PHYSIOLOGICAL AND BEHAVIORAL DIFFERENCES BETWEEN HIGH AND LOW FIT FEMALES

by

SARA ASHTON GARCIA

(Under the Direction of Nathan T. Jenkins)

ABSTRACT

Individuals with poor metabolic flexibility are at elevated risk for cardiometabolic disease. Developing sensitive tests to detect poor metabolic flexibility before clinically significant metabolic disease has been established is vital to reduce disease risk. The aim of this study was to comprehensively assess fitness status and determine if high-intensity interval rowing exercise is sensitive enough to detect differences in metabolic flexibility between fitness statuses. The two groups significantly differed in terms of aerobic fitness, body composition, BMI, mitochondrial capacity, and amount of vigorous activity completed weekly. The groups were similar in all other assessed areas (dietary habits, quality of life, muscular strength, sedentary patterns, etc.). Consistent with the hypothesized outcome, groups were significantly different in substrate utilization during the high-intensity interval rowing exercise protocol. These findings suggest that the high-intensity interval rowing exercise protocol used in the present study was sufficient to detect differences in metabolic response between fitness statuses.

INDEX WORDS: Fitness status; exercising metabolism; female; metabolic flexibility; high-intensity interval exercise; fitness assessment; fat oxidation.

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DEDICATION

I would like to dedicate my thesis work to my family and friends. A great deal of thanks to my parents, Pete and Candice Garcia. None of this would have been possible without your love, support, and encouragement throughout my life, especially over the past few years. You both made sure I had every opportunity to chase my dreams fully. You taught me to be disciplined, determined, and brave. Thank you both for being my biggest cheerleaders and believing in me when I did not believe in myself. Thank you for wiping my tears in the lowest moments and celebrating with me in the victories. I will forever be indebted to you both. I love you, Mama and Dad!

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TABLE OF CONTENTS

Page

LIST OF TABLES

Page

LIST OF FIGURES

Page

APPENDICES

Page

CHAPTER 1

INTRODUCTION

Historically, metabolic flexibility studies have compared individuals with established metabolic disorders to healthy individuals. Recent research has switched to measuring metabolic flexibility before clinically significant metabolic disease has been established (Smith et al. 2018). Metabolic flexibility is defined as the body's ability to match substrate utilization to the substrate availability or efficiently switch between carbohydrate and fat metabolism (Smith et al. 2018). When the body cannot efficiently use available fuel sources, displaying poor metabolic flexibility, metabolic diseases are likely to arise. Type 2 diabetes, dyslipidemia, and many other diseases are rooted in this disordered fuel storage and utilization pattern, making it a current, highly problematic concern for the world today. Therefore, determining sensitive and practical approaches for measuring metabolic flexibility prior to the onset of the disease is a prolific area for research.

Substrate availability can be altered in many ways to test an individual's metabolic flexibility, and the literature supports a wide range of these tests. A high-fat meal challenge is commonly used. A metabolic flexibility meal challenge assessment aims to significantly alter the availability of a given substrate, carbohydrates or fats, and see how the metabolism responds. However, these meal challenges have often been used primarily in diseased populations and have proven not to be as sensitive in a healthy population (Olenick et al., 2022). In addition to feeding, exercise requires the coordination of all metabolic pathways to support rapidly changing energy needs. (Smith et al., 2018). Physical activity and exercise increase the metabolic rate well above

the resting state. The strain placed on one's metabolism during exercise requires metabolic flexibility. Using exercise as a test of metabolic flexibility is a relatively new concept. San-Milian & Brooks (2018) tested metabolic flexibility during a graded cycling test. Olenick et al. (2022) compared a cycling high-intensity interval exercise bout to the more common high-fat meal assessment. Not only did the exercise protocol provide results consistent with the high-fat meal challenge but appeared to give more insight into metabolic differences between the young, healthy groups. As such, exercise protocols might be the preliminary observational test needed to detect poor metabolic flexibility before disease onset.

Previous literature has shown that various lifestyle behaviors can improve metabolic flexibility. It would be beneficial to know which specific physiological and behavioral factors positively influence metabolic flexibility. Aerobic fitness is vital to metabolic flexibility (Bergouignan et al., 2013; Goodpaster & Sparks, 2017). Additionally, previous literature has shown that aerobic fitness increases fat oxidation during high-intensity interval exercise (HIIE) (Aslankeser & Balci, 2017; Hetlelid et al., 2015). Lean individuals of higher fitness status tend to exhibit higher fat oxidation rates in response to exercise and high-fat meals (Olenick et al., 2022). Females with high aerobic fitness have greater fat oxidation rates than males with similar fitness statuses (Olenick et al., 2022). On the contrary, low fitness status appears to be more detrimental to metabolic flexibility in females than males (Olenick et al., 2022). Additionally, Amaro-Gahete et al. (2019) found age, sex, activity intensity, and other behavioral factors influence the complex relationship between physical activity and metabolic flexibility. Given the novelty of an HIIE test of metabolic flexibility, there is a lack of understanding on what physiological and behavioral factors influence exercising metabolic flexibility during this testing protocol.

This study aimed to assess the influence of fitness characteristics on exercising metabolic flexibility during a high-intensity interval rowing protocol in young, healthy females. To do this, we comprehensively assessed fitness status based on the five components of physical fitness: cardiovascular endurance, muscular strength, muscular endurance, flexibility, and body composition (American College of Sports Medicine, 2018). Additionally, we aimed to obtain background information on participants' behavioral patterns and other physiological characteristics, such as microvascular function and mitochondrial capacity. With this information, we hope to broaden the knowledge of which elements of fitness are correlated with greater metabolic flexibility during an HIIE rowing test. Additionally, we hope to confirm that the HIIE test of metabolic flexibility is sensitive enough to detect differences in an otherwise healthy population.

CHAPTER 2

LITERATURE REVIEW

1 . Defining metabolic flexibility

The body's energy systems rely on three main substrates, all of which can be consumed through diet. Amino acids, fatty acids, and glucose are the substrates available to the body to metabolize and create fuel. Fatty acids and glucose are the substrates of focus when it comes to measuring metabolism (Storlien et al., 2004). In an individual with a healthy metabolism, the body can efficiently store and utilize available energy sources. When the body cannot efficiently use and store available fuel sources, metabolic disruption occurs. This disordered pattern of fuel storage and utilization underlines type 2 diabetes, dyslipidemia, and metabolic syndrome (Storlien et al., 2004). Therefore, proper metabolic function, utilizing and storing substrate, is important to overall health.

Metabolic flexibility is a measure of the individual's ability to match substrate usage to substrate availability and serves as an adaptation to maintain fuel homeostasis most efficiently (Smith et al., 2018, Storlien et al., 2004). Poor metabolic flexibility is the diminished ability to switch substrate usage to optimize availability and demand, typically seen as a characteristic of metabolic disorders (Smith et al., 2018). As results showed by Kelley et al. (1999), in a metabolically flexible individual, the reaction to consuming a high-fat meal would be increasing rates of fat oxidation. Similarly, in an insulin-stimulated condition, there would be a shift toward glucose as the primary source of fuel. An individual with poor metabolic flexibility would have

an absent or blunted shift to fat oxidation, following the consumption of a high-fat meal. This disordered pattern of fuel usage and storage is indicative of poor metabolic flexibility.

Metabolic flexibility is regulated by skeletal muscle, adipose tissue, and cellular mitochondria. Skeletal muscle plays a significant role in energy expenditure, as it responds to insulin with glucose uptake. Additionally, it is a large storage site for glucose, along with the liver. Adipose tissue helps regulate metabolism by preventing large changes in circulating fatty acid levels (Frayn et al., 2002). In states of metabolic disease, adipose tissue has a diminished response to insulin and a disordered lipase response (Storlien et al., 2004). Mitochondrial function regulates the conversion of nutrients to substrates that can be processed in the citric acid cycle (Muoio et al., 2014). Reduced fatty acid uptake in mitochondria and muscle cells can lead to disordered fat oxidation during feeding and exercise. This inappropriate uptake can lead to dyslipidemia and subsequent insulin resistance, indicative of metabolic inflexibility (Galgani et al., 2008). Therefore, mitochondria and skeletal muscle health play a crucial role in metabolic flexibility.

Many scientists had contributed to the literature related to metabolic flexibility before the term “metabolic flexibility” had been coined. Falta & Boller (1931) were the first to hypothesize that insulin resistance was a possible underlying mechanism of type 2 diabetes. At the time, this was a novel concept, as people were yet to understand the cause of this disease. This finding set the scene for future research in this area that would discover insulin resistance is indeed a mechanism of type 2 diabetes and can be altered by health behaviors.

While “metabolic flexibility” is relatively new in research, the underlying concept has been observed for decades. The first research to name this phenomenon comes from Kelley et al. (1999). This study explored differences in metabolic flexibility between obese and lean

individuals. They found lean individuals were able to make better use of available substrates following an overnight fast, marked by an increased rate of fat oxidation. Additionally, following infusions of insulin, they had a reduction in fat oxidation. The obese experimental group did not show increased fat oxidation following an overnight fast. This group also did not have downregulation of fat oxidation following the insulin infusion. As seen in the data, the lean group was better able to adapt to the available fuel sources, hence they are described as metabolically flexible.

2 . Assessment of metabolic flexibility

In the literature, many different measures of metabolic flexibility are utilized. Commonly, a respiratory quotient (RQ) is used. RQ represents the difference between oxygen consumption and carbon dioxide exhalation. RQ is an indicator of mitochondrial metabolism and can be representative of whole-body metabolism, as well. An RQ close to 0.70 indicates primarily fat oxidation, whereas values closer to 1.0 indicate glucose oxidation. Compared to baseline values, the degree to which the RQ value shifts after substrate alteration gives insight into an individual's metabolic flexibility. RQ allows metabolic flexibility to be studied by collecting respiratory gases.

The stoichiometric equations formulated by Frayn et al. (1983) can be used to measure total fat and carbohydrate oxidation. Using indirect calorimetry, oxygen and carbon dioxide levels are collected and measured. The oxidation of carbohydrates (CHO) can be described as: (CHO Oxidation($g*min^{-1}$) $=4.55VCO_2 – 3.21VO_2$). Fat oxidation can be calculated as: (Fat Oxidation($g*min^{-1}$) $=1.67VO_2 – 1.67VCO_2$) (Fryan, 1983. San-Millan & Brooks, 2017). This allows researchers to know how many grams of carbohydrates and fats are being oxidized, rather than the relative proportion of each, as in the RQ.

Substrate usage can be altered in many ways to test an individual's metabolic flexibility, and the literature supports a wide range of these tests. A high-fat meal challenge is commonly used. In these meal challenges, the goal is to significantly increase the availability of a given substrate, carbohydrates or fats, and see how the metabolism responds. If an individual is able to shift to an increased amount of fat oxidation in response to consuming a high-fat meal, this is a marker of metabolic flexibility. For a meal containing a large portion of carbohydrates, a healthy metabolism can increase carbohydrate oxidation to use the available carbohydrates for fuel. Another common test is to stimulate a fasted individual with insulin. A response of reduced fat oxidation and increased dependence on glucose is another positive sign of metabolic flexibility.

As mentioned in the previous section, a common practice of measuring metabolic flexibility is during states of purposefully altered substrate availability. Kelley et al. (1999) tested during a fasted and insulin-stimulated state. In an individual with a healthy metabolism, substrate usage is very different in the fasted state, even after a short overnight fast. In the fasted state, there is a decrease in plasma glucose and fatty acids. In a healthy individual, the metabolism would shift towards fat oxidation to maintain fuel homeostasis. Whereas in a less metabolically flexible individual, we would not expect to see as large of a shift towards fat oxidation (Kelley et al., 1999). This blunted shift is due to metabolic disorder.

Measuring how the metabolism responds to caloric deficit and excess, is only one way to test metabolic flexibility. Another important stressor of metabolism is physical activity. Caloric deficit and excess responses are primarily reliant on endocrine and mitochondrial shifts. Exercise also requires a shifting of skeletal muscle pathways to support the enormous amount of energy needed (Smith et al., 2018). Physical activity and exercise increase the metabolic rate well above what is seen in a resting state. The strain placed on one's metabolism during exercise requires

metabolic flexibility. When the body can efficiently make use of the available substrates, there is a likelihood of improved metabolic outcomes and performance.

Using exercise as a test of metabolic flexibility is a relatively new concept. San-Milian & Brooks (2018) tested metabolic flexibility during a graded cycling test. The study used indirect calorimetry to measure fat oxidation during the exercise bout. When comparing professional cyclists, moderately active individuals, and participants with metabolic disease, they found athletes to have the highest rates of fat oxidation. Amaro-Gahete et al. (2019) recorded maximal fat oxidation and the intensity of exercise it took to elicit this response in all individuals. This was their indicator of metabolic flexibility, as well. Using this, they found many of the observed differences between their test groups were moderated by cardiorespiratory fitness.

Recent work in our laboratory (Olenick et al., 2022) compared a novel cycling, high-intensity, interval exercise bout to the more common high-fat meal assessment. Not only did the exercise protocol provide results consistent with the high-fat meal challenge but appeared to give more insight between groups. The exercise bout illustrated differences between fitness statuses. Whereas the high-fat meal test showed no difference between trained and untrained. Results included highly fit women having the greatest rates of fat oxidation when compared to high-fit men and low-fit men and women.

3 . Effect of sex on metabolic flexibility

As touched on in the previous section, sex has been shown to have some effect on metabolic flexibility. However, many studies show conflicting results. Some results argue the differences in metabolic flexibility between the sexes are rooted in body composition differences. For example, when controlled for lean body mass, differences, such as females experiencing increased fat oxidation, are no longer apparent (Santosa & Jensen, 2015). However,

this is not always the case. Olenick et al. (2022) found results indicated highly fit women have the greatest rates of fat oxidation when compared to high-fit men and low-fit men and women. However, low-fit women exhibited the lowest rates of metabolic flexibility, showing that fitness may have an amplified effect in women compared to men. Contrary to other studies, these differences remained present, even when controlled for total body mass. Olenick et al.'s (2022) data suggest body composition is not the only cause of metabolic differences between males and females. With conflicting results, it is important to control for body composition differences when studying those of different sexes.

There is research to suggest sex differences may result from the hormonal and biological differences between males and females. Estrogen and testosterone are hormones found in both males and females. Typically, males have much lower levels of estrogen and higher levels of testosterone. Both play a role in the reproductive systems of the sexes. Some studies suggest that these hormones may pose more or equal influence on metabolic flexibility and body composition (Eaton et al., 2019).

A case where this is evidenced is in a study of a clinical population. Polycystic ovarian syndrome (PCOS) patients have disordered hormonal patterns. They often present with higher-than-normal levels of testosterone, luteinizing hormone, and follicle-stimulating hormone than healthy women. The study found metabolic inflexibility was correlated with disordered hormonal status, independent of body composition. Both the lean and obese PCOS patients exhibited poor metabolic flexibility when compared to the control groups. Indicating while body composition may have an effect, it appears hormones also play a role (Gebara et al, 2019).

To further expand on this topic, a study from MacCannell et al. (2021) found that sex and hormonal control did appear to affect metabolic flexibility. Using a well-controlled mouse

model, they found that male rats responded differently to a high-fat diet than women. To tie in the effect of body composition, they used both obese and lean rats. The rats were divided into four groups based on sex and body composition. All male rat groups had diminished metabolic flexibility, thus a less adaptive response than the female rats. Once again this suggests there are innate hormonal and morphological differences to explain sex disparities in metabolic flexibility, not body composition alone.

Establishing that males and females are innately different emphasizes the importance of a female-focused study. The field of kinesiology research is oversaturated with male-focused, or male-centered research. 66% of participants in sport and exercise research were males (Cowley et al, 2021). Thus, well over half of what we know in kinesiology, has only been shown in males. Knowing that females and males are inherently different due to biology and behaviors, it would be inappropriate to assume findings would be the same in females. Further, only 6% of exercise and sports research has been done in females only, and 31% of studies consisted of only male participants (Cowley et al, 2021). Thus, women have been grossly underrepresented in exercise science research.

4 . Effect of physical fitness on metabolic flexibility

Physical fitness is defined as a combination of cardiorespiratory endurance, muscular endurance, muscular strength, flexibility, and body composition (American College of Sports Medicine, 2018). Fitness status and physical activity are known to have a positive effect on metabolic flexibility. As such, those with higher fitness status and activity levels typically exhibit a greater level of metabolic flexibility. Additionally, it supports the notion that exercise can be used as an intervention in cases of metabolic inflexibility (Smith et al., 2018). It also further supports that physical inactivity is a leading cause of metabolic diseases.

Bergouignan et al. (2013) compared physical activity levels, to an individual's metabolic flexibility. They found that there was a strong correlation between higher levels of habitual physical activity and metabolic flexibility. Additionally, the physically inactive group experienced a decline in metabolic flexibility. Importantly, only those who were previously sedentary were assigned to physical activity and only those who were active were assigned to bed rest. Therefore, they could not conclude that more sedentary time would worsen metabolic flexibility in already metabolically inflexible groups. However, they were able to suggest a correlation between physical activity and metabolic flexibility. It is important to note the study did not examine markers of physical fitness in the participants, but solely compared them based on physical activity levels.

Battaglia et al. (2012) studied exercise and the response to a high-fat diet in obese and lean individuals. They found that 10 days of moderate aerobic exercise improved fat oxidation of skeletal muscle in both lean and obese individuals. In fact, obese individuals saw improvements comparable to the lean group. In another study out of the Bergouinan laboratory, they conducted an intervention. They reported that one month of exercise, following nationally recommended guidelines, improved metabolic flexibility in an insulin-stimulated condition (Bergouinan et al. 2013). This suggests routine exercise does have a role in metabolic flexibility. However, again, this study did not quantify an area of physical fitness.

Blanc et al (2000), performed the reverse intervention. They used an oral glucose tolerance test to test metabolic flexibility in a group of men and women. The subjects were tested both before and after 7 days of bed rest. Following the week of bed rest, both sexes trended toward metabolic inflexibility. Both previously mentioned interventions show metabolic flexibility can see increases or decreases within a short period following both physical activity

and inactivity. Rynders et al. (2018) hypothesized that breaks in sedentary time would have similar, or greater, improvements in metabolic flexibility as a daily bout of exercise. They were not able to conclude in their review but state the importance of future studies investigating this idea.

Amaro-Gahete et al. (2019) found a relationship between physical activity and metabolic flexibility in women. However, it seemed like this effect was moderated by cardiorespiratory fitness. Additionally, they did find a correlation between certain sedentary behaviors and the exercise intensity it took to elicit maximal fat oxidation in young men and women, but not the middle-aged cohort. Their findings point out the complexity of the relationship between physical activity and metabolic flexibility. It appears many factors like age, sex, activity intensity, and other behaviors influence this relationship. The fact that their results were moderated by cardiorespiratory fitness levels emphasizes the importance of fitness measures when studying metabolic flexibility.

As mentioned in the previous section, Olenick et al. (2022) found there was a significant effect of aerobic fitness status on metabolic flexibility during an exercise bout. Those who had a higher aerobic fitness status, determined by the highest rate of oxygen consumption (VO_{2peak}), displayed greater rates of metabolic flexibility. Moreover, high-fit women had the greatest rate of fat oxidation. Like Amaro-Gahete et al. (2019), we see that many factors influence the effect of exercise and physical activity on metabolism. However, the limitation is that Olenick et al. (2022) only quantified fitness as cardiorespiratory endurance.

Recent research has investigated resistance training as a treatment for type 2 diabetes, a form of metabolic inflexibility. The Stasser and Schobersberger (2011) review paper found that resistance training was sufficient in decreasing glycated hemoglobin levels (HbA1C) in type 2

diabetes patients. Since decreased HbA1C reduction is a marker of better metabolic health, it seems resistance training and improved muscular strength could have potential implications on metabolic flexibility. However, the authors pointed out that most patients saw a decrease in adipose tissue and an increase in lean body mass. Therefore, making it is difficult to say if metabolic health was improved by resistance training and muscular strength, or only due to the resulting body composition changes. Additionally, this study did not measure cardiorespiratory fitness, which could have potential implications.

Shoemaker et al. (2022) compared sarcopenic and non-sarcopenic patients. Sarcopenia is defined as age-related progressive loss of muscle mass and strength. Their results showed that the sarcopenic patients showed significantly different levels of metabolic flexibility during rest, feeding, and exercise. However, they were unable to conclude whether the loss of muscle strength preceded the loss of metabolic flexibility or vice versa. Regardless, it is important to note the relationship between muscle strength and metabolic flexibility.

Often, physical activity, sedentary time, and exercise time are self-reported. While many of these self-report recalls are well supported, it can be difficult to view this as an objective measure. Therefore, it can be beneficial for researchers to report other objective measures, like a maximal rate of oxygen consumption (VO_{2max}). This allows for a better understanding of the population's aerobic fitness level, which we would expect to be influenced by exercise and physical activity patterns. While an individual may be physically active, the person may still have poor endurance or strength. Therefore, it is important to make a distinction between physical activity and fitness. Physical activity is any movement that requires more energy than resting. Fitness is the state of being physically strong and healthy.

5 . Assessing individual fitness level

Many of the aforementioned studies have reviewed the effect of cardiorespiratory fitness or physical activity levels on metabolic flexibility. Although cardiorespiratory, or aerobic, fitness is important, this classification neglects the other aspects of what defines fitness. Physical fitness is the combination of cardiorespiratory (aerobic) fitness, muscular strength, muscular endurance, flexibility, and body composition (American College of Sports Medicine, 2018). Therefore, fitness tests, like the Presidential Fitness Gram and Military Fitness Test, include exercises to measure all five aspects of physical fitness. On the other hand, as seen in previous sections, the field of exercise physiology has, historically, quantified fitness as the maximal rate of oxygen uptake (VO_{2max}). While cardiorespiratory, or aerobic, fitness is a large contributor to overall fitness, it fails to comprehensively assess all metabolic systems. Metabolic flexibility is a measure of these metabolic systems, so it is important to accurately capture all systems. A comprehensive fitness assessment allows for this holistic view.

Considering this, many labs and industries have adapted more comprehensive measures of fitness (Blizon et al., 2001). The Henrich Lab at Kansas State University has employed the use of several fitness measures in its studies. Most of these tests do not require extensive amounts of equipment or time and give a better picture of one's fitness status. Examples of endurance tests include pushups, sit-ups, squats, and pullup repetitions in a minute. In Cosgrove et al, (2019) these tests proved feasible, as they were able to detect significant differences following exercise training. A standard sit and reach test can be used to measure flexibility. Muscular strength can be measured in many ways. Maximal voluntary contraction (MVC) with a dynamometer is a validated objective measure. A 1 repetition-maximum (1-RM) can be used as an objective

strength measure, as well. When comparing individuals of varying body compositions, it is usually best to normalize the data to fat-free mass (FFM) or total body weight (Jaric et al., 2002).

Comprehensively capturing fitness is an important factor in comparing trained and untrained individuals. Therefore, it is best to get the broadest view. Testing each aspect of fitness not only gives a comprehensive picture of an individual's fitness level but can give insight into their training regimens. If an individual has a very high aerobic capacity, this could confirm the validity of their self-reported training regimen. This is important as training modality can be an important factor in exercise studies.

Aside from these practical assessments, other scientific measures can give insight into individual fitness and health. Skeletal muscle mitochondrial oxidative capacity can be assessed by measuring rates of muscle oxygen saturation during periods of ischemia and muscular stimulation (Sumner et al., 2019). The muscular response of $m\dot{V}O_2$ and metabolism during the periods of ischemia following stimulation represents an increase in the cellular respiration (McCully & Hamaoka, 2000). Fitness levels are well documented as regulating mitochondrial respiration (Zoll et al., 2002). Drake et al. (2015) showed that exercise training was correlated with improvements in the quality and quantity of mitochondria. Therefore, mitochondrial capacity can serve as a surrogate marker of training and fitness levels.

Exercise is popular for its ability to improve cardiovascular health. Therefore, measures of vascular function can give insight into individual fitness and health levels, as well. Many assessments of vascular function can be made, in a variety of different ways. A validated, non-invasive measure is microvascular reactivity. Using a series of ischemia periods, one can measure rates of muscle oxygenation and vasodilation (Willingham et al, 2016). Another set of health indicators. Tew et al. (2009) showed that aerobic fitness status protected against age-

related declines in vasodilator function. As with mitochondrial capacity, it seems that microvascular function may serve as a surrogate marker of training and fitness levels.

As described above, previous studies have primarily investigated the role of one aspect of physical fitness alone (Amaro-Gahete et al., 2019; Olenick et al., 2022; Shoemaker et al., 2002). When studying metabolic flexibility, the individual's fitness level, health parameters, training background, and physical activity patterns can explain group disparities. This once again suggests that a comprehensive measure of fitness is vital to this topic. With HIIE tests of metabolic flexibility being a newer approach, there is less literature studying the effect of physical fitness. Cardiorespiratory (aerobic) fitness, muscular strength, muscular endurance, flexibility, body composition, mitochondrial capacity, and microvascular function should all be considered markers of fitness (American College of Sports Medicine, 2018; Drake et al., 2015; Tew et al., 2009). However, they are often neglected in the literature, as aerobic fitness status is primarily used. This is a gap we hope to bridge in the current study. The aim of this study was to comprehensively assess fitness status and determine if high-intensity interval rowing exercise is sensitive enough to detect differences in metabolic flexibility between fitness statuses. We hypothesize that our comprehensive assessment of fitness will result in groups that significantly differ in terms of exercising metabolic flexibility. Based on previous literature, we hypothesize females with a higher comprehensive fitness level will exhibit increased rates of exercising metabolic flexibility.

CHAPTER 3

METHODS

1. Ethical Approval

This study was approved by the WCG Institutional Review Board (study no. 1323806, approved on 24JAN2022), with written informed consent being obtained prior to any experimental procedures. The study conformed to the standards set by the Declaration of Helsinki, except for registration in a database.

2. Participant Characteristics

Potential participants were recruited via word-of-mouth and by flyers from the Athens, GA and University of Georgia communities, and completed a questionnaire to initially screen participants based on the below inclusion and exclusion criteria. Inclusion criteria included adults that chronically participated in a high-intensity exercise regimen (3-4 times/wk for >1 y) or did not participate in an exercise regimen (<2 h/wk of physical activity for >1 y) and were aged between 18-35 years. Exclusion criteria included biological male sex, weight loss or gain exceeding 5% in the past three months, plans to begin a weight loss or exercise program during the study, age outside of 18-35 years, those with chronic hypertension (systolic BP ≥ 140 mmHg or diastolic BP ≥ 90 mmHg), diabetes, or other chronic diseases, thyroid medication, non-oral contraception, lipid-lowering medication, blood pressure medication, antipsychotics, supplement use (other than a daily multivitamin/mineral), or tobacco use. If a potential participant reported supplement use, they were instructed to follow a 2-wk washout phase before testing. No participants reported supplementation use; thus, no washout period was warranted. All normally

mensurating participants completed the sessions within days 2-10 following the self-reported onset of their menstrual cycle (Sims & Heather, 2018). For participants who were on oral contraceptive pills, testing sessions took place during days 2-7 of the placebo week of their medication (Mattu et al., 2019). Only individuals on oral contraceptive pills were included in the study population, all other forms of prescription contraception were excluded.

3. Study Design and Protocol

Participants completed two trials consisting of 1) baseline testing to comprehensively assess fitness status, and 2) high-intensity interval exercise using a rowing ergometer to assess exercising substrate oxidation. Testing visits took place at least 48 hours apart, all visits were completed within 6 days. Participants were asked to avoid exercise for 48 hours and caffeine and alcohol 24 hours before both visits. All participants were instructed to follow their normal dietary routine, other than the specific guidance given below, for the duration of the study. Both visits are described in detail below.

3.1. Baseline Visit

Participants received a pre-visit meal recommendation that consisted of 50% carbohydrate, 35% fat, and 15% protein, equating to 25% of the estimated resting energy expenditure (Mifflin et al., 1990). Participants were given guidance on food selection to meet the prescribed energy content and macronutrient composition for recommended meals. Participants were requested to consume the pre-visit meal 2 hours prior to arriving at the Integrative Cardiovascular Physiology Laboratory for testing.

First, participants' height, weight, resting blood pressure, and body composition (via dual-energy X-ray absorptiometry, Horizon® DXA System, Hologic, Inc., Marlborough, MA, USA) were measured. After these measurements, participants completed a series of four

questionnaires: 1) a 24-hour food recall (Subar et al.. 2001), 2) a 7-day sedentary activity time recall, combing the Measure of Older Adults Sedentary Time (MOST) and the Sedentary Behavior (SBQ) questionnaires (Prince et al., 2017), 3) a 7-day physical activity recall, combing the International Physical Activity (IPAQ)-Short form questionnaire and a 7-day exercise recall, and 4) the modified 16-question Quality-of-Life Scale (QOLS) (Burakhardt & Anderson, 2003).

Muscular strength of the quadriceps muscle was then assessed using a Biodex System 4 dynamometer (Model 850-230, Universal Pro Single Chair Assy, Biodex Medical Systems, Inc., Shirley, NY, USA) by measuring the maximal voluntary isometric contraction of the dominant leg. The maximum force from three trials at three different angles (90, 75, & 60 degrees) was recorded. The joint angle was manually calibrated before each repetition to ensure the dynamometer axis was correctly positioned and all restraining straps were tightened to reduce leverage. A warmup was provided to avoid injury, along with a practice trial. Verbal encouragement was provided throughout, with a 30-second rest in between each repetition and a 3-minute break in between each angle (Sosnoff et al., 2010).

Participants then completed a standard sit-and-reach test to assess the flexibility of the lower back and hamstrings. Sit and reach trials were conducted using the Baseline Sit N' Reach Trunk Flexibility Box (Fabrication Enterprises Inc., White Plains, NY). Participants sat with straight knees and soles of both feet against the box. Participants were instructed to overlap both hands and push the marker as far as possible, without bending at the knees. The distance of the marker was recorded, according to the YMCA scale. Three trials were completed, and the average reach was recorded in inches (Liemohn et al., 1994).

Upper body muscular endurance was then measured as the maximal number of modified pushups that could be completed in a continuous effort, without rest. Participants began in a

prone, planked position, with their knees resting on the floor. Participants were instructed to lower their chest and thighs to the floor, then push back up to full elbow extension (Baumgartner et al., 2002). A continuous effort was defined as less than a 3-second rest between push-ups.

To conclude this visit, participants completed a maximal exercise test to determine the highest rate of oxygen consumption (VO_{2peak}) on a rower ergometer (AssaultRowerElite, Assault Fitness, Carlsbad, CA, USA). Prior to test start, participants were given a familiarization brief. Participants were instructed on proper rowing form, according to the four-phase technique: catch, drive, finish, and recovery (Ruffaldi et al., 2009). All participants were coached through several practice strokes. Following familiarization, participants were fitted with a mask to collect respiratory gasses for analysis via indirect calorimetry (TrueOne 2400, Parvo Medics, Sandy, UT, USA), a heart rate monitor (Polar, Polar Electro Inc., Lake Success, NY, USA), and a two muscle oxygen monitors (Moxy Monitor, Hutchinson, MN, USA). One Moxy Monitor was placed on their right Vastus Lateralis, approximately 2/3 of the way down from the greater trochanter to the patella, and the second was placed on their Biceps Brachii, approximately 1/2 of the way from the humeral head to the coronoid fossa. Additionally, Moxy Monitors were secured using elastic pre-wrap and an elastic bandage to reduce transient light. Participants started with a low pace of 3:00 (500 meters every 3 minutes) for 3 minutes followed by a pace increase every 2 minutes until volitional exhaustion or participants could no longer sustain the prescribed pace, *Table 1*. When a participant could not keep a given pace, they were asked to complete a 1-minute all-out effort prior to cessation. At the cessation of exercise, subjects were asked to report their rating of perceived exertion (RPE) and blood lactate was measured (Lactate Plus, Nova Biomedical, Waltham, MA, USA). VO_{2peak} was confirmed by satisfying three of the following requirements, (i) an RER $\geq$ 1.10, (ii) a plateau in oxygen consumption (change < 100 $mL \cdot min^{-1}$

in the last 30 s stage), (iii) a maximum heart rate ≥ 85% of the age-predicted maximal heart rate, (iv) RPE ≥ 18, and (v) blood lactate ≥ 7 mmol (Wasserman et al., 2004).

3.2. High-Intensity Interval Exercise Visit

The evening before this visit, participants received a dinner recommendation that consisted of 50% carbohydrate, 20% fat, and 20% protein, equating to 30% of their estimated resting energy expenditure (Mifflin et al., 1990). For this visit, participants were instructed to eat their pre-fasting meal the evening prior to their visit. They arrived to the Integrative Cardiovascular Physiology Laboratory after an overnight fast (~10-12 hours). Upon arrival to the lab, the participants' body weight was measured.

First, skeletal muscle mitochondrial oxidative capacity was assessed by measuring the changes in Continuous-Wave Near-Infrared Spectroscopy (CW-NIRS; PortaMon, Artinis Medical Systems, Einsteinweg, The Netherlands) signals during periods of ischemia (Sumner et al, 2019). Each participant laid supine on a padded table with both legs fully extended (0° of flexion) with a CW-NIRS optode placed on the right Vastus Lateralis, approximately 2/3 of the way down from the greater trochanter to the patella and secured using elastic pre-wrap and an elastic bandage to reduce transient light. The knee extensors were stimulated percutaneously by two rectangular electrodes (2 x 4 in) placed over the belly of the Vastus Lateralis (Theratouch 4.7, Rich-mar, Inola, OK, USA) proximal and distal to the CW-NIRS probe. A rapid inflating pneumatic cuff (Delfi V34, Medical Innovations Inc., Vancouver, BC, CA and D.E. Hokanson Inc., Bellevue, WA, USA) was placed proximal to the CW-NIRS optode with enough separation to prevent mechanical influence from inflation. CW-NIRS signals were sampled at 10 Hz and laser diodes at three wavelengths (905, 850, and 760 nm) corresponding to the absorption wavelengths of oxygenated hemoglobin. Resting measurements of mVO_2 were assessed by

inflation (250–300 mmHg) for 30 seconds. To assess exercise mVO_2, 30 s of twitch neuromuscular electrical stimulation (NMES; biphasic pulse, duration/interval = 200/50 μs) was administered at 6.0 Hz. The intensity was adjusted for each subject to produce twitch contractions at the maximal tolerable level. To measure the rate of recovery of muscle oxygen uptake back to resting levels, four mitochondrial oxidative metabolism tests were performed consisting of a series of six brief occlusions (5 s on/ 5 s off of 250-300 mmHg) following 30 s of twitch NMES.

Microvascular reactivity, as a maker of microvascular function, was measured in the forearm muscles using CW-NIRS (Willingham et al., 2016). The right arm was extended and positioned at an angle of ~80° from the torso. A rapid inflation pneumatic cuff (Hokanson SC5, D.E; Hokanson Inc., Bellevue, WA, USA) was positioned immediately proximal to the olecranon process to provide a stimulus of forearm ischemia. The CW-NIRS probe was placed distal to the occlusion cuff on the forearm. Following 2-min of continuous baseline recording, the forearm cuff was inflated (~220 mmHg) for 5 min. Upon cuff deflation, recording continued for 3 min. CW-NIRS signals were sampled at 10 Hz and laser diodes at three wavelengths (905, 850, and 760 nm) corresponding to the absorption wavelengths of oxygenated hemoglobin. Adipose tissue thickness was measured via ultrasound (Logiq E; GE Medical Systems, Chicago, IL, USA) with an average of three measurements being recorded. Adipose tissue thickness was measured on the Vastus Lateralis, Bicep Brachii, and forearm.

Lastly, participants completed a bout of high-intensity interval exercise on a rower ergometer. Participants were fitted with a mask to collect respiratory gasses for analysis via indirect calorimetry (TrueOne 2400, Parvo Medics, Sandy, UT, USA), a heart rate monitor (Polar, Polar Electro Inc., Lake Success, NY, USA), and a two muscle oxygen monitors (Moxy

Monitor, Hutchinson, MN, USA). One Moxy Monitor was placed on their right Vastus Lateralis, approximately 2/3 of the way down from the greater trochanter to the patella, and the second was placed on their Biceps Brachii, approximately 1/2 of the way from the humeral head to the coronoid fossa. Additionally, Moxy Monitors were secured using elastic pre-wrap and an elastic bandage to reduce transient light. Participants began with a warmup at a rate of perceived exertion of 11/20 (Borg, 1970) for 3 minutes. Participants received verbal feedback during the warmup, to keep their heart rate at approximately 55% of their age-predicted max heart rate. The warm-up was followed by a 3-minute rest period. During the high-intensity bouts, participants were instructed to perform with maximum effort. Each bout was 3 minutes long and was followed by a 3-minute rest bout. During rest, participants remained seated on the rower. Lactate (Lactate Plus, Nova Biomedical, Waltham, MA, USA), and glucose (OneTouch UltraMini, LifeScan, Inc., Milpitas, CA, USA) were measured via fingerstick at baseline, the end of every high and low bout, immediately post-, 3 minutes post-, and 10 minutes post-exercise. RPE was taken at all these time points, as well.

4 . Group Categorization

Following data collection, the females were scored in each of the following categories: cardiorespiratory endurance, lower-body muscular strength, upper-body muscular endurance, body composition, flexibility, microvascular function, and mitochondrial capacity. The scoring is shown in *Table 2.* Cardiorespiratory endurance, upper body muscular endurance, and flexibility scores were adapted from previously reported normative values (Liguori, G., 2018). Body composition scores were adapted from the American Council on Exercise (American Council on Exercise, 2009). Lower body strength, mitochondrial capacity, and microvascular function were scored relative to the current data set. Range values were established then equal distance

categories were created between the variable's range. A summation of each variable's score was calculated for each participant; this summation represented the fitness status score with higher scores representing greater fitness level. Following the scoring of each participant in all categories, we performed a median split based on the total score to create two groups: high fitness status and low fitness status.

5. Data Analyses

Exercising muscle oxygen monitor signals provided included: muscle oxygen saturation percent (SmO_2%) and total hemoglobin (tHb). Moxy Monitor data was exported and analyzed as 10-second averages. Skeletal muscle oxygenation rates were averaged for the duration of each interval (H1-H4) and recovery period (L1-3 and 10-min recovery).

For the mitochondrial oxidative capacity data, CW-NIRS signals provided included optic density (OD) of oxygenated hemoglobin (O_2Hb), deoxygenated hemoglobin (HHb), $Hb_{difference}$ ($Hb_{difference} = O_2Hb - HHb$), and total hemoglobin ($tHb = O_2Hb + HHb$). The tissue saturation index (TSI%) was calculated as the ratio of absorbance at 850 nm – (850 nm + 760 nm) x 100 to produce a percentage value (Sanni &McCully, 2019). CW-NIRS signals were analyzed using Matlab-based analysis software MATLAB® R2018b (MathWorks Inc., Natick, MA), and a rate constant for the return of muscle oxygen uptake to resting levels was calculated as previously described (Sumner et al., 2020). Microvascular reactivity CW-NIRS signals were analyzed using an electronic spreadsheet (Excel, Microsoft Corp., Redmon, WA, USA) and were assessed by comparing changes in O_2Hb, HHb, and TSI% at rest, during occlusion, and during reactive hyperemia (RH) phases (Willingham et al., 2016).

The macronutrient oxidation rate was assessed for the entire HIIE trial using equations developed by Frayn (Frayn, 1983): fat ($g \cdot min^{-1}$) = ($1.67 * VO_2$ ($L \cdot min^{-1}$)) – ($1.67 * VCO_2$ ($L \cdot min^{-}$

^{1})) and carbohydrate ($g \cdot min^{-1}$) = (4.55*VCO_2 ($L \cdot min^{-1}$)) – (3.21*VO_2 ($L \cdot min^{-1}$)). Oxidation values calculated as negative values were replaced with a zero. For HIIE sessions, macronutrient oxidation rates were averaged for the duration of each interval. Total grams of substrate oxidized were calculated by multiplying the average rate of substrate oxidation by time duration and summed for total exercise (H1-H4), recovery (L1-3 and 10-min recovery), and full session.

6. Statistical Analyses

Student's *t*-tests were conducted to assess the statistical significance between groups (high- vs low-fitness status) on demographics, VO_{2peak}, lower body muscular strength, upper body muscular endurance, flexibility, skeletal muscle mitochondria oxidative capacity, microvascular function, total fitness score, 24-hour food recall, sedentary time questionnaire, physical activity time questionnaire, quality of life scale, and calculations of total fat and carbohydrate oxidized during HIIE. A one-way repeated-measures ANOVA was conducted to assess the statistical significance between groups (high- vs low-fitness status) for HIIE data with student's *t*-tests post hoc analysis. Effect size was determined by partial eta squared (η_p^2), where a value of 0.01 represents a small effect, 0.06 represents a medium effect and >0.14 represents a large effect. Statistical significance was accepted at $P \leq 0.05$. Data are presented as means ± SD. All statistical analyses were performed with JMP®, Version 16 (SAS Institute Inc., Cary, NC, 1989–2021).

7. Power Analysis

Due to the exploratory nature of this study and the lack of data on exercise metabolism during HIIE, a power analysis was based on studies with similar outcome measures. Previous data of sex and fitness status comparison on exercising substrate utilization used a sample of 10 – 12 adults per sex, with a fitness status comparison between groups of 5 participants (Olenick et

al., 2022). Studies examining mitochondrial capacity via CW-NIRS have used 9 adults for a between-group comparison (Erickson et al., 2013). Therefore, to detect differences between groups of similar magnitudes across the proposed study's outcomes using an alpha of 0.05, we aimed to recruit 7 females per group (14 total). Our final participant total was 15 females (8 high- and 7 low-fitness status).

Table 1. Rower Highest Oxygen Uptake (VO_{2peak}) Protocol

Pace	Time
3:00	3 minutes
2:45	2 minutes
2:30	2 minutes
2:15	2 minutes
2:00	2 minutes
1:50	2 minutes
1:45	2 minutes
1:40	2 minutes
1:35	2 minutes
1:32	2 minutes
1:30	As long as possible

Table 2. Fitness Scoring Rubric

Points Earned:	1	2	3	4	5
VO_{2peak} (ml/kgBW/min)	<33.8	33.9-37.7	37.8-39.9	40-44.9	45+
Lower Body Muscular Strength (nm/kgFFM)	1-1.9	2-2.9	3-3.9	4-4.9	5+
Upper Body Muscular Endurance (# of Pushups)	7-11	12-22	23-29	30-35	36+
Sit & Reach (in)	0-16.9	17-18.9	19-20.9	21-23.9	24+
Body Fat (%)	32+	25-31.9	21-24.9	14-20.9	10-13.9
Microvascular Function ($T_{1/2}$)	22+	18-21.99	14-17.99	9-13.99	5-8.99
Mitochondrial Capacity (OD/sec)	0-0.49	0.5-0.99	1-1.49	1.5-1.99	2+

CHAPTER 4

RESULTS

1. Participant Demographics

15 healthy, non-smoking females were recruited (Table 3). Participants' weight did not significantly change during their time in the study ($P = 0.3011$). Due to technical difficulties, one low-fitness status participant's mitochondrial capacity was not assessed, data presented represents high-fitness status n = 8 vs. low-fitness status n = 6. Due to failure to self-report, one high-fitness status participant's 24-hour food recall was not assessed, data presented represents high-fitness status n = 7 vs. low-fitness status n = 7.

2. Participant Fitness Characteristics

The high-fitness status group had higher relative VO_{2peak} (ml/kgBW/min and ml/kgFFM/min) compared to the low-fitness status group ($P < 0.05$, Table 4). The high-fitness status group had higher mitochondrial capacity (OD/sec) compared to the low-fitness status group ($P < 0.05$, Table 4). There were no significant differences between groups, except for days with vigorous activity ($P = 0.0225$, Table 7), in the 24-hour food recall, the 7-day sedentary activity time recall, the 7-day physical activity recall, and the modified 16-question QOLS ($P > 0.05$, Tables 5-8, respectively).

3. Fat and Carbohydrate Oxidation

There was no significant group effect for relative fat oxidation (g/kgFFM/min) during HIIE (group, $P = 0.0954$, $\eta_p^2 = 0.0193$, Figure 1a). There was a significant time effect for relative

fat oxidation (g/kgFFM/min) during HIIE (time, $P < 0.0001$, $\eta_p^2 = 0.0457$, Figure 1a). Total grams of fat oxidized did not differ between the groups ($t(13) = 1.22$, $P = 0.2426$, Figure 1b).

Overall, there was a significant group effect for relative carbohydrate oxidation (g/kgFFM/min) during HIIE (group, $P < 0.0001$, $\eta_p^2 = 0.2247$, Figure 1c). Relative carbohydrate oxidation (g/kgFFM/min) was significantly higher in the high-fitness status group during High 1 ($P = 0.0121$), High 2 ($P = 0.0067$), Low 2 ($P = 0.0474$), High 3 ($P = 0.0374$), Low 3 ($P = 0.0457$), and High 4 ($P = 0.0199$) compared to the low-fitness status group, Figure 1c. There was a significant time effect for relative carbohydrate oxidation (g/kgFFM/min) during HIIE (time, $P < 0.0001$, $\eta_p^2 = 0.7988$, Figure 1c). The total grams of carbohydrates oxidized did not differ between groups ($t(13) = -1.41$, $P = 0.1811$, Figure 1d).

4. Glucose and Lactate Response

Overall, there was a significant group effect for blood glucose (mmol/L) during HIIE (group, $P = 0.0257$, $\eta_p^2 = 0.0377$). Post-hoc analysis revealed groups did not statistically differ at any one time point ($P > 0.05$). There was a significant time effect for blood glucose (mmol/L) during HIIE (time, $P = 0.0024$, $\eta_p^2 = 0.01747$). Overall, there was no significant group effect for blood lactate (mmol/L) during HIIE (group, $P = 0.6063$, $\eta_p^2 = 0.0021$). There was a significant time effect for blood lactate (mmol/L) during HIIE (time, $P = 0.0027$, $\eta_p^2 = 0.1725$).

5. Skeletal Muscle Oxygenation

There was a significant group and time effect for skeletal muscle oxygenation levels of the biceps brachii (SmO_2%) during HIIE (group, $P = 0.0050$, $\eta_p^2 = 0.0539$; time, $P < 0.0001$, $\eta_p^2 = 0.5330$, Figure 2a). Post-hoc analysis revealed groups did not statistically differ at any one time point ($P > 0.05$). There was not a significant group effect for skeletal muscle oxygenation levels of the vastus lateralis (SmO_2%) during HIIE (group, $P = 0.1343$, $\eta_p^2 = 0.0156$, Figure 2b). There

was a significant time effect for skeletal muscle oxygenation levels of the vastus lateralis (SmO_2%) during HIIE (time, $P < 0.0001$, $\eta_p^2 = 0.2727$, Figure 2b).

6. Rate of Perceived Exertion

Overall, there was a significant group effect for RPE (Borg 6-20) during HIIE (group, $P = 0.8087$, $\eta_p^2 = 0.0005$). Post-hoc analysis revealed groups did not statistically differ at any one time point ($P > 0.05$). There was a significant time effect for RPE (Borg 6-20) during HIIE (time, $P < 0.0001$, $\eta_p^2 = 0.01747$). Upon further analysis, all high bouts had statistically higher rates of perceived exertion than periods of inactivity ($P < 0.05$).

Table 3. Participant Demographics

	High-Fitness Status (n = 8)	Low-Fitness Status (n = 7)	P-value
Age (y)	21.3 (3.0)	22.7 (4.5)	$P = 0.4294$
Height (cm)	163.79 (8.28)	166.64 (7.47)	$P = 0.4981$
Weight (kg)	59.18 (7.37)	73.37 (12.11)	$P = 0.0155$
BMI (kg/m^2)	22.39 (2.54)	26.23 (3.08)	$P = 0.0200$
Body Fat (%)	24.83 (3.52)	32.30 (4.78)	$P = 0.0041$
Fat-Free Mass (kg)	44.46 (5.81)	49.34 (7.15)	$P = 0.1681$
Waist-to-Hip Ratio	0.74 (0.05)	0.76 (0.04)	$P = 0.3659$
Systolic Blood Pressure (mmHg)	115.9 (19.0)	117.0 (10.0)	$P = 0.8907$
Diastolic Blood Pressure (mmHg)	70.8 (13.1)	73.4 (16.1)	$P = 0.6301$
Exercise Frequency (h/wk)	7.8 (5.3)	4.5 (3.7)	$P = 0.1920$

Note: y, years; cm, centimeter; m, meter; kg, kilogram; %, percent; mmHg, millimeters of mercury; h, hours; wk, week.

Table 4. Participant Fitness Characteristics

	High-Fitness Status (n = 8)	Low-Fitness Status (n = 7)	P-value
VO_{2peak} (L/min)	2.46 (0.25)	2.27 (0.55)	*P* = 0.3864
VO_{2peak} (ml/kgBW/min)	41.88 (3.48)	30.83 (4.75)	*P* = 0.0002
VO_{2peak} (ml/kgFFM/min)	55.70 (4.06)	45.55 (5.81)	*P* = 0.0016
Lower Body Muscular Strength (nm/kgFFM)	4.02 (0.91)	3.67 (0.66)	*P* = 0.4117
Upper Body Muscular Endurance (# of Pushups)	34.9 (11.8)	26.7 (8.9)	*P* = 0.1588
Sit & Reach (in)	20.23 (1.44)	20.88 (2.39)	*P* = 0.5312
Microvascular Function ($T_{1/2}$),	10.40 (4.31)	11.77 (5.08)	*P* = 0.5809
Mitochondrial Capacity (OD/sec)	1.42 (0.59)	0.72 (0.19)	*P* = 0.0167
Fitness Score	24.50 (2.00)	18.14 (2.79)	*P* = 0.0002

Note: L, liters; kg, kilogram; BW, total body weight; FFM, fat-free mass; nm, newton meters; in, inches; $T_{1/2}$, time for the O_2 signal to reach 50% peak levels; OD, optical density.

Table 5. 24-hour Food Recall

	High-Fitness Status (n = 7)	Low-Fitness Status (n = 7)	P-value
Calories (kcal/d)	1665.1 (463.1)	1514.4 (509.0)	*P* = 0.5730
Carbohydrate (g/d)	203.7 (55.9)	174.1 (67.0)	*P* = 0.3876
Fat (g/d)	61.5 (37.8)	53.2 (25.9)	*P* = 0.6409
Protein (g/d)	74.2 (26.2)	84.8 (26.1)	*P* = 0.4656

Note: kcal, kilocalorie; g, grams; d, day.

Table 6. 7-day Sedentary Activity Time Recall

	High-Fitness Status (n = 8)	Low-Fitness Status (n = 7)	P-value
Watching TV or Videos?	93.8 (63.0)	132.9 (81.8)	*P* = 0.3183
Using a computer?	273.0 (165.8)	295.7 (173.6)	*P* = 0.7997
During the last 7 days, how much time did you usually spend sitting on a week/weekend day?	360.0 (111.1)	437.1 (157.7)	*P* = 0.2882
Sitting and driving in a car, bus, or train?	45.0 (41.0)	62.1 (56.4)	*P* = 0.5084
Sitting reading a book or magazine?	62.8 (28.2)	81.4 (33.4)	*P* = 0.2611

Note: All times expressed in minutes.

Table 7. 7-day Physical Activity Recall

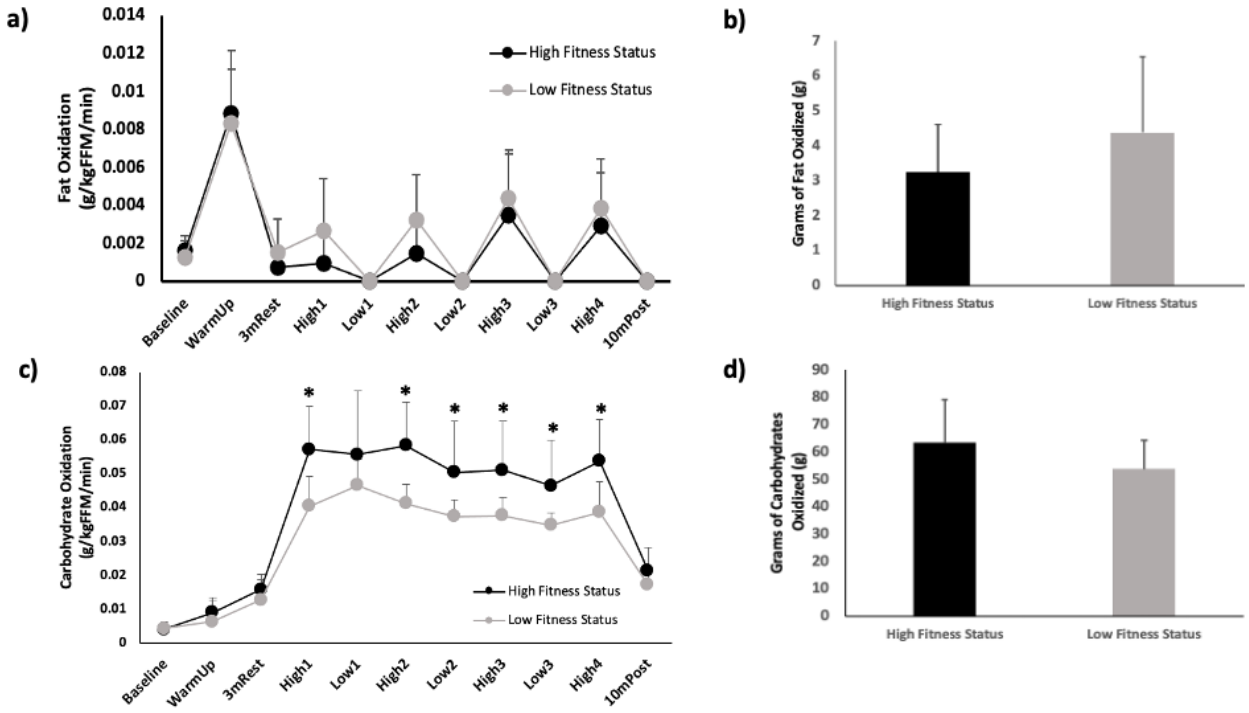
	High-Fitness Status (n = 8)	Low-Fitness Status (n = 7)	P-value
Days with VIG activity	3.9 (0.6)	2.3 (1.6)	*P* = 0.0225
Minutes per day VIG activity	90.0 (63.6)	60.0 (34.6)	*P* = 0.2877
Days with MOD activity	3.0 (1.3)	3.1 (2.3)	*P* = 0.8815
Minutes per day MOD activity	47.5 (32.3)	62.9 (38.2)	*P* = 0.4171
Days walked more than 10 minutes	5.5 (1.9)	5.6 (1.1)	*P* = 0.9309
Minutes spent walking on those days	66.9 (31.5)	59.3 (36.8)	*P* = 0.6737
Minutes spent sitting on a weekday	360.0 (90.7)	345.7 (165.6)	*P* = 0.8360

Note: VIG, vigorous; MOD, moderate.

Table 8. 16 Question Quality of Life Scale

	High-Fitness Status (n = 8)	Low-Fitness Status (n = 7)	P-value
Total Score	100.6 (5.0)	97.4 (6.8)	*P* = 0.3117

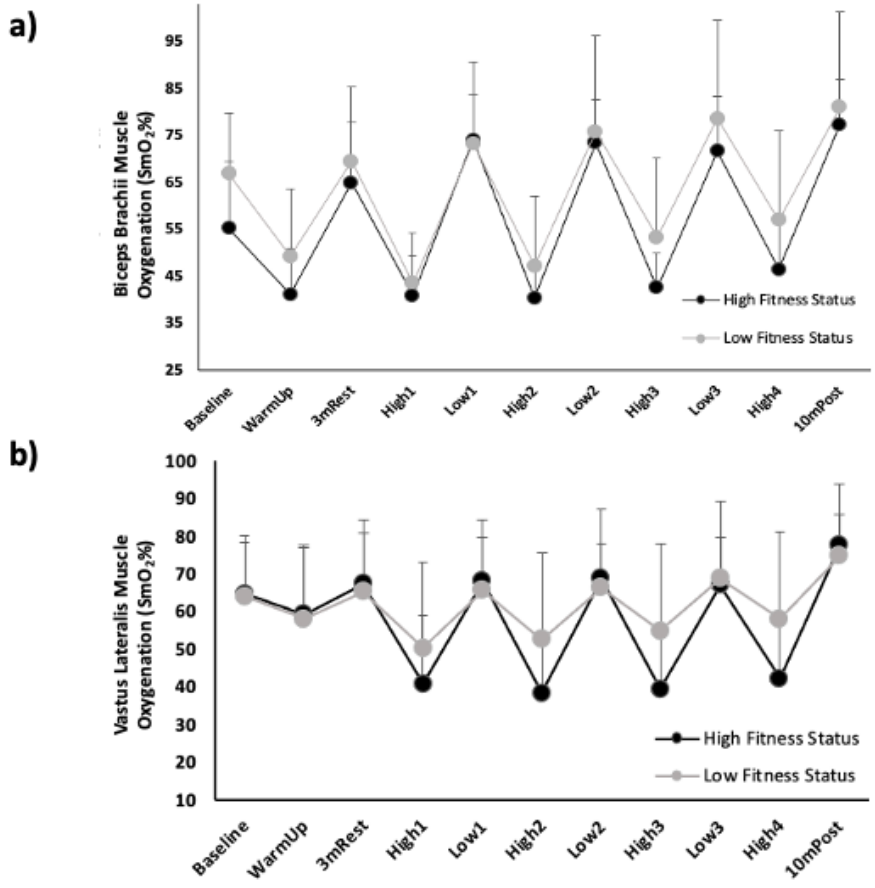
Figure 1. Fat and carbohydrate oxidation response to HIIE by fitness status



Note: (a) Relative fat oxidation (g/kgFFM/min) response during HIIE (ANOVA: group, *P* = 0.0954, η_p^2 = 0.0193; time, *P* < 0.0001, η_p^2 = 0.0457; n = 15). (b) Total fat oxidized (g) during

full session HIIE ($t(13) = 1.22$, $P = 0.2426$, high-fitness status n = 8 vs low-fitness status n = 7). (c) Relative carbohydrate oxidation (g/kgFFM/min) response during HIIE (ANOVA: group, $P < 0.0001$, $\eta_p^2 = 0.2247$; time, $P < 0.0001$, $\eta_p^2 = 0.7988$; n = 15). (d) Total carbohydrate oxidized (g) during full session HIIE ($t(13) = -1.41$, $P = 0.1811$, high-fitness status n = 8 vs low-fitness status n = 7).

Figure 2. Skeletal muscle oxygenation response to HIIE by fitness status



Note: (a) Biceps Brachii muscle oxygen saturation percent ($SmO_2\%$) response during HIIE (ANOVA: group, $P = 0.0050$, $\eta_p^2 = 0.0539$; time, $P < 0.0001$, $\eta_p^2 = 0.5330$; n = 15). (b) Vastus Lateralis muscle oxygen saturation percent ($SmO_2\%$) response during HIIE (ANOVA: group, $P = 0.1343$, $\eta_p^2 = 0.0156$; time, $P < 0.0001$, $\eta_p^2 = 0.2727$; n = 15).

CHAPTER 5

DISCUSSION

The purpose of this study was to comprehensively assess fitness status and determine if high-intensity interval rowing exercise was sensitive enough to detect differences between high- and low-fitness status females. Previous research has suggested that lean individuals of higher aerobic fitness, tend to exhibit higher rates of metabolic flexibility in response to both exercise and high-fat meals. Therefore, we hypothesized that a higher comprehensive fitness status would be sufficient to cause a significant increase in terms of metabolic flexibility during a high-intensity interval exercise bout on the rowing ergometer between the groups. Our results suggest that the metabolic response to exercise was different between the two groups. These findings are similar to that of Olenick et al. (2022). They also found that HIIE was sensitive enough to detect metabolic differences between high and low-fit females. The subjects in Olenick et al. (2022) were of a similar demographic to our participants in terms of age, aerobic fitness, and body fat percentage. Tentolouris et al. (2011) found contrasting results to our study, as their two groups did not differ in macronutrient oxidation despite differences in fitness, as it relates to body composition.

As previously stated, the HIIE test was sensitive enough to detect differences in metabolic response in the clinically healthy study population. This finding is consistent with previous literature (Olenick et al., 2022). However, to our knowledge, this is the first study to attempt to assess differences in metabolic flexibility during a maximal intensity HIIE protocol. Olenick et al. (2022) used a protocol with high-intensity bouts averaging 69% of the participants'

VO_{2peak}. Rates of and changes in fat oxidation were used as an indicator of metabolic flexibility. Additionally, San-Millan and Brooks (2018) assessed metabolic flexibility by monitoring participants' fat oxidation response during a ramped-intensity protocol. Participants in the present study were exercising at high-intensity bouts that averaged 78.32 (4.12)% of their VO_{2peak}. At this intensity, there is significant fuel contribution in the form of carbohydrate oxidation, even in the fittest of individuals (San-Millan and Brooks, 2018). Due to the significant contribution of carbohydrate oxidation, the relative contribution of fat oxidation becomes minimal. In the present study, this made it difficult to assess differences in fat oxidation between the two groups. The groups did not significantly differ in terms of relative fat oxidation, or changes in fat oxidation between bouts ($P > 0.05$). However, the high-fitness status group had increased rates of relative carbohydrate oxidation in response to maximal exercise. This potentially suggests they were able to make better use of circulating and stored muscle glycogen. Metabolic flexibility is defined as the individual's ability to store, traffic, and utilize substrates in the face of changes in metabolic demand (Smith et al., 2018, Storlien et al., 2004). At an intensity that demanded significant fuel contribution in the form of carbohydrates, it seems that our high-fitness status group met this demand to a greater capacity. However, with the current state of the literature, it is difficult to definitively say this is a marker of improved metabolic flexibility. Although, the greater metabolic capacity of the high-fitness status group during maximal exercise is of important note.

In the present study, our groups differed in terms of aerobic fitness, body composition, and mitochondrial capacity. They were not statistically different in the 4 other assessed fitness categories. Additionally, the females in both groups consumed a similar number of daily calories and had a similar amount of sedentary and physical activity time, aside from days of vigorous

activity per week. Most participants were recruited from a large college campus. As such, they were similar in terms of breaking sedentary patterns with walking breaks to get to classes and various locations on campus.

Note, once again, that the groups were similar in all areas except aerobic fitness, body composition, and mitochondrial capacity and did differ significantly in terms of exercising substrate utilization. Our results potentially suggest that a lower aerobic fitness status, lower mitochondrial capacity, and poorer body composition are sufficient to cause marked alterations in metabolic response during high-intensity interval exercise on a rowing ergometer. Amaro-Gahete et al. (2019) findings pointed out the complexity of the relationship between physical activity and metabolic flexibility. Their results showed many factors like age, sex, activity intensity, and other behaviors influence this relationship. Longitudinal studies would be needed to determine the long-term implications of physical inactivity in the studied population. From a longitudinal study, it would be interesting to examine how habitual physical inactivity trends with metabolic changes over the life span.

Our groups did not significantly differ in terms of microvascular function. de Jongh et al. (2004) compared a group of lean and obese women, slightly older than our study participants. Their findings suggested that impaired microvascular function was present in obese individuals. This is inconsistent with our findings, as our high fitness status group had a normal average body fat percentage, while our low fitness status group had an average body fat percentage classed as obese. Despite this body composition difference, they did not significantly differ in terms of microvascular function. Collectively, various lines of evidence suggest once again that many factors interact to determine one’s metabolic, mitochondrial, and microvascular health.

As far as the feasibility of the interval rowing exercise test, it was sufficient to cause shifts in substrate oxidation. Additionally, it was sensitive enough to detect differences in metabolic response in a clinically healthy population. Since "maximal" was not defined by a certain wattage or $\%VO_{2max}$, but rather interpreted by the participants, there were potential implications to our results. During the interval visit, on average, participants' VO_{2peak} was either equal to or higher than their baseline visit determined VO_{2peak}. Additionally, RPE, heart rate, and lactate data all indicated a near-maximal effort was given during the interval visit (Wasserman et al., 2004). The average RPE value immediately post-HIIE was (Borg 6-20; 17.4 ± 1.7). The average blood lactate value immediately post-HIIE was (mmol/L; 8.89 ± 2.64). The average heart rate value post-HIIE was (bpm; 183.9 ± 10.2). None of the aforementioned values significantly differed between the two groups ($P > 0.05$). Most of the participants reported some familiarity with the rower ergometer. However, only 3 participants (all in the high-fit group) reported routinely using the rower. Although those new to the rowing ergometer may not have been as mechanically efficient, we were testing for maximal effort. The recorded parameters suggest the participants were still performing at maximal effort, even though it was a novel exercise.

5.1 Limitations

A potential limitation of our study is the lack of diet control. We did recommend a macronutrient-controlled dinner and pre-workout meal. However, it is impossible for us to be sure the participants followed this recommendation, aside from verbal confirmation at their visit. There have been studies that have controlled the participants' diets for 1-4 weeks leading up to the testing sessions, which allows for a more controlled experimental design (Branis et al., 2015), although such rigorous laboratory settings have limited real-world applicability. All participants

in our study did confirm they followed the recommended food intake protocol before study visits.

Additionally, our results can only be applied to the narrow age range studied, as well as the female sex. We aimed to recruit females between the ages of 18-35 years. Our actual recruited range was 18-29 years (with a mean of 21.9 ± 3.6 years). We also excluded those with any health or metabolic conditions, and those on certain medications, such as antipsychotics. Therefore, the results can only be assumed to hold true in a young, healthy female population.

Another limitation of our current study is that we did not definitively test for circulating hormone levels. While we attempted to control for menstrual cycle timing by using the onset of menses as our physiological indicator of time during phase, differences in phase length, ovulation, and/or irregularities in cycle were not assessed (Sims & Heather, 2018).

Due to the limitations of self-report, we ultimately made the decision to split the groups post-hoc. Preliminary recruitment was based on high-intensity exercise 3-4 days a week for the high-fit group. Less than 2 hours of structured exercise per week was the classification for the low fitness status group. However, this preliminary classification did not account for differences in sedentary time and different perceptions of exercise intensity. These limitations contributed to our decision to do a median split on the groups post-hoc. However, we still resulted in groups that were significantly different in terms of aerobic fitness, body composition, and mitochondrial capacity. There were no significant differences in other aspects of physical fitness or sedentary time between the two groups. Most participants were recruited from a large college campus. Therefore, by way of getting around campus, the females were of low aerobic fitness but not considered sedentary individuals. Future research should aim to recruit groups of females that are

significantly different in the other 4 assessed categories of physical fitness: muscular strength, muscular endurance, microvascular function, and flexibility.

5.2 Conclusions

Obesity, along with cardiovascular and metabolic diseases have been popular areas of public interest and research. Risk factors for each of these health concerns, as well as how they relate to aging, have been important areas of exploration. Regular exercise can lead to significant differences in metabolic, micro- and macrovascular health, and muscular health. All of which can potentially reduce the risk of obesity, cardiovascular, and metabolic diseases. The recent pandemic has brought even more attention to these areas, and once again emphasized the importance of metabolic health in reducing mortality rates. As such, research has shifted to find methods ample to detect differences in metabolic health. Exercising tests of metabolic flexibility have proven to be sensitive enough to detect differences in metabolic flexibility that traditional meal challenges have not (Olenick et al., 2022). The aim of this study was to determine if a comprehensive assessment of physical fitness would positively influence exercising metabolic flexibility during a novel HIIE rowing protocol, in a group of young, healthy females. From the results of our study, it appears that a comprehensively assessed fitness status is sufficient to significantly alter carbohydrate oxidation during HIIE at ~78% of VO_{2peak}. This increased carbohydrate oxidation may be suggestive of an increased metabolic capacity during maximal-intensity exercise for the high-fitness status group. Additionally, our results support previous literature that suggests an HIIE test is sufficient to detect metabolic differences in a young, healthy, female population. Further research following chronic physical inactivity across the lifespan, as well as the interaction of other aspects of physical fitness and exercise intensity, is needed to address the remaining questions.

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Appendix A

IRB Approved Consent Form

IRB APPROVED
Jan 24, 2022

University of Georgia – Informed Consent Form

Assessment of metabolic function in young, healthy adults.

TITLE: Fitness Status and Exercise Metabolism

PROTOCOL NO.: PROJECT00004783
IRB Protocol #20216696

SPONSOR: University of Georgia

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STUDY-RELATED PHONE NUMBER(S): (706)-263-4019
240-595-7299 (24 hours)

STUDENT CO-INVESTIGATOR: Sara Garcia, B.S.Ed
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Researcher's Statement

You are being asked to take part in a research study. The information in this form will help you decide if you want to be in the study. Please ask the researcher(s) if there is anything that is not clear or if you need more information.

- The purpose of this research study is to assess the effects of fitness status on metabolism during exercise in young, healthy adults.
- You will complete a health questionnaire regarding your current health conditions and potential recent exposure to COVID-19. We will also take your temperature by using an infrared forehead thermometer. We will only permit you to enter the lab and proceed with further data collection if the questionnaire determines that you have no recent exposure to COVID-19 and your forehead temperature is lower than 100.4 degrees.
- Your involvement in the study is voluntary, and you may choose not to participate or stop at any time without penalty or loss of benefits to which you are otherwise entitled.
- This study's participation involves two visits, including a baseline and an interval exercise visit. The total time involvement for these two visits will be approximately 3.5 hours, with at least 48 h between each visit.
- Participation also involves measurement of body composition using a Dual Energy X-ray Absorptiometry, an x-ray scan of the entire body. The muscle function test uses a mild electrical shock to contract the muscle. Microvascular function testing uses a short (5 minute) blood pressure cuff on the arm. We will also take blood samples with finger sticks at multiple time points during the interval exercise test.
- Risks associated with participation include mild pain associated with finger sticks, feelings of physical fatigue or discomfort during exercise tests, and x-ray exposure during body composition testing. X-ray exposure is less than a typical doctor's x-ray exam for broken bones.
- You will receive information about your body composition, resting blood pressure, and cardiorespiratory fitness level.
- As an alternative to participating in this study, you can get your body composition and cardiorespiratory fitness level tested at any local sport performance testing facility.

If you are interested in participating in the study, please read the additional information on the following pages, and feel free to ask questions at any point.

IRB APPROVED
Jan 24, 2022

Study Procedures

We are recruiting adults (18-35 years old) who participate in a regular, high-intensity exercise routine with less than 30% fat mass or who do not participate in a structured exercise program (less than2h of exercise per week) with a normal or overweight BMI. If you do not meet the weight status inclusion criteria, you will be put on a waitlist until it is deemed safe for your participation. All study procedures will be at the Department of Kinesiology located within the Ramsey Student Center.

Baseline Visit

This visit will take about 1.5 h to complete. You will receive dietary recommendations before this visit to include dinner and breakfast recommendations. You will be asked to avoid exercise for 48 h prior and caffeine and alcohol 24 h before the visit. For this visit, you will arrive at the laboratory 2 hours after your recommended breakfast time. If you do not follow pre-visit diet and exercise recommendations, you will be asked to reschedule this visit. During this visit, we will measure your height, weight, blood pressure, body composition, lower body strength, muscle flexibility, and muscular endurance (describe below). Additionally, you will complete dietary and health behavior questions and a maximal effort exercise test (described below).

- **Height and weight measurement:** We will measure your height and weight using a standard scale.
    - If you are not participating in a structured exercise program, and your BMI results in a reading of greater than 29.9 kg/m^2 your visit will end, and you will be put on a waitlist until it is deemed safe for your participation.
- **Body composition:** We will measure body composition by Dual Energy X-ray Absorptiometry (DEXA). This test measures muscle mass, fat mass, and bone density. It involves an x-ray scan of the entire body. During the scan, you will lie still on the exam table for approximately 5 minutes.
    - For all participants, if your body composition scan results in a reading of greater than or equal to 30% fat mass your visit will end, and you will be put on a waitlist until it is deemed safe for your participation.
- **Blood Pressure:** We will take your blood pressure twice with a standard blood pressure machine, with one-minute between the measurements.
- **Dietary and Health Behavior Questionaries**: You will complete a series of 4 surveys. The first will ask you to recall and log all foods and beverages you have consumed in the previous 24 hours. The second and third will ask you to recall time you spend participating in sedentary and physically active activities. The fourth survey will ask questions related to your overall quality of life. Each survey will take ~5 minutes to complete.
- **Lower Body Strength Test**: You will perform a maximal effort knee extension to determine the strength of your dominant leg. The maximum force from three trials at three different angles (90, 75, & 60 degrees) will be recorded. A warmup will be provided to avoid injury, along with a trial for practice. Verbal encouragement will be provided throughout, with a 20 sec rest in between each repetition and a 5-min break in between each angle. The warmup trial will take place immediately before the actual test followed by a 10-minute rest.
    - NIRS device: A NIRS device will be strapped on your thigh for the test. The NIRS device shines low intensity light into muscle and measures the light which returns to the device, like a pulse oximeter. The light is used to measure oxygen in the muscle.
- **Muscle Flexibility Test:** You will perform a standard sit and reach test to determine the level of flexibility of your lower back and hamstring muscles. You will perform three trials. Verbal encouragement will be provided throughout, with a 30 sec rest in between each repetition.
- **Muscular Endurance Test:** To measure muscular endurance, you will perform the maximal number of push-ups you can complete in one minute. Verbal encouragement will be provided throughout, with a warmup immediately before the test.
    - NIRS device: A NIRS device will be strapped on your arm for the test. The NIRS device shines low intensity light into muscle and measures the light which returns to the device, like a pulse oximeter. The light is used to measure oxygen in the muscle.
- **Exercise until exhaustion test:** The test will be performed on a rowing ergometer where you will row at a slow pace for 3 minutes as a warmup, followed by a gradual intensity (rowing pace) increase every 2 minutes. The test will end when you can no longer continue. The air you inhale will be room air; the air exhaled will be collected by a hose attached to an analyzer box where oxygen and carbon dioxide in your breath will be measured. You will be asked to wear a facemask with valves that direct expired air into the box.
    - NIRS device and heart rate monitor: A heart rate monitor will be placed on your torso for the duration of the test. A NIRS device will be strapped on both your thigh and arm for the test. The NIRS device shines low intensity light into muscle and measures the light which returns to the device, like a pulse oximeter. The light is used to measure oxygen in the muscle.
    - We will measure blood lactate by finger stick immediately following conclusion of the test.

You will then schedule the next visit for at least 48 hours later. You will be asked to continue your normal dietary and exercise habits but will receive instructions before your next visit.

Interval Exercise Visit

This visit will take about 2 h to complete. You will receive dietary recommendations before this visit to include dinner recommendations. You will be 10-12h fasted before this visit. After you eat your dinner meal, you will be asked to only drink clear water until your visit. You will be asked to avoid exercise for 48 h prior and caffeine and alcohol 24 h before the visit. If you do not follow pre-visit diet and exercise recommendations you will be asked to reschedule this visit. We will begin with a short COVID-19 screening with a no-touch temperature recording, and then you will wash your hands.

- **Muscle Function Test:** We will put a NIRS device on your thigh for the test. We will measure resting metabolism by inflating a blood pressure cuff to stop blood flow to the leg. This test will last 30 seconds and will be done 1-3 times. A familiarization shock (15-60 sec) will be administered by starting at the lowest setting and increasing to elicit muscle movement. A short period of muscle contraction using mild electrical shock (15-60 sec) is performed during the test. The stimulation level will make the muscle move but should not be painful. Following the stimulation, we will inflate the blood pressure cuff to cut off blood flow for 5-15 seconds. This will be done 6-22 times to measure the muscle's recovery from the exercise. This entire process will be repeated four times. This test's final step is to measure the recovery of oxygen in the muscle after inflating a blood pressure cuff to stop blood flow for 30 sec. We will measure how fast the blood returns to your muscle after the cuff pressure is released. The muscle function test should take ~45 min.
- **Microvascular Function Test:** This test will measure and determine how your blood vessels respond to short periods of blood flow obstruction. We will place a blood pressure cuff near your elbow. We will place a NIRS device on your forearm to measure smaller blood vessels. We will measure the arm's resting oxygen saturation during 2 minutes of no cuff inflation. We will inflate the cuff to obstruct blood flow for 5 minutes. The measurements will be taken for another 3 minutes after the cuff pressure is released (blood flow return).
- **Interval exercise test:** This exercise test will consist of a rowing workout. The entire exercise test will be ~40 minutes total including 5 minutes seated rest, 25 minutes of interval exercise, and 10 minutes of seated recovery. We will confirm that you fully understand the exercise protocol before we start. To measure your effort during exercise we will ask for your rate of perceived exertion on a scale from 6-20. We will also measure your blood lactate and glucose values by finger sticks. You will complete a warm-up followed by four, 3-minute long, high intensity exercise bouts, separated by three, 3-minute long rest bouts.
    - Warm up: You will row at a self-selected light intensity (rate of perceived exertion: 11) for 3 minutes for the warm-up.
    - High intensity bouts: You will be instructed to row at maximal effort for 3-minutes.
    - Low intensity bouts: You will remain seated on the rowing ergometer for 3 minutes between high intensity exercise bouts.
    - Blood lactate and glucose: We will measure blood lactate and glucose by finger stick at rest, and at the end of each interval, and in recovery. A total of 10 finger sticks will be performed. You will be asked if you would like these to be on the same or different fingers.
    - NIRS device and heart rate monitor: A heart rate monitor will be places on your torso for the duration of the test. A NIRS device will be placed on your thigh and arm for the test.

The Baseline Visit will come first, and the Interval Exercise Visit will follow.

Risks and discomforts

We will collect personal information, including your name, contact information, and details about your health and medical history as part of your participation in this study. However, we will reduce any risk associated with collecting this information by not sharing your information with anyone outside of the study team, and your information will be de-identified in the study's database.

To maintain your safety, all researchers follow additional safety practices to reduce the transmission of COVID-19 and other unknown pathogens. Safety practice includes a pre-visit screening of participants and researchers, regular cleaning of all research spaces and equipment with registered disinfectants before and after each participant, proper use of personal protective equipment for researchers and participants, and the use of social distancing (when applicable). Although the study team will attempt to reduce the risk of COVID-19 infection during your participation, there is still a risk that you may become ill with this infection. This could lead to severe respiratory or other organ failure, and death. These complications would be more likely if you have one of the higher risk health conditions. Please review your health history with the study team member to see if you have one of these conditions.

The body composition test uses x-rays to measure your body composition; risks associated with a body composition scan are less than a typical doctor's x-ray exam (for broken bones). If you do not want to have a body composition scan, there are alternative methods to measure your body composition such as skinfold (pinch) or an electric scale that estimates your body fat. There are no known risks with these alternative procedures.

Finger sticks may feel uncomfortable, and you may experience mild pain associated with the needle sticks. Less common symptoms include bruising or fainting. We will minimize risks of infection by using standard sterilization procedures before and after each finger stick.

You may experience discomfort associated with the blood pressure cuff on the arm.

You may experience some discomfort from the muscle function test. This discomfort is due to the occlusion of blood to the thigh and for individuals who have never received electrical stimulation therapy, making their muscles contract involuntarily. These tests do not have any known long-term risks associated with them. The discomfort will subside immediately after the test is over.

You may experience feelings of physical exhaustion and fatigue during and just after the exercise tests. You may experience nausea and light-headedness as a result of these tests. We will provide water from a nearby fountain; however, we recommend you bring your own water bottle. Feelings of fatigue and discomfort with exercise are normal and subside within a few minutes. You may stop exercising and withdraw from further testing without penalty if you find the exercise to be too uncomfortable. If you experience severe pain or discomfort after exercising in a study visit, you should contact a medical professional, then contact the research team.

Benefits
While you may not directly benefit from this study, we will give you a copy of your body composition, resting blood pressure, body mass index, and cardiorespiratory fitness level. The results from this study will provide new information regarding metabolic function of females, at different fitness statuses. The knowledge gained may help give greater insight on how to examine metabolic health through exercise.

Alternatives
As an alternative to participating in this research study, you can get your body composition and cardiorespiratory fitness level tested at any local sport performance testing facility.

Compensation
If you are traveling from off-campus, we will provide you a parking pass for a parking spot in lot E04, the East Campus Parking Deck.

Cost to participants
It will not cost you anything to take part in this research study.

Privacy/Confidentiality
We will attempt to ensure your personal information is kept confidential. You will be assigned a study ID code for all data collection and analysis that will be store on a secured electronic drive in the Integrative Cardiovascular Physiology Laboratory. Your name and contact information will be stored separately on a secured drive in the Integrative Cardiovascular Physiology Laboratory. Your name and contact information will only be used during the time of data collection to contact you about visit scheduling. Any directly identifiable information will be destroyed once data collection is completed. Your name will not appear in any published article. We will keep all de-identified information collected for three years after data collection has ended in an electronic database stored on a secured drive in the Integrative Cardiovascular Physiology Laboratory. Only approved study team members will be granted access to this information. Researchers will not release identifiable results of the study to anyone other than individuals working on the project without your written consent unless required by law or if you were injured and needed care. After identifiers are removed, the information may be shared with other researchers and/or used for future studies without additional consent. There will be no unused blood stored. Additionally, data will not be used for commercial profit. Research will not include whole genome sequencing. This research involves the transmission of data over the internet. This information will be sent to you by email and includes 1) a screening survey, 2) informed consent, and 3) pre-visit diet instructions. Every reasonable effort has been taken to attempt to ensure the effective use of available technology; however, confidentiality during online communication cannot be guaranteed. The risk of breach of confidentially has been mitigated, but not eliminated.

Your private information may be shared with individuals and organizations that conduct or watch over this research, including:

- The research sponsor
- People who work with the research sponsor
- Government agencies, such as the Food and Drug Administration
- WCG IRB, the Institutional Review Board (IRB) that reviewed this research

IRB APPROVED
Jan 24, 2022

Taking part is voluntary

Your participation in this research project is completely voluntary, and you may choose not to participate or to stop at any time without penalty or loss of benefits to which you are otherwise entitled. If you are a student, your decision to participate or not will have no bearing on your grades or class. If you are an employee, your decision to participate in the research will not affect your employment or employment evaluations. Researchers reserve the right to terminate your participation for any reason, for example, a change in medical condition that would make the study unsafe for you. If you withdraw or the researcher terminates your participation, the information and data collected that can be identified as yours will be kept as part of the study and may continue to be analyzed unless you make a written request to remove, return, and/or destroy said information.

Research injuries or illnesses

The researchers will exercise all reasonable care to protect you from harm as a result of your participation. If any research-related activities result in an injury, the sole responsibility of the researchers will be to arrange for your transportation to an appropriate health care facility. If you think you have suffered a research-related injury, pain, or unusual soreness, you should seek immediate medical attention and then contact Dr. Nathan Jenkins right away at jenkinsn@uga.edu. If you suffer a research-related injury, your medical expenses will be your responsibility or that of your third-party payer. However, you are not precluded from seeking to collect compensation for injury related to malpractice, fault, or blame on the part of those involved in the research.

If you have any questions

The main researcher conducting this study is Dr. Nathan Jenkins, a professor at the University of Georgia. Please ask any questions you have now. If you have questions, concerns, or complaints later, or you believe you have been injured by your participation. you may contact Sara Garcia at sara.garcia@uga.edu, or Dr. Nathan Jenkins at jenkinsn@uga.edu or at by telephone at (706)-263-4019 or (240)-595-7299 (24 hours).

This research is being overseen by WCG IRB. An IRB is a group of people who perform an independent review of research studies. You may talk to them at 855-818-2289 or researchquestions@wcgirb.com if:

- You have questions, concerns, or complaints that are not being answered by the research team.
- You are not getting answers from the research team.
- You cannot reach the research team.
- You want to talk to someone else about the research.
- You have questions about your rights as a research subject.

Research Subject's Consent to Participate in Research

To voluntarily agree to take part in this study, you must sign on the line below. Your signature below indicates that you have read or had read to you this entire consent form and have had all of your questions answered.

_____	_____	_____
Name of Researcher	Signature	Date
_____	_____	_____
Name of Participant	Signature	Date

Please keep one copy and return the signed copy to the researcher.

Appendix B

Screening Questionnaire

Screening Document to be delivered via web-based service (Qualtrics).

Introduction Page: Thank you for your interest in our study. The Kinesiology Department at the University of Georgia is conducting a research study to learn more about the effects of fitness status on exercise metabolism in adults. We are recruiting adults (18-35 years old) who are healthy with no musculoskeletal disorders (ex. Osteoporosis) or chronic health conditions.

- Participation in this study involves two visits: a baseline visit and an interval exercise visit. Total time for participation is ~ 3.5 hours with at least 48 h between visits.
- The baseline visit will consist of a body composition scan*, muscular strength/endurance/flexibility assessment, series of physical activity and health habit questionnaires and a maximal effort rowing exercise test.
- The interval exercise visit will consist of a muscle and vascular function tests, and high intensity rowing exercise.
- The exercise testing visits involve high effort exercise and blood collection via finger sticks (11 total).
- Risks associated with participation include mild pain associated with finger sticks, mild discomfort from mild electrical stimulation to contract the muscle, feelings of physical fatigue or discomfort during exercise tests, and *x-ray exposure during body composition testing. X-ray exposure is less than a typical doctor's x-ray exam for broken bones.
- You will receive information about your body composition, resting blood pressure, cardiorespiratory fitness level.

To see if you might qualify for this study, you will be asked questions about your health history and present health condition. Some of these questions may be sensitive, such as questions about health and medication use. You do not have to answer any question that you do not want to answer. You may stop this questionnaire at any time. If you do not qualify for this study, the information you give me will be destroyed. Would you like to proceed?

If yes: Page advances to the following questions.
If no: Webpage terminates and no questions are asked.

Screening Questions:
1. Are you between the ages of 18-35 years?
A. Yes B. No
If no, the webpage terminates and no more questions are asked.
2. Have you participated in a high-intensity exercise training regimen (3-4x a week) for at least 1 year?
A. Yes B. No
2.1. Do you participate in a structured exercise program more that 2 hours a week?
A. Yes B. No
Demographics
3. What is your full name?
4. What is your phone number?
5. What is your email address?
6. What is your preferred contact method?
A. Phone B. Email

7. What is your age?
8. What is your height (inches)?
9. What is your weight (pounds)?
10. What is your race?
11. What is your biological sex?
A. Male B. Female

Health History
12. Have you had surgery of any kind in the past 12 months?
A. Yes B. No
13. Do you currently use tobacco products or have you ever used tobacco products?
A. Yes B. No
If yes,13.1. Have you used tobacco products within the last 12 months?
A. Yes B. No
14. Do you have or have you ever had type 1 diabetes, type 2 diabetes requiring insulin therapy, coronary artery disease, fatty liver disease (alcoholic or non-alcoholic), renal disease, celiac disease, any cancer?
A. Yes B. No
15. Do you have asthma or exercise-induced asthma?
A. Yes B. No
16. Do you take any medications?
A. Yes B. No
If yes, 16.1. Do you take any medications known to alter hormonal or metabolic processes, including steroids, hormone therapies, anti-depressants, anti-psychotics, lipid-lowering drugs, or anti-hyperglycemic medications, anti-inflammatory medications?
A. Yes B. No
16.2. Do you take any medications known to alter blood pressure or blood volume responses to exercise, thereby making exercise potentially unsafe, including anti-hypertensive medications or diuretics?
A. Yes B. No
17. Are you taking a multi-vitamin supplement?
A. Yes B. No
If yes, 17.1. Are you willing to abstain from these during your participation in the study?
A. Yes B. No
18. Are you currently taking any hormones for performance enhancement such as Arimidex, DHEA or Chrysin?
A. Yes B. No
19. Do you have any blood clotting disorders?
A. Yes B. No
20. Do you have sickle cell disease?
A. Yes B. No
21. Do you have any musculoskeletal disorders, such as osteoporosis or sarcopenia?
A. Yes B. No
22. Has your doctor ever said you have a heart condition and that you should only do physical activity recommended by a doctor?
A. Yes B. No

23. Do you feel pain in your chest when you do physical activity?
A. Yes B. No
24. In the past month, have you had a chest pain when you were not doing physical activity?
A. Yes B. No
25. Do you lose balance because of dizziness or do you ever lose consciousness?
A. Yes B. No
26. Do you have a bone or joint problem that could be made worse by a change in your physical activity?
A. Yes B. No
27. Do you know of any other reason why you should not take part in physical activity?
A. Yes B. No
If yes, please comment:
COVID-19
28. Are you sick?
A. Yes B. No
If yes, 28.1. Please describe illness.

29. Do you have a fever (temperature of at least 100.4°F), cough, shortness of breath, chills, sore throat, muscle pain, vomiting or diarrhea, or new loss of taste or smell?
A. Yes B. No
30. Have you had any of the above symptoms, even if they were mild, in the past 2 weeks?
A. Yes B. No
If yes, 30.1. When did they stop?

31. In the past 2 weeks, have you had close contact with anyone who has had fever (temperature of at least 100.4°F), cough, shortness of breath, chills, sore throat, muscle pain, vomiting or diarrhea, or new loss of taste or smell?
A. Yes B. No
32. In the past 2 weeks (14 days) have you had close contact with anyone who has had a possible COVID-19 infection?
A. Yes B. No
33. Have you been tested for COVID-19 in the last two weeks (14 days)?
A. Yes B. No
If yes, 33.1. What was the date of your test:
33.2. What was test result?
A. Positive B. Negative
34. Have you traveled outside your community in the past 2 weeks (14 days)?
A. Yes B. No
35. Do you routinely utilize public transportation?
A. Yes B. No
36. Are you practicing social distancing by wearing a mask when you leave the house and do you stay 6 feet from people when in public places?
A. Yes B. No
37. Do you work in a healthcare facility or as a first responder?
A. Yes B. No
38. Do you work in the meat packing industry or in critical infrastructure?

A. Yes B. No
39. Do you live in a congregate living settings or have you had close contact with someone who does (e.g., residents in long-term care facilities, prisons and shelters)?
A. Yes B. No
40. Within the past 2 weeks (14 days) have you, or a member of your household, attended an event/ location that has been identified as a COVID-19 outbreak location?
A. Yes B. No
41. Have you received both doses of a COVID-19 vaccine?
A. Yes B. No

Closing Statement – after completion of questionnaire:

"Thank you for your response. If you qualify for the study you will be contacted within 48 hours via your preferred contact method. Thank you!"

- If all inclusion and exclusion criteria are met: A study member will contact the potential participant within 48 hours to describe the study and provide informed consent so they can decide if they would like to continue to participate. The informed consent will be sent, via email, prior to this conversation. The study will be described in depth and the informed consent document will be explained to the study participants over the phone or Zoom. Please note that our standard practice is to sit down with the participant and walk them through all the parts of the document in extensive detail. This aspect will be revised to be done over the phone or Zoom. The process takes approximately 30-45 minutes. It is our practice to ensure that participants are fully informed on all aspects of the study before they provide their written consent (i.e., we do not only rely on them having read the IC, we devote significant time and effort to also explain the entire document). After remotely conducting the informed consent, we will request participants electronically sign the document and return it via email prior to Visit 1.
    - If they want to participate, we will schedule Visit 1.

Appendix C

Physical Activity Questionnaire

Physical Activity Questionnaire

Date: _____ Participant ID: _____ Visit: _____ Time: _____

Instructions: The questions will ask you about the time you spent being physically active in the **last 7 days**. Please answer each question even if you do not consider yourself to be an active person. Please think about the activities you do at work, as part of your house and yard work, to get from place to place, and in your spare time for recreation, exercise or sport.

Think about all the **vigorous** activities that you did in the **last 7 days**. **Vigorous** physical activities refer to activities that take hard physical effort and make you breathe much harder than normal. Think *only* about those physical activities that you did for at least 10 minutes at a time.	
1. During the **last 7 days**, on how many days did you do **vigorous** physical activities like heavy lifting, digging, aerobics, or fast bicycling?	_____ **days per week** No vigorous physical activities ***Skip to question 3***
2. How much time did you usually spend doing **vigorous** physical activities on one of those days?	_____ **hours per day** _____ **minutes per day** Don't know/Not sure

Think about all the **moderate** activities that you did in the **last 7 days**. **Moderate** activities refer to activities that take moderate physical effort and make you breathe somewhat harder than normal. Think only about those physical activities that you did for at least 10 minutes at a time.	
3. During the **last 7 days**, on how many days did you do **moderate** physical activities like carrying light loads, bicycling at a regular pace, or doubles tennis? Do not include walking.	_____ **days per week** No moderate physical activities ***Skip to question 5***
4. How much time did you usually spend doing **moderate** physical activities on one of those days?	____ **hours per day** _____ **minutes per day** Don't know/Not sure

Think about the time you spent **walking** in the **last 7 days**. This includes at work and at home, walking to travel from place to place, and any other walking that you have done solely for recreation, sport, exercise, or leisure.

5. During the **last 7 days**, on how many days did you **walk** for at least 10 minutes at a time?	_____ **days per week**
6. How much time did you usually spend **walking** on one of those days?	_____ **hours per day** _____ **minutes per day** Don't know/Not sure

The last question is about the time you spent **sitting** on weekdays during the **last 7 days**. Include time spent at work, at home, while doing course work and during leisure time. This may include time spent sitting at a desk, visiting friends, reading, or sitting or lying down to watch television.	
7. During the **last 7 days**, how much time did you spend **sitting** on a **week day**?	_____ **hours per day** _____ **minutes per day** Don't know/Not sure

Instructions: Please answer the questions about structured exercise completed in the **past 7-days**.

1. In the past 7 days, how many hours of cardio did you complete?	
2. In the past 7 days, how many hours of strength training did you complete?	
3. List all other types of exercise completed in the past 7 days.	
4. Is this a typical week exercise regimen for you?	
5. How long have you been performing this training regimen? (i.e. "I have been participating in this training regimen for __ years." "I have been participating in CrossFit for 3 years." "I have been participating in bodybuilding for 2 years." Etc.)	
6. Do you participate in any sports? - If yes, which ones and how many hours a week? - Do you play competitively? - If yes, where do you place in competitions?	

Appendix D

Sedentary Time Questionnaire

Sedentary Time Questionnaire
Date: _____ Participant ID: _____ Visit: _____ Time: _____

Instructions: The following questions are about activities you did over the past week while sitting, reclining or lying down. Do not count the time you spent in bed sleeping or napping.

On a typical WEEKDAY/WEEKEND DAY in the past week, how much time do you spend sitting, reclining or lying down and. . .

Sedentary Item	Time
1) Watching television or videos? (count time spent watching television, DVDs, and online videos)	Hours: Minutes:
2) Using a computer? (count time spent on things such as computers, laptops, Xbox, PlayStation, IPod, IPad, or other tablet, or a smartphone, YouTube, Facebook, or other social networking tools, and the internet).	Hours: Minutes:
3) During the last 7 days, how much time did you usually spend sitting on a week/weekend day? (Include time spent at school or work, at home, while doing course work, and during leisure time. This may include time spent sitting at a desk, visiting friends, reading or sitting or lying down to watch television.)	Hours: Minutes:
4) Sitting and driving in a car, bus, or train?	Hours: Minutes:
5) Sitting reading a book or magazine? (Only include reading during your free time. Include reading done using electronic formats. Include time spent reading as part of your homework, but do not include time spend reading at work, during class time or while exercising).	Hours: Minutes:

Appendix E

Quality of Life Scale (QOLS)

Quality of Life Scale (QOLS)

Date: _____ Participant ID: _____ Visit: _____ Time: _____

Instructions: Please read each item and circle the number that best describes how satisfied you are at this time. Please answer each item even if you do not currently participate in an activity or have a relationship. You can be satisfied or dissatisfied with not doing the activity or having the relationship.

	Delighted	Pleased	Mostly Satisfied	Mixed	Mostly Dissatisfied	Unhappy	Terrible
1. Material comforts home, food, conveniences, financial security	7	6	5	4	3	2	1
2. Health - being physically fit and vigorous	7	6	5	4	3	2	1
3. Relationships with parents, siblings & other relatives-communicating, visiting, helping	7	6	5	4	3	2	1
4. Having and rearing children	7	6	5	4	3	2	1
5. Close relationships with spouse or significant other	7	6	5	4	3	2	1
6. Close friends	7	6	5	4	3	2	1
7. Helping and encouraging others, volunteering, giving advice	7	6	5	4	3	2	1
8. Participating in organizations and public affairs	7	6	5	4	3	2	1
9. Learning- attending school, improving understanding, getting additional knowledge	7	6	5	4	3	2	1
10. Understanding yourself-knowing your assets and limitations - knowing what life is about	7	6	5	4	3	2	1

11. Work - job or in home	7	6	5	4	3	2	1
12. Expressing yourself creatively	7	6	5	4	3	2	1
13. Socializing-meeting other people, doing things, parties, etc	7	6	5	4	3	2	1
14. Reading, listening to music, or observing entertainment	7	6	5	4	3	2	1
15. Participating in active recreation	7	6	5	4	3	2	1
16. Independence, doing for yourself	7	6	5	4	3	2	1

Appendix F

24-Hour Food Recall

24H Food Recall Questionnaire

Date: _____ Participant ID: _____ Visit: _____ Time: _____

Instructions: Please write down all food and drinks consumed.

Meal	Food	Amount	Calories
Breakfast			
Lunch			
Dinner			
Snacks			

*Calories were calculated by a researcher