one hour. Resting VO$_2$ and RER were measured every 15 minutes for 5-minute periods for a total of five samples over the duration of the hour.
The subjects were not permitted to stand/move around. Magazines or other reading material were provided upon request. A wheelchair was utilized for travel to the restroom, if requested.

VO₂max was assessed using open-circuit indirect calorimetry employing a Parvo Medics TrueOne 2400 metabolic measuring system (Parvo Medics, Sandy, UT). Subjects performed a graded exercise test (GXT) on an electrically-braked cycle ergometer (Monark 839E, Stockholm, Sweden). The test subjects were given a 5-minute warm-up. The subjects then began the GXT. Subjects pedaled at their self-selected cadence starting at 50 watts. Power output was increased in 50 watt increments up to 200 watts, and in 25 watt increments thereafter until volitional fatigue. Heart rate was measured at the end of every stage using a standard heart rate monitor around the chest with corresponding wristwatch (Ekho, Dallas, TX).

During each of the three exercise trial periods, gas collection was completed using a Hans Rudolph T-Shape Two-Way Non-Rebreathing Valve and stabilizing headgear and nosepiece. Gas analysis was completed using a Parvo Medics TrueOne 2400 metabolic measurement system. Heart rate was collected at rest and every 5 minutes throughout the exercise. To collect a resting sample, the subject was seated quietly in a chair. A 5-minute gas sample was collected and analyzed. Next, the subject was given a 5-minute warm-up followed by the commencement of the exercise trial (time = 0 min). Gas analysis was conducted every 15 seconds during the exercise bout until completed. The subject was then given a 5-minute cool down plus 10 minutes to change attire and utilize the restroom. The postexercise recovery collection period then began.
once the subject was seated. Gas collection/analysis was conducted at 15-minute intervals for 5-minute periods for a total duration of three hours postexercise.

Subjects were assigned to exercise trial days (60-minute bout or two 30-minute bouts) in random order. Each exercise bout will be performed at 65% VO$_2$max to maintain an equal amount of work performed between trials. Subjects were given a 5-minute warm-up period before commencing each exercise bout, however, the warm-up time was not included in the total measured time of the exercise bout so total work performed would not be affected. Subjects were able to listen to music of their choice while conducting exercise. Subjects drank water ad libitum.

**60-minute Trial.**

The subjects exercised for 60 minutes at 65% VO$_2$max, followed by a 5-minute cool down plus 10 minutes to change attire (Figure 1). Immediately after, the subject remained sedentary for three hours, seated. Reading material was provided upon request. Water was consumed ad libitum, however, consuming food or nutritional beverages was prohibited.

**30-minute Trials 1 and 2.**

The subjects performed two 30-minute bouts of exercise at 65% VO$_2$max, each followed by a 5-minute cool down plus 10 minutes to change attire (Figure 2). The first 30-minute bout and cool down was immediately followed by a three-hour sedentary period with the same limitations as described for the 60-minute trial. The subjects were then given a 2-hour break. During the break, the subjects were able to consume food, however, it was recommended to refrain from consuming high-fat foods and simple sugars. It was recommended subjects choose items that have a lower caloric value but
remain nutrient-dense, such as fruit, vegetables, whole grains, etc. Upon return to the laboratory, the subjects completed the second 30-minute bout plus three-hour sedentary period in the same manner as stated above.

Figure 1. 60-minute Trial Protocol. Experimental protocol for single exercise session, one 60-minute bout of exercise at 65% VO\(_2\)max, followed by a 3-hour postexercise recovery period. Each participant completed the two exercise trials in randomized order.

Data Analysis

The following equations were used to determine the rates of substrate oxidation (Peronnet & Massicotte, 1991):

\[
\text{CHO oxidation (g/min)} = [4.59 \times \text{VCO}_2 \text{ (L/min)}] - [3.23 \times \text{VO}_2 \text{ (L/min)}]
\]

\[
\text{Lipid oxidation (g/min)} = [1.69 \times \text{VO}_2 \text{ (L/min)}] - [1.70 \times \text{VCO}_2 \text{ (L/min)}]
\]

Resting control trial values were calculated using the last three of five sampling periods, omitting the first 30 minutes of the one-hour resting trial. This was done to correct for high RER values produced as a result of excitement. To control for time during exercise and allow for comparison, the final two sampling periods (30 minutes) of the 60-minute trial were used for analysis.
Figure 2. 30-minute Trials Protocol. Experimental protocol for split exercise sessions, two 30-minute bouts of exercise at 65% VO\textsubscript{2}max, each followed by a 3-hour postexercise recovery period. Each participant completed the two exercise trials in randomized order.

Statistical analyses were performed using both Microsoft Excel for Mac 2011, Stata 12.0, SPSS and Vassar Stats. Statistical significance (\(\alpha\)) was set at \(p < 0.05\).

A comparison was made between the sum of the two 30-minute bout postexercise recovery periods (sum of split exercise session substrate oxidation rates) and the 60-minute bout postexercise recovery period. Areas under the curve were calculated and analysis was conducted using a paired t-test. Factor analyzed were lipid oxidation and CHO oxidation rates (g/min).

Statistical analyses were conducted using analysis of variance with repeated measures with post hoc comparisons using the Bonferroni correction to determine significance between control values and each of the timepoints for the three exercise...
trials. Comparisons were made of the following: lipid oxidation, CHO oxidation, RER and VO₂.

Statistical analyses using area under the curve and one-way analysis of variance with repeated measures were conducted to determine significance between each of the three postexercise periods. Comparisons were made of the following: lipid oxidation, CHO oxidation, and RER.
CHAPTER IV

RESULTS AND DISCUSSION

Presentation of the Findings

Subject characteristics

Physical characteristics of study participants (n=7) are listed in Table 1. The mean age (years) was 27.7 ± 4.7 and VO\textsubscript{2}max (ml/kg min) was 44.6 ± 3.6. The mean body fat (%) was 24.6 ± 6.8.

Table 1.

Characteristics of Study Participants

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>27.7 ± 4.7</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>168.5 ± 4.9</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>64.7 ± 6.8</td>
</tr>
<tr>
<td>BMI (kg/m\textsuperscript{2})</td>
<td>22.6 ± 2.4</td>
</tr>
<tr>
<td>Body Fat (%)</td>
<td>24.6 ± 6.8</td>
</tr>
<tr>
<td>VO\textsubscript{2}max (L/min)</td>
<td>2.9 ± 0.4</td>
</tr>
<tr>
<td>VO\textsubscript{2}max (ml/kg min)</td>
<td>44.6 ± 3.6</td>
</tr>
<tr>
<td>VO\textsubscript{2}max (mL/kg FFM min)</td>
<td>59.1 ± 4.6</td>
</tr>
<tr>
<td>65% VO\textsubscript{2}max (ml/kg min)</td>
<td>29.0 ± 2.4</td>
</tr>
<tr>
<td>65% VO\textsubscript{2}max (watts)</td>
<td>131.9 ± 20.2</td>
</tr>
<tr>
<td>HRmax (bpm)</td>
<td>179.9 ± 2.7</td>
</tr>
</tbody>
</table>

Values are means ± SD; n = 7 females.

Substrate Oxidation

A significant difference in both lipid oxidation (p < 0.0001) and CHO oxidation (p < 0.005) was found between the 60-minute trial postexercise recovery period and the sum of the split sessions (two 30-minute trial recovery periods) (Figure 3).
Figure 3. Substrate Oxidation During the Postexercise Recovery Period. Substrate oxidation (g/min) between one vs. two bouts of exercise. Values are AUC ± standard error. * Significant difference in CHO oxidation between 60-minute single trial and sum of two 30-minute trials (p < 0.0001); # significant difference in lipid oxidation between 60-minute single trial and sum of two 30-minute trials (p < 0.005).

Significant differences in CHO oxidation were found between exercise and control, and between exercise and postexercise timepoints (p < 0.017) (Figure 4a). A significant difference in lipid oxidation was found between exercise and postexercise (p < 0.046). No significant differences in lipid or CHO oxidation were found between the control trial and any of the timepoints during three postexercise recovery periods (Figure 4a-d). Strong trends were evident. An increase in lipid oxidation was seen between resting control and postexercise recovery (Figure 4c). A trend in CHO oxidation is seen as well, showing a decrease between control and the recovery period (Figure 4d).
Figure 4a.

Figure 4b.
Figure 4c.

Figure 4d.
Figure 4. Substrate oxidation (Lipid and CHO) vs. Time. Values are substrate oxidation rate (g/min) ± standard error. (a) Substrate oxidation during rest, exercise, and postexercise recovery for all three exercise trials, and during resting control. Timepoint 0 = rest. Timepoints 15 and 30 are samples of 30 minutes of exercise. For the 60-minute trial, data from the second 30 minutes of exercise were used. Timepoints 45 through 210 are data representing three-hour postexercise period. a: Significant difference in CHO ox between exercise timepoints, control, and all postexercise timepoints (p < 0.017); b: Lipid oxidation significantly lower than exercise during 30-min Trial 2 (p < 0.046). (b) Substrate oxidation during the postexercise recovery period only. No significant differences found between timepoints. (c) Lipid oxidation and (d) CHO oxidation during the postexercise recovery period, shown individually.

Figure 5. Substrate Oxidation Between Trials. Comparison of substrate oxidation (g/min) during the postexercise recovery period of individual trials. Values are AUC ± standard error. No significant differences were found in lipid oxidation or CHO oxidation between the three individual trials.
Utilizing area under the curve and a one-way analysis of variance with repeated measures, no significant differences in lipid oxidation or CHO oxidation were found when comparing the three postexercise recovery periods (Figure 5).

**VO₂**

VO₂ (L/min) significantly increases during exercise (p < 0.001) and returns near to baseline during the postexercise recovery period (Figure 6a). No significant differences in VO₂ were found between the control trial and any of the three postexercise recovery periods (Figure 6b), however a trend is evident, showing a decrease in VO₂ during the postexercise recovery period of the second 30-minute trial and 60 minute trial.

![Graph showing VO₂ vs. Time (Rest, Exercise and Postexercise)](image)

Figure 6a.
Figure 6. Oxygen Consumption (VO₂) vs. Time. Values are means ± standard error. (6a) VO₂ during rest, exercise, and postexercise recovery for all three exercise trials, and during resting control. Timepoint 0 = rest. Timepoints 15 and 30 are samples of 30 minutes of exercise. For the 60-minute trial, data from the second 30 minutes of exercise were used. Timepoints 45 through 210 are data representing three-hour postexercise period. * Significant difference between exercise timepoints and each postexercise timepoint, and the control trial (p < 0.001). (6b) VO₂ during the postexercise recovery period only. a: Significant difference in VO₂ between timepoints (p < 0.014). No significant differences found between control and postexercise timepoints.

RER.

RER during the resting control trial was 0.85 (Figure 7). For each of the three exercise trials (30-minute trial one, 30 minute trial two, and the 60 minute trial), RER was 0.86. During exercise, RER increased to 0.93, 0.95 and 0.90 for each trial, respectively. Upon onset of the postexercise sampling period, RER decreased to 0.78,
0.81 and 0.76 for each trial, respectively, and increased to 0.82, 0.80 and 0.83, respectively.

Figure 7. RER vs. Time. Values means ± standard error. RER during rest, exercise, and postexercise recovery for all three exercise trials, and during resting control. Timepoint 0 = rest. Timepoints 15 and 30 are samples of 30 minutes of exercise. For the 60-minute trial, data from the second 30 minutes of exercise were used. Timepoints 45 through 210 are data representing three-hour postexercise period. a and b: 30 min Trial 1 – Like letters denote a significant difference between exercise and postexercise timepoint (p < 0.049). c: 30 min Trial 2 – Like letters denote a significant difference between exercise and postexercise timepoint (p < 0.048); d: Significantly different from 165 min timepoint (p < 0.001); e: Timepoints are significantly different (p < 0.031). f and g: 60 minute trial – Like letters denote a significant difference between exercise and postexercise timepoint (p < 0.049). No significant differences were found between control and postexercise.

Significant differences were found between exercise and several postexercise timepoints (p < 0.049) (Figure 7). Although there were no significant differences found between the control trial and any of the three postexercise recovery periods, an overall
decrease is seen. No significant differences in RER were found when comparing between the three postexercise recovery periods.

Discussion of the Findings

These results may indicate that split exercise bouts of identical workload may lead to an overall greater postexercise lipid oxidation when combined, compared to a single exercise session. When combined, the split exercise bouts had a higher total postexercise lipid oxidation than the single session of equal work (p < 0.000).

To analyze the trend established by the results, an increase in lipid oxidation is seen during all three trials, lasting approximately three hours postexercise (or the majority of the duration of the sampling period) before returning to near baseline conditions. Overall duration of increased lipid oxidation during the day containing split exercise sessions was nearly double.

Although no significant difference in lipid oxidation rates were found between control and postexercise across time, or between the postexercise portion of the three trials, a strong trend is seen and meaningful differences must be addressed (Figure 4c). Overall, the three postexercise periods have a higher lipid oxidation rate than during resting control. Further analysis shows the first 30-minute trial having the smallest rise in postexercise lipid oxidation, the second 30-minute trial to have a higher lipid oxidation rate than the first trial, and the 60-minute trial to have the highest.

Through the first 60 minutes of postexercise sampling, the 60-minute trial remains consistently higher than the 30-minute trials, and then looks similar to the second 30-minute trial until the end of the three-hour sampling. The first 30-minute trial remains
consistently lower than both other exercise trials. The last hour of postexercise sampling shows all three trials to be similar, returning to baseline by the last sample timepoint.

The opposite is true for CHO oxidation. Although no significant differences were found between control and postexercise across time, or between the postexercise portion of the trials, when comparing the rates, a trend is observed here as well. All three postexercise periods have a lower oxidation rate than during resting control. To analyze the response further, the last 105 minutes of all three trials are nearly identical (Figure 4d). However, at the onset of recovery, the 30-minute trials have a higher CHO oxidation rate than the 60-minute trial, the differences most noted between 30 and 60 minutes into recovery sampling (timepoints 75, 90 and 105 on Figure 4d).

There was no significant change in VO$_2$ between the control and postexercise trials, however fuel selection did change. It has been noted in previous studies that a prior bout of exercise may lead to a depressed RER at rest (Henderson & Alderman, 2014), which aligns with these findings. Although no significant differences in RER between the control and any postexercise timepoints were found, existing trends must be noted, especially considering the complimentary trends seen in substrate oxidation. The RER of the 60-minute trial is lower than both 30-minute trials through the first hour of the postexercise period, the second 30-minute trial being generally lower than the first. The second 30-minute trial then shows the lowest RER during the last 90 minutes of the recovery sampling period when compared to the other two trials.

In addition, the majority of the postexercise RER’s were significantly different than exercise, although resting control was not. The mean resting control RER was higher (0.85) than all of the postexercise timepoint means. A larger sample size
could be expected to increase the probability of a difference between control and postexercise.

Although not all studies have reached the same conclusion, if females utilize more IMTG during exercise (Tarnopolsky et al., 1990; Wu & O’Sullivan, 2011), and IMTG is restored via adipose triglyceride lipolysis and subsequent FFA uptake from blood, this could explain why a rise in lipid oxidation is noted during recovery (Henderson et al., 2007; Roepstorff et al., 2006b). In addition, females have an increased postexercise plasma triglyceride clearance, with no significant difference found between exercise intensities (Henderson et al., 2010). These findings could explain why less lipid oxidation is seen during the first 30-minute trial, as less fuel is being used when compared to the duration of the 60-minute trial.

It has been also established that above-baseline levels of norepinephrine during postexercise recovery leads to an increased rate of lipolysis of triglyceride in adipose tissue, however, much less significantly in females (Henderson, 2014). Due to the shorter duration of the individual split sessions without alteration of intensity, less fuel is consumed, and an attenuated norepinephrine response could account for the lower rate of lipid oxidation during the single bouts (Imamura et al., 2004). In addition, it is unclear on whether or not the second 30-minute bout elicited the same postexercise norepinephrine response as the first, which could or could not account for the higher postexercise lipid oxidation rate seen.

Also, the first 30-minute bout was conducted fasted, while the second session was conducted approximately 2 hours postprandial. This could account for the consistent trend seen in the results, showing a higher rate of lipid oxidation during the second 30-
minute session recovery period, as it has been show that lipid oxidation may increase after a prior bout of exercise that is conducted postprandially (Henderson & Alderman, 2014; Paoli et al., 2011).

Imamura et al. (2004) had found a significant difference in EPOC between two exercise trials conducted at 60% VO$_2$max, one with a duration of 30 minutes and the other lasting 60 minutes. A significant increase in serum glycerol was noted immediately following both trials, as well as 30 minutes into the postexercise recovery period of the 60-minute trial. However, compared to a one-hour resting control period, there was only a significant rise in postexercise FFA following the 60-minute bout of exercise. This, along with the presented findings, may suggest that a single 30-minute bout of exercise at 65% VO$_2$max may not be a sufficient enough duration to significantly affect whole body lipid oxidation, however, a combination of two split sessions may indeed enhance whole body lipid oxidation to a greater total, as seen in the results of this investigation.

Strong trends in the data presented in this study must be addressed. The reliability of utilizing gas samples to obtain data may raise question to reliability of the data, as measuring the R$_a$ of glycerol and FFA to discern substrate oxidation rates would be more accurate. A larger sample size may have resulted in less variability between subjects and more significant findings between postexercise substrate oxidation rates and RER when compared to the resting control trial. However, due to a violation of sphericity, conservative adjustments were utilized when running the tests of within-subjects effects, to include a Bonferroni post-hoc analysis. A larger sample size would be beneficial and may negate the possibility that a type II error occurred ($\beta = 0.80$).
CHAPTER V

SUMMARY, CONCLUSIONS AND RECOMMENDATIONS

Summary and Conclusions

It may be inferred from the findings that a single 30-minute bout of exercise may not be a sufficient enough duration to significantly effect whole body lipid oxidation. However, if two bouts are conducted in one day, and total work is equal to a single 60-minute bout of exercise at the same intensity, total lipid oxidation could be expected to be greater. Because the combined postexercise periods of the split sessions are nearly double in length, providing a greater use of lipids, it could be concluded that split sessions may allow females to maximize potential alterations to resting metabolism and sequester positive health benefits (Henderson, 2014).

Research Recommendations

Although there are no significant differences between control substrate oxidation and postexercise substrate oxidation during single 30-minute exercise sessions, a trend exists, and it has been noted that split exercise sessions may elucidate an increase in lipid oxidation, even though the duration is short. A larger sample size could show significance in the apparent trends. It could be suggested that a higher intensity would lead to an increase in postexercise lipid oxidation rate derived from extramuscular
sources if split exercise sessions are chosen (Børsheim & Bahr, 2003; Imamura et al., 2004; Phelain et al., 1997).

There are several future studies that could reveal differences in lipid oxidation and lipolysis during exercise and postexercise recovery period. A similar study incorporating the affects of high-intensity interval training is recommended. Many positive responses to high-intensity interval training (HIIT) have been consistently established, such as increases in various mitochondrial proteins, improvements in blood pressure and endothelial function (Gaesser & Angadi, 2011). An investigation comparing sex differences and the postexercise response to sustained moderate-intensity exercise would be an appropriate follow-up.

Investigations using postmenopausal women may show a difference in lipolysis postexercise. Altitude may also have an effect different than the effects illustrated here or in previous studies. Additionally, it has been shown that L-carnitine supplementation shifts fuel selection toward lipid during rest, preventing weight gain (Stephens et al., 2013). It is unknown what the effects are during the postexercise recovery period, but the effects may be beneficial to body composition maintenance or fat mass loss.
REFERENCES


bouts of different intensity. *Journal of the American College of Nutrition*, 16(2), 140-146.


April 11, 2014

Jennifer Barker
260 E. Lassen Avenue #10
Chico, CA 95973

Dear Jennifer Barker:

As the Chair of the Campus Institutional Review Board, I have determined that your research proposal entitled "LIPID OXIDATION IN FEMALES DURING THE POSTEXERCISE RECOVERY PERIOD: ONE VS. TWO BOUTS OF EXERCISE" has been granted clearance through an expedited review. This clearance allows you to proceed with your research.

I do ask that you notify our office should there be any further modifications to, or complications arising from or within, the study. In addition, should this project continue longer than the authorized date, you will need to apply for an extension from our office. When your data collection is complete, you will need to turn in the attached Post Data Collection Report for final approval. Students should be aware that failure to comply with any HSRC requirements will delay graduation. If you should have any questions regarding this clearance, please do not hesitate to contact me.

Sincerely,

John Mahoney, Ph.D., Chair
Human Subjects in Research Committee

Attachment: Post Data Collection Report

cc: John Azevedo (330)
To Project Participant:

You are invited to take part in a research project conducted by Jennifer L. Barker at California State University, Chico. In this study we hope to learn more about lipid oxidation (fat use/burning) during the postexercise recovery period, specifically in females.

**Purpose.** We hope that our research will lead to an increased understanding of sex differences regarding fuel use during the postexercise recovery period. We will be analyzing the duration of fat oxidation (fat burning) after conducting either one or two bouts of exercise to see how total fat oxidation is affected. This may lead to information that could better strategize exercise prescriptions, potentially promoting fat loss and a decreased risk of cardiovascular disease.

**Participants.** You were selected to participate in this study because you met all desired criteria for qualification. Criteria include:

- Female
- Age: 19-35 years
- Hours of endurance activity/week, preferably cycling and/or running 5-10 hours/week, 4+ exercise sessions/week
- BMI: <28
- Must be eumenorrheic (normal menstruation)

Exclusion criteria:

- Body fat less than 12%
- Medications that effect energy metabolism
- Diabetics/pre-diabetics
- Smoking
- Excess alcohol consumption
- Subjects with orthopedic and/or cardiovascular contraindications to exercise

**Procedures.** The subject will meet a total of three times at the California State University, Chico campus. Initial prescreening will include body composition analysis utilizing the BodPod, BMI, a time matched sedentary control period, and determination of VO₂max utilizing a cycle ergometer (VO₂max will also be scaled per kg body weight, and to kg lean body mass). The subject will be required to fast for 12 hours before each exercise trial and refrain from alcohol and caffeine consumption for 24 hours before each trial. The subject will be expected to perform a cycle exercise at a moderate intensity on two additional separate occasions during the follicular phase of their menstrual cycle, either 1.) within a 3-8 day window, or 2.) one month apart. During one trial, the subject will exercise for 60 minutes at a moderate intensity (65% VO₂max). During the second trial, the subject will perform two 30 minute bouts of exercise at a moderate intensity (65% VO₂max), with a three hour sedentary period plus two additional hours in between each 30 min bout. Trials will be randomized.

**Risks.** The subject understands that the exercise may be uncomfortable and there are risks associated with any exercise. Although very rare, the risk of muscle or ligament strain, sprain or broken bones can occur due to exercise. The subject understands that they will be wearing headgear with a mouthpiece for each trial, as well as a nose clip on the nose to assure that all expiratory airflow goes into the mouthpiece and spirometer for gas collection/analysis.
subject understands that this may cause some discomfort while exercising on a cycle ergometer (e.g. excessive production of saliva, mouth may become dry).

We do not expect any adverse medical effects to occur. In the event of an injury or illness as the result of participation in this research project, an enrolled/eligible student may seek basic medical and/or mental health care within the scope of the services of the Student Health Center (SHC), as authorized by the Trustees of the California State University, during the Student Health Center’s normal operating hours, or see a personal/outside health care provider for care and treatment. For care beyond the scope of services of the SHC, subjects must seek care and treatment from an outside/personal health care provider. A non-student subject is only eligible to receive basic first-aid care from the SHC during its normal operating hours and will need to seek care beyond first-aid from an outside/personal health care provider. In all cases, in the event of need for emergency medical care, 911 will be called. Any and all incurred health care costs associated with participation in this research project are the responsibility of the subject.

Confidentiality. Reports resulting from this study will not identify you as a participant. All information gathered in this study will remain confidential and be given out only with your permission or as required by law. If you give us permission by signing this consent form, we will protect your confidentiality. All files will be kept in the adviser’s or other department office, although anonymous data files may be used at the primary investigator’s home during the project period. After publication of this study, all personally identifiable information will be destroyed.

Benefits. There will be an end of thesis random drawing for a $40 gift card to Fleet Feet Sports or North Rim Adventure Sports (winner’s choice).

Right to refuse or withdraw. By signing this consent form you indicate that you have read the form and agree voluntarily to participate in the study. If you choose not to take part there will be no penalty or loss of benefits to which you are entitled. If you agree to take part, you are free to withdraw from it at any time. Likewise, no penalty or loss of benefits to which you are otherwise entitled will occur.

If you have any questions about this research at any time, please call the primary investigator Jennifer L. Barker at 419-656-9409 or write her at jbarker27@yahoo.com or 260 E Lassen Ave, Apt 10, Chico, CA 95973. A free copy of the published results/conclusions of the study may be requested by the subject.

I agree to participate in LIPID OXIDATION IN FEMALES DURING THE POSTEXERCISE RECOVERY PERIOD: ONE VS. TWO BOUTS OF EXERCISE as set out above.

Participant Name

______________________________
Participant Signature ________________________________ Date

Investigator Name

______________________________
Investigator Signature ________________________________ Date

THIS PROJECT HAS BEEN REVIEWED BY THE CALIFORNIA STATE UNIVERSITY, CHICO INSTITUTIONAL REVIEW BOARD FOR THE PROTECTION OF HUMAN SUBJECTS IN
RESEARCH. ADDITIONAL CONCERNS AND COMPLAINTS, OR QUESTIONS REGARDING YOUR RIGHTS AS A RESEARCH PARTICIPANT, SHOULD BE DIRECTED TO THE DEAN OF GRADUATE STUDIES AND RESEARCH (Phone number: 323-343-3798).
CALIFORNIA STATE UNIVERSITY, CHICO
GRADUATE THESIS: LIPID OXIDATION IN FEMALES DURING THE
POSTEXERCISE RECOVERY PERIOD: ONE VS. TWO BOUTS OF EXERCISE

MEDICAL AND EXERCISE HISTORY

NAME__________________________
DATE__________________________

BIRTHDATE___________________     AGE_____    HEIGHT_______
WEIGHT______

*Investigator use only: Calculated BMI: ______   Body composition: ______   Subject ID# ______

1. How many sessions of exercise do you have per week? (circle one) 1-2 3-4 5-6 7+

2. On average, what is the duration of a typical exercise session for you? (circle one) 10-20 30-60 60+ min/session

3. Describe the intensity of your exercise (circle one)
   1 = none
   2 = light (e.g. casual walking, golf)
   3 = moderate (e.g. brisk walking, jogging, cycling, swimming)
   4 = heavy (e.g. running, high intensity sport activity)

4. What types of exercise do you engage in? (circle all that apply)
   1 = walking
   2 = jogging/running
   3 = swimming
   4 = cycling
   5 = team sports (i.e. basketball, softball, soccer, etc.)
   6 = racquet sports
   7 = weight training
   8 = cardiovascular machines (i.e. elliptical, stair climber, etc.)
   9 = other ________________________________________________________________

5. How much time per week is dedicated to each type of exercise circled in #4 above?
   1 = walking minutes/week: ______
   2 = jogging/running minutes/week: ______
   3 = swimming minutes/week: ______
   4 = cycling minutes/week: ______
   5 = team sports minutes/week: ______
   6 = racquet sports minutes/week: ______
   7 = weight training minutes/week: ______
   8 = cardiovascular machines minutes/week: ______
   9 = other minutes/week: ______
6. Do you measure your heart rate during exercise?  1 = yes  2 = no

7. How long have you had a regular exercise program? ________Months _______Years

8. What condition or shape do you consider yourself to be in now (in terms of physical fitness)?
   1 = poor
   2 = fair
   3 = good
   4 = excellent

9. Do you smoke?  1 = yes  2 = no

10. Do you drink alcohol?  1 = yes  2 = no

11. If yes, how many drinks per week (circle one)?  1-2  3-5  5-7  7-10  10+

12. Has a close blood relative had or died from heart disease or related disorders (Heart Attack, Stroke, High Blood Pressure, Diabetes etc.)?
   1=Mother
   2=Father
   3=Brother - Sister
   4=Aunt - Uncle
   5=Grandmother - Grandfather
   6=None
   If yes- Give ages at which they died or had the problems.

13. Indicate which of the following apply to you (circle all that apply).
   1 = high blood pressure
   2 = high blood fats or cholesterol
   3 = cigarette smoking
   4 = known heart disease or abnormalities
   5 = family history of heart disease (parents or siblings before age 50)
   6 = sedentary lifestyle
   7 = stressful lifestyle at home or at work
   8 = diabetes mellitus
   9 = gout (high uric acid)
   10 = obesity

14. Any medical complaints now (illness, injury, limitations, neurological symptoms)?
   1 = yes  If yes, describe completely___________________________________________________________
   2 = no  ____________________________________________________________  ____________________
   ____________________________________________________________  ____________________

15. Any major illness in the past?
   1 = yes  If yes, describe completely___________________________________________________________
   2 = no  ____________________________________________________________  ____________________
   ____________________________________________________________  ____________________

69
16. Any surgery or hospitalization in the past?
   1 = yes If yes, describe completely
   2 = no

17. Are you currently taking any medications (prescription or over-the-counter: including birth control)?
   1 = yes If yes, list drugs and dosages
   2 = no

18. Have you ever had any neurological problems?
   1 = yes If yes, describe completely
   2 = no

19. Do you now have, or have you ever had, any of the following? (circle all that apply)
   1 = heart murmurs
   2 = any chest pain at rest
   3 = any chest pain upon exertion
   4 = pain in left arm, jaw, neck
   5 = any palpitations
   6 = fainting or dizziness
   7 = daily coughing
   8 = difficulty breathing at rest or during exercise
   9 = any known respiratory diseases
   10 = any bleeding disorders or problems with bleeding
   Please describe fully any items you circled:

20. Do you now have, or have you ever had, any of the following? (circle all that apply)
   1 = any bone or joint injuries
   2 = any muscular injuries
   3 = muscle or joint pain following exercise
   4 = limited flexibility
   5 = any musculoskeletal problems which might limit your ability to exercise
   Please describe fully any items you circled:
APPENDIX D
Hey, ladies!
Interested in being a part of a great study?

Did you know men and women burn extra fat after conducting a bout of aerobic exercise? Did you know women only do this for 3 hours, where men have increased fat burn for 24 hours?

We will be analyzing the duration of fat oxidation (fat burning) in females after conducting either one or two bouts of exercise on a cycle ergometer (exercise bike) to see how total postexercise fat oxidation is affected.

We hope that our research will lead to an increased understanding of sex differences regarding fuel use during the postexercise recovery period. This may lead to information that could better strategize exercise prescriptions, potentially promoting fat loss and a decreased risk of cardiovascular disease.

One lucky participant will win a $40 gift card to Fleet Feet or North Rim Adventure Sports—their choice!

Do you qualify?

- Female
- Age: 19-35 years old
- Current Exercise habits: 5+ hours of endurance exercise/week
- 4+ exercise sessions/week
- No smoking/excessive alcohol consumption
- Normal menstrual cycle

Contact information
Primary Investigator: Jennifer Barker
Yolo, Room 271
jbarker8@mail.csuchico.edu
Hi! I want to first say it is great that you are interested in becoming a participant in my thesis! For those that complete the study, I will raffle off a $40 gift card to either Fleet Feet or North Rim Adventure Sports, your choosing! Here is a bit of background information:

I will be analyzing the duration of fat oxidation (fat burning) after conducting either one or two bouts of exercise to see how total fat oxidation is affected. This may lead to information that could better strategize exercise prescriptions, potentially promoting fat loss and a decreased risk of cardiovascular disease. I will be meeting with participants in the mornings, and each subject will meet with me three times to conduct exercise on a cycle ergometer (exercise bike). The exercise will consist of one 60-minute bout at a moderate intensity, and two 30-minute bouts at a moderate intensity. To qualify for participation, the inclusion criteria are as follows:

1. Female recreational athletes (i.e. no pros), age 19-35
2. Current exercise habits: Cycling (preferred) or running, 5+ hours a week, 4+ sessions a week
3. No smoking or heavy drinking
4. Have a normal menstrual cycle
5. BMI and body fat percentage will be tested during the first meeting to finalize inclusion criteria.

Exclusion criteria include:

1. Body fat less than 12% (we will test this during preliminary testing)
2. Medications that effect energy metabolism
3. Diabetics/pre-diabetics
4. Subjects with orthopedic and/or cardiovascular contraindications to exercise

I will start data collection Monday, May 19th, and will run through end of August ideally, but longer if necessary (depending on how many participants I get/don't drop out). It is important that we conduct preliminary testing and both exercise trials during days 3-8 of your menstrual cycle, where day 1 is the first day of your period. This is to standardize hormone levels between all subjects. Let me know when this falls in the month so we can set up an appointment for you to come in and begin preliminary testing.

It is important that you fast for 12 hours before each of the three meetings (preliminary testing, and both exercise trials), and refrain from alcohol and caffeine consumption for 24 hours before each meeting.

During the first meeting, I will have you fill out a medical/exercise questionnaire and read/agree to the consent form. We will assess your body composition using the BodPod, get a resting gas sample to get some baseline metabolic data, and then conduct a VO2max
test. We will use this to find your moderate work rate for the two exercise trials. It is important that you bring or wear compression shorts and a sports bra for the body composition assessment in the BodPod.

The two separate exercise trials will consist of one 60-minute bout of moderate cycling, and two 30-minute bouts of moderate cycling in the same day spaced 5 hours apart. You will not know which you will be conducting first. We will analyze your fuel consumption (fat oxidation) for three hours after the exercise is completed using gas analysis.

Ideally, I'd like to have you conduct the preliminary testing and the first trial within the first 3-8 day window of your menstrual cycle, at least 48 hours apart. Even more ideal would be to conduct all three meetings within the window (although this is not likely). If you can conduct two, that is great, if not, then that is also fine - whatever works for you. We will then space the remaining meetings out to the next month, within the same days 3-8 window of your cycle, as long as we get all three meetings in! Let me know what works best for you!

Please send me your full name and a good phone number in case I need to reach you. Additionally, please respond with some dates so I can book the lab. Thank you, and I look forward to hearing from you!

Sincerely,

Jennifer Barker
jbarker27@yahoo.com
419-656-9409
LinkedIn: http://lnkd.in/bwyExWR