AN ACUTE METABOLIC EVALUATION OF EXERCISE AND NUTRITION IN WOMEN

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ABSTRACT

Hailee Lorrain Wingfield: An Acute Metabolic Evaluation of Exercise and Nutrition in Women
(Under the direction of Abbie Smith-Ryan)

The purpose of the current study was to examine the effect of aerobic endurance exercise (AEE), high-intensity interval training (HIIT), and high-intensity resistance training (HIRT), combined with pre-exercise carbohydrate (CHO) or protein (PRO) ingestion on resting energy expenditure (REE) and respiratory exchange ratio (RER) in women. Participants completed six sessions, of three exercise modalities: AEE, HIIT, and HIRT, and two acute nutritional interventions: 25 grams of CHO and PRO. Measurements were taken at baseline, immediately post, 30min post and 60 min post. Post exercise, HIIT elicited the largest increase in REE compared to AEE ($p<0.0001$) and HIRT ($p<0.0001$). HIIT and HIRT resulted in significantly lower RER ($p<0.0001$-$p=0.0004$) compared to AEE post exercise. PRO intake significantly elevated REE ($p<0.01$) and reduced RER ($p=0.0012$) to a greater extent than CHO beginning 30min post exercise. Integrating HIIT and pre-exercise PRO intake may have positive implications on energy expenditure and fat utilization in women.

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CHAPTER I

INTRODUCTION

Aerobic exercise has been the primary mode of exercise prescribed to individuals in order to improve health and maintain weight (1). According to the American College of Sports Medicine, all healthy adults are encouraged to participate in at least 30 minutes of moderate-intensity aerobic physical activity five times per week, or 20 minutes of vigorous-intensity physical activity three times per week. These guidelines provide the minimum amount of exercise needed in order to decrease the risk of developing chronic disease, but they are not detailed enough to provide an exercise prescription for all adults. While the most specific guidelines exist for aerobic exercise, activities such as resistance training and interval training have been recently reported to be more time-efficient modes of exercise to stimulate rapid changes in energy expenditure (2–6) as well as body composition (7), which are two influential variables used in predicting health and disease risk.

Energy expenditure (EE) is a major factor in weight loss and weight maintenance (8). Several studies have used EE to assess the effectiveness of various modes of exercise (8–10). For example, Jamurtas and colleagues (8) demonstrated that resting energy expenditure (REE) can be significantly elevated 24-48 hours after acute bouts of weight lifting or running. Furthermore, comparable intensity bouts of resistance and aerobic exercise, 70% VO_{2max} and 70% 1-repetition maximum (RM) respectively, have

demonstrated similar increases in 24-hour EE in non-obese men (9). These two studies demonstrate that aerobic and resistance training elicit similar EE responses. However, when examining oxygen uptake in untrained women at 30-minutes post exercise, a bout of circuit weight training resulted in significantly higher REE values than did a bout of treadmill exercise for a similar duration and intensity (10). Additional research evaluating different types and intensities of resistance and aerobic exercise as effective modes for enhancing EE could be beneficial for exercise prescription. More so, additional research emphasizing the effects in women could provide greater insight into metabolic sex differences.

In addition to different modes of exercise, continuous and interval exercise protocols have also been examined to determine potential EE and the rate of substrate oxidation differences. Two studies by Goto and colleagues (11,12) demonstrated that repeated bouts of moderate-intensity (60% VO_{2max}) endurance exercise contributes to greater fat oxidation compared with a single bout of cycling with equivalent exercise durations. Gosselin and associates (4) also found that despite subjects performing similar amounts of work at 90% VO_{2max}, altering work-to-rest ratios can significantly alter caloric expenditure responses to interval exercise. These three studies provide evidence that interval exercise may produce higher EE values than continuous exercise. However, future studies are needed to determine appropriate work-to-rest ratios to maximize REE.

Appropriate exercise intensity must also be explored in order to create more specific guidelines. Gore and Withers (13) found exercise intensity to be the major determinant of excess post-exercise oxygen consumption (EPOC) variance. This is in conjunction with Thornton and Potteiger's (14) finding that at 85% of an 8-RM, a high-

intensity resistance exercise bout produced a greater EPOC volume and magnitude than a bout of resistance exercise at 45% 8-RM. According to Bielsinki and colleagues (15), intense exercise (180 minutes at 50% VO_{2max}) stimulates fat oxidation and REE for extended periods of time following the exercise. More so, a high intensity, short duration cycle bout produced a significantly higher REE than low intensity, short- and long-duration bouts (6). When examining resistance training intensity, Paoli et al. (5) found shorter, high-intensity resistance training sessions (80-85% 1-RM) to be more effective than traditional resistance training sessions (70-75% 1-RM) at increasing REE and reducing respiratory exchange ratio (RER), representing enhanced fat oxidation. In addition, Howley et al. (16) suggested that with all other factors held constant, shorter rest periods during resistance training result in greater EPOC. It is apparent from these studies that the exercise intensity is an important aspect of exercise prescription, energy expenditure, and fuel utilization.

Along with exercise, nutrition plays an important role in weight control. Nutritional composition and timing are two major factors that can affect the impact of exercise (17–20). Bullough and associates (17) suggested that energy intake, exercise, and their combined effects influence resting metabolic rate. Also, exercise performed in the post-absorptive state saves more carbohydrate and oxidizes more fat in the body, without affecting REE (20). Hackney and colleagues (18) demonstrated protein ingestion prior to a heavy resistance training bout can increase REE by elevating RMR up to 24 hours after the exercise session. In addition, macronutrient intake during and after moderate-intensity exercise was found to influence substrate oxidation in active young

women (19). Further studies could lead to more specific recommendations regarding the effects of nutrient timing and composition on energy expenditure and fuel utilization.

It is apparent that sex differences exist in metabolic fuel utilization. Additional research regarding macronutrient intake and metabolic efficiency is needed. Smith and McNaughton (21) demonstrated that during a 30-minute cycling bout, men and women had the same EE when body mass was accounted for, but Isacco et al. (22) stated there is substantial evidence demonstrating the influence of sex on substrate utilization during exercise, signifying that men typically rely less on fat metabolism than women. The initiation of these differences become apparent after the onset of puberty, as a result of fluctuations in ovarian hormones. Multiple studies (22–25) have concluded no observable differences in whole body carbohydrate or lipid oxidation during moderate, prolonged exercise among the normal menstrual cycle phases. While some studies showed greater lipid and lesser carbohydrate rates of oxidation during the luteal phase of the menstrual cycle (25–29), there is still conflicting evidence on observable differences in substrate utilization during exercise in different menstrual cycle phases (22). Due to available conflicting evidence in regards to sex and associated hormones, the menstrual cycles should be controlled for when studying women. More so, additional data evaluating nutrient timing in women are warranted.

In addition to exercise, nutrition, and sex differences, cortisol may also influence metabolic fuel utilization. Cortisol is released by the adrenal cortex and regulates many physiological systems (30,31). Previous research has determined that cortisol is an important regulator of fuel utilization during exercise, and has been shown to significantly increase with exercise of high intensity (30,31). These studies provide

evidence that increased cortisol levels may determine fuel utilization with high-intensity exercise.

While there are various studies examining exercise and nutrition, we are unaware of any previous studies that have explored the combined effects of exercise intensity and macronutrient alterations to stimulate REE and RER. Research exclusively evaluating women is especially lacking. For example, current recommendations regarding exercise prescription and dietary intake to augment REE and fat oxidation in women are unclear. Therefore, this study aims to determine exercise mode, intensity, and nutritional composition that best maximize REE and fat oxidation in women.

Purpose

1. The primary purpose of this study was to determine the effect of acute exercise on resting energy expenditure (REE) from baseline to immediately post- (IP), 30 minutes post- (30min) and 60 minutes (60min) post-exercise in women.
    a. The effect of a single acute bout of aerobic endurance exercise (AEE), high-intensity interval running (HIIT), and high-intensity resistance training (HIRT) on energy expenditure was evaluated.
2. The secondary purpose of this study was to determine the influence of pre-exercise carbohydrate (CHO) or protein (PRO) ingestion, on REE time course response and respiratory exchange ratio (RER) in women.
3. An exploratory purpose of this study was to determine the influence of exercise modality on post-exercise cortisol concentrations.

Research Questions

1. Does the intensity and mode of exercise have a greater influence on post-exercise REE?
2. Does a pre-exercise supplementation with carbohydrate or protein elevate post-exercise REE or modify RER?
3. Does the intensity and mode of exercise have an influence on post-exercise cortisol levels?

Research Hypothesis

1. Single acute bouts of HIIT and HIRT will produce a similar REE, which will be higher than for a single acute bout of AEE.
2. PRO ingestion before an exercise bout will result in a higher REE and lower RER than CHO ingestion.
3. Cortisol concentrations will be higher after a bout of HIIT and HIRT than after a bout of AEE.

Delimitations

1. Twenty recreationally active women were recruited to participate in this study.
2. Participants were between the ages of 18-35.
3. REE was estimated by the Weir (32) formula, using gas samples obtained from indirect calorimetry.
4. RER was measured using indirect calorimetry.
5. Exercise modes consisted of aerobic endurance exercise (AEE), high-intensity interval running (HIIT), and high-intensity resistance training (HIRT).

6. Exercise intensities were 45-55% HRR for AEE, 85-95% HRR for HIIT, and 80-85% 6-8RM for HIRT.

Limitations

1. Subject recruitment took place throughout various departments on the University of North Carolina at Chapel Hill campus, as well as through available physical activity and exercise science classes. Therefore, subject selection was not truly random.
2. Indirect calorimetry was used to measure REE and RER, rather than whole body direct calorimetry, which is considered the gold standard.
3. No major dietary modifications were made, which could have a potential effect on the acute fuel utilization measurement.
4. This was an acute intervention, and post-exercise measurements were only conducted for 60 minutes, so it is difficult to predict longer term resting energy expenditure and fuel utilization.

Assumptions

Theoretical

1. Subjects provided accurate health and exercise history on the enrollment questionnaire.
2. Subjects provided accurate dietary intake information on the dietary intake log.
3. Subjects followed pre-testing exercise and dietary guidelines.
4. Subjects maintained consistent exercise and nutrition habits throughout the duration of the study.

Statistical

1. The population from which the sample was drawn was normally distributed.
2. The order of exercise bouts and nutritional components was randomly assigned.
3. The variability of the sample in the experiment was equal or nearly so (homogeneity of variance).

Definition of terms

Aerobic Endurance Exercise (AEE) – an exercise strategy that involves a warm-up period, a long, slow endurance run (45-55% HRR), and a cool-down period. The entire session will last around 20 minutes.

High-intensity Interval Training (HIIT) – an exercise strategy that involves a warm-up period, ten 60-second, maximum-intensity aerobic efforts (85-95% HRR) separated by 60-second moderate recovery intervals (35-40% HRR), and a cool-down period. The period of alternating effort and recovery intervals will last between 15-20 minutes.

High-intensity Resistance Training (HIRT) – an exercise strategy that involves a warm-up period, several short, maximum-intensity resistance training efforts (6-8RM) separated by moderate recovery intervals (20-second rest between sets; 150-second rest between exercises), and a cool-down period. The period of alternating effort and recovery intervals will last around 20 minutes.

Recreationally Active – 1-5 hours per week of recreational and/or structured aerobic and/or resistance exercise, excluding competitive athletes

Resting Energy Expenditure (EE) – energy expended before exercise and during the post-exercise recovery period as measured using indirect calorimetry

Respiratory Exchange Ratio (RER) – a non-invasive technique used to estimate the percent contribution of fat or carbohydrate to energy metabolism before, during, or after exercise that is the ratio of carbon dioxide output to the volume of oxygen consumed (33)

Significance of study

This study attempted to determine energy expenditure differences among aerobic endurance exercise, high-intensity interval training, and high-intensity resistance training bouts. It attempted to determine RER and REE differences among carbohydrate and protein nutritional interventions. Multiple studies have examined these variables, but to date no literature has been found that examines all of these variables together, and more specifically no evaluations exist in women. The results of the present study may be further incorporated into exercise prescriptions to maximize metabolic adaptations and caloric expenditure in women, potentially resulting in chronic weight loss and maintenance.

CHAPTER II

REVIEW OF LITERATURE

Introduction

Data evaluating the combined metabolic effects of exercise and nutrition in women are limited. Several studies (4,5,13,34) serve as the basis for the current study, which examined the acute effects of exercise and nutrition on REE and RER from baseline through 1-hour post-exercise.

Exercise protocols similar to those used in the above studies have been integrated within the present study to establish appropriate guidelines for exercise mode and intensity. Specifically, data from Gore and Withers (13) demonstrated an increase in EPOC from a 20-minute treadmill run at 50% VO_{2max}; Gosselin et al. (4) reported increased EE with work-to-rest ratios of one (i.e., 60-second work period, 60-second rest period); and lastly, adapted from Paoli et al. (5), who reported an increased REE with high-intensity resistance training (HIRT) compared to traditional resistance training. Therefore, the present study compared the effects of a 30-minute treadmill run at 45-55% HRR, ten cycles of 60-second work and rest periods at 85-95% HRR, three sets of 6-8RM with 20-second and 150-second rest periods between sets and exercises.

Research by Shimada et al. (20) and Hackney et al. (18) provides a rationale for the nutrition protocol that were used in the current study to evaluate REE and RER. Shimada and colleagues (20) reported that exercise performed in the post-absorptive state

saves more carbohydrate (CHO) and oxidizes more fat in the body. In regards to nutrition content, Hackney et al. (18) found that post-exercise REE was higher when protein was ingested pre-exercise, when compared to carbohydrate ingestion. These two articles helped shape the nutrient timing and content for this study, which sought to determine if carbohydrate or protein ingestion prior to exercise influences post-exercise REE and RER.

Due to the varying nature of study population and design, the purpose of this review is to evaluate exercise and nutrition separately, in order to provide an extensive foundation for which the proposed project was designed. Protocols of AEE, HIIT, and HIRT will be discussed first, followed by the effect of CHO and protein (PRO) on REE and RER, and a final evaluation of fuel utilization considerations in women.

Exercise and Energy Expenditure

Energy expenditure (EE) from exercise may play a role in weight loss and maintenance (8). Based on previous literature (4–6,10–12), mode of exercise may have an influence on varying REE. The majority of existing data evaluates the acute effects of varying modes of exercise, including AEE and HIRT, but not HIIT. The current study compared the acute effects of these modes on REE, which gives insight to cumulative chronic effects in women, such as body composition and oxygen uptake improvements. This study employed three exercise modes to evaluate which mode, if any, was more effective for maximizing caloric expenditure.

Aerobic Endurance Exercise (AEE)

Several studies have examined the effect of endurance exercise bouts on EE and EPOC (13,21,35,36). Due to the broad definition of aerobic exercise, research investigating the influence on REE has varied substantially in time, intensity, and mode. Imamura and colleagues (35) demonstrated a greater EPOC response as a result of a longer duration exercise bout (60 minutes of cycling at 60% of maximal oxygen uptake (VO_{2max}) in comparison to a shorter duration exercise bout (30 minutes of cycling at 60% VO_{2max}) in young women. In moderately trained men and women, Smith and McNaughton (21) employed cycling sessions at 40%, 50%, and 70% of VO_{2max}, and found 3-hour post-exercise EPOC to be determined by the exercise intensity; as exercise intensity increased, EPOC did as well. Similarly, after having male subjects perform exercise bouts of 20, 50, and 80 minutes of treadmill exercise at 30, 50, and 70% VO_{2max}, Gore and Withers (13) found exercise intensity, and not duration, to be the major determinant of EPOC variance. In addition, Warren et al. (36) found that when exercise duration, intensity, and modality were altered, only intensity significantly affected post-exercise fat metabolism and EPOC. These studies provide evidence in support of the role of exercise intensity has on outcome variables such as EPOC and REE, leading to a hypothesis that high intensity exercise would produce a greater EPOC and REE than low intensity exercise.

The effect of endurance exercise bouts on RER has also been examined (37,38). Chad and Quigley (37) reported a greater response in oxygen uptake (VO_2) and a reduction in RER three hours following a 30 minute cycle bout at 50% VO_{2max} compared to 70% VO_{2max} in women. These results suggest greater estimated fatty acid oxidation

with a lower intensity. However, Kuo and colleagues (38) discovered post-exercise fatty acid oxidation to be the same with cycling protocols of 89 minutes at 45% VO_{2peak} and 60 minutes at 65% VO_{2peak} in men and women. These two studies present conflicting results, therefore a definite hypothesis regarding intensity and RER cannot be made.

High-Intensity Interval Training (HIIT)

Research examining acute bouts of high-intensity interval training (HIIT) is limited. However, several studies have investigated the chronic effects of HIIT on EE or REE. While chronic data is important, the acute effect of HIIT may provide insight in determining physiological changes that stimulate chronic adaptations. Due to its high level of muscle fiber recruitment and potential stress on type II fibers, Gibala and McGee (3) suggest that HIIT stimulates rapid changes in skeletal muscle capacity. In addition to neuromuscular benefits, improvements in EE have also been found with HIIT. Sedlock et al. (6) demonstrated that a bout of high-intensity (75% VO_{2max}), short-duration cycling produced a significantly higher total net EE than when performed during shorter and longer durations at a lower intensity (50% VO_{2max}). Gosselin and associates (4) found that despite subjects performing similar amounts of work, altering work-to-rest ratios can significantly alter caloric expenditure responses to interval exercise. In this study, four different HIIT protocols were evaluated, in comparison to one continuous aerobic bout, in their influence on energy expenditure. Each HIIT protocol had a different work-to-rest ratio, but all consisted of work periods totaling approximately ten minutes, and were performed at 90% VO_{2max}, while the continuous bout lasted 20 minutes and was performed at 70% VO_{2max}. HIIT protocols of 90 seconds work with 30 seconds active

rest, and 60 seconds work with 30 seconds active rest resulted in lower total caloric energy expenditure when compared to HIIT protocols of 30 seconds work with 30 seconds active rest, 60 seconds work with 60 seconds of active rest, and 20 minutes of continuous work. These studies suggest that HIIT protocols should include high-intensity work periods in combination with equally timed rest periods to maximize REE.

Two studies by Goto and colleagues (11,12) demonstrated that three repeated bouts of 10-minute moderate-intensity (60% VO_{2max}) endurance exercise in men contribute to greater fat oxidation compared with a single 30-minute bout of cycling. When compared to low-intensity (50% VO_{2max}), short- and long-duration bouts, a high-intensity (75% VO_{2max}) short-duration cycle bout produced a significantly higher total net caloric expenditure in male triathletes (6). Despite the differing intensities and work-to-rest ratios, all of these studies demonstrate that HIIT generally produces a greater EE, which may be utilized for exercise prescription.

High-Intensity Resistance Training (HIRT)

High-intensity resistance training (HIRT) is another exercise modality used in recent research to stimulate gains in REE and RER. When examining resistance training intensity in men, Paoli et al. (5) found shorter, HIRT sessions (80-85% 1-RM) to be more effective than traditional resistance training sessions (70-75% 1-RM) at increasing REE and decreasing RER, representing enhanced fat oxidation. Similarly, Thornton and Potteiger (14) found that at 85% of an 8-repetition maximum (RM), a HIRT bout produced a greater EPOC volume and magnitude than a bout of resistance exercise at 45% of an 8-RM. These two studies provide a framework for similar research to work

from, and present evidence that HIRT is more effective than more traditional resistance training protocols for enhancing REE.

Resistance exercise has also been compared to endurance exercise to evaluate REE differences. In examining different exercise modes, Jamurtas and colleagues (8) demonstrated that REE can be significantly elevated 24-48 hours after acute bouts of weight lifting (60 minutes at 70-75% of 1-RM) and running (60 minutes at 70-75% VO_{2max}). Furthermore, comparable intensity bouts of resistance and aerobic exercise, 70% VO_{2max} and 70% 1-repetition maximum (RM) respectively, have demonstrated similar increases in 24-hour EE in non-obese men (9). These two studies demonstrate aerobic and resistance training elicit similar REE responses.

Research involving resistance training order and type, as well as set number have also been conducted. Farinatti et al. (39) found that changing the order of resistance training exercises by participating in large to small muscle groups or small to large muscle groups, has no effect on the overall VO_2 or EE of an exercise bout of the upper body. Haddock and Wilkin (40) demonstrated that while keeping the same intensity, if the volume of resistance training is increased from one set to three sets, excess post-exercise energy expenditure, the increase in REE in the post-exercise period, does not significantly increase in previously trained women. In men, 1-hour EPOC magnitude was found to increase with shortened interval durations during a circuit weight training bout. However, from circuit weight training collectively, the exercise and recovery caloric costs were slightly increased for a protocol that incorporated increased rest intervals (41). When examining oxygen uptake in untrained women at 30-minutes following a bout of circuit weight training (3 sets of 15 repetitions at 65% 1-RM), REE values were

significantly higher than a bout of treadmill exercise for a similar duration and intensity (10). These three studies examine different aspects of HIRT, and provide some insight as to potential ways training can influence outcomes such as REE and EPOC.

Summary

Taking into account all of the exercise literature presented, hypotheses can be made regarding AEE, HIIT, and HIRT modes on REE. In general, interval exercise results in higher REE values than continuous exercise. When compared to low-intensity, high-intensity exercise has been shown to produce higher REE values. From these conclusions, it was hypothesized that a HIIT or HIRT exercise bout would have a greater net REE than an AEE bout. However, research comparing REE in aerobic and resistance exercise are still conflicted. Therefore, further research was needed to evaluate these two modes.

Exercise and Nutrition

In addition to exercise, nutrition has also been previously shown to influence REE and RER. By examining the effect of carbohydrate and protein on REE and RER, the current study investigated which macronutrient would maximize caloric expenditure and fat oxidation acutely, potentially leading to decreased fat mass, increased fat free mass, chronic disease prevention, and overall health.

The Influence of Nutrient Timing and Content on EE and RER

Numerous studies have evaluated the potential role of nutrition before, during, or after exercise, on REE and RER. Bullough and associates (17) suggested that energy intake, exercise, and their combined effects influence resting metabolic rate (RMR). Shimada et al. (20) reported that exercise performed in the post-absorptive state saves more carbohydrate and oxidizes more fat in the body, without affecting 24-hour EE; while Bahr and Sejersted (42) discovered a prolonged EPOC in fasted subjects when compared to fed subjects, which was hypothesized not to be caused by increased rates of glycogen resynthesis or an increase of the thermic effect of food. In contrast, in male runners, RER was increased by ingesting a CHO drink during a 2-hour treadmill run at 65% VO_{2max}, when compared to ingesting milk, which increased essential amino acid concentrations (43). Bielinski and colleagues (15) fed male subjects a mixed meal (55% CHO, 18% PRO, and 27% fat) after a 4-hour resting period and 30 minutes post-exercise (3-hour treadmill exercise at 50% VO_{2max}) on two separate occasions. It was found that lipid oxidation was greater after the exercise bout and post-exercise meal compared to after the rest bout and post-rest meal, suggesting intense exercise stimulates lipid oxidation and resting energy expenditure for a prolonged period. These studies provide conflicting evidence on how REE and RER are affected by nutrient timing.

In its position stand, the International Society of Sports Nutrition (ISSN) summarizes nutrient timing and composition. Kerksick et al. (34) states that when ingesting only PRO prior to exercise, muscle protein synthesis will be enhanced. If PRO and CHO are both ingested, muscle protein synthesis is enhanced further. In addition, if CHO and PRO are consumed regularly, greater gains in strength and positive body

composition changes have been demonstrated to occur in comparison to CHO consumption alone. The body composition changes can be a result of increased REE, as well as enhanced fat oxidation, which can lead to losses of fat mass, and potential gains in fat free mass. In developing its position, the ISSN evaluated numerous studies that examined nutrient timing and composition.

Several studies have manipulated subject diets in order to observe the influence on exercise outcomes. Hackney and colleagues (18) demonstrated that protein ingestion prior to a heavy resistance training bout can increase REE by elevating RMR up to 24 hours after the exercise session in men and women. In addition, Patterson and Potteiger (19) manipulated diets two days prior to exercise to determine the effects on substrate oxidation. Subjects consumed a 2-day low-CHO diet (20% CHO, 40% PRO, 40% fat) or an isocaloric 2-day moderate-CHO diet (55% CHO, 15% PRO, 30% fat), then performed a moderate-intensity treadmill exercise bout at 55% VO_{2max}. Indicating a positive influence of macronutrient intake during and after exercise on substrate oxidation, fat oxidation was greater with the low-CHO diet in active young women. These two studies provide evidence that a meal or snack with higher PRO content will increase fat oxidation in both men and women.

Summary

While many nutritional studies exist, a limited number evaluate REE and RER with exercise. However, based on the literature presented, one could hypothesize a lesser post-exercise RER when more PRO is ingested than CHO before exercise, however more specific research is needed, especially in women.

Influence of Sex on Metabolism

The current study only evaluates the effects of exercise and nutrition on metabolism in women, contributing to the existing body of fuel utilization literature predominately focused in men. However, in order to use women as participants, a general knowledge base needed to be formed to understand potential differences in men and women.

The vast amount of applied physiology research has been completed in men due to convenience and the ability to control for hormones. Differences between men and women in metabolic fuel utilization are influenced by variances in hormones. While Smith and McNaughton (21) demonstrated similar EE responses for men and women during a 30-minute cycling bout, the majority of evidence refutes this. For example, multiple studies have suggested men rely less on fat metabolism than women (22,44–48). Higher estrogen levels, such as those seen in women, result in increased fat oxidation, which results in a decreased carbohydrate usage and more glycogen spared during exercise (49). It is therefore reasonable to assume sex differences do exist in fuel utilization.

In examining the menstrual cycle, research is conflicted on its effect on REE. Some studies provide evidence that the menstrual cycle does augment REE with exercise, augmenting fat oxidation and blunting CHO oxidation during the luteal phase of the menstrual cycle (26–29,50–54). In contrast, several studies (23–25) have found the menstrual cycle to have no effect on EE. The divide in literature illustrates the need for further research in this area.

Summary

From these studies that evaluate sex and metabolism, it was concluded that women burn more calories from fat in comparison to men. With this knowledge, more specific exercise guidelines and prescriptions can be made for women to maximize this effect, leading to positive chronic changes. Research regarding REE differences throughout menstrual cycle phases is still conflicted, so further research is still needed.

Conclusion

A summary of various populations and study designs regarding exercise mode, nutrition, and sex metabolism has been presented. Most influential to the present study is exercise research by Gosselin et al. (4), Gore and Withers (13), and Paoli et al. (5), and nutrition research by Shimada et al. (20) and Hackney et al. (18). While these studies were important in forming hypotheses for this study, the majority of this research was completed in men, so the results may not be generalized to women without further investigation.

The discussed studies give insight into a variety of AEE, HIIT, and HIRT protocols. There is still room for future research in these areas; for example, the intensity and duration of each exercise mode at which caloric expenditure and health benefits are maximized, and how this translates to chronic benefits. Literature examining acute bouts of HIIT is also lacking, and should be further investigated. In addition, it is still unclear if all results can be generalized across sexes, due to hormonal differences between men and women. Therefore, while a variety of exercise and nutrition metabolism literature exists, research focusing specifically on women is lacking.

This review examined research completed on the effects of modes of exercise to be used in this study, AEE, HIIT, and HIRT, and pre-exercise nutrition on REE and RER, as well as sex differences in metabolism. To summarize, interval and high-intensity exercise result in higher REE values than continuous and low-intensity exercise, respectively. However, further research is still needed to determine if aerobic or resistance exercise produces greater REE values. In regards to the nutrition literature, a pre-exercise ingestion of PRO results in greater fat oxidation, when compared to CHO ingestion, but evidence is still conflicted on the effects of nutrient timing on REE and RER. Lastly, because of higher estrogen levels, women oxidize more fat during exercise than men, so there is a need for more consistent control among studies using women. The present study addressed all of these components, added to the limited studies in women, and provided ideas for future research.

CHAPTER III

METHODOLOGY

Experimental Design

Each subject made seven visits to the Applied Physiology Laboratory. The first visit consisted of a body composition assessment for descriptive measures, a heart rate measurement to calculate heart rate reserve, and a maximal strength assessment to calculate weight loads for exercise sessions. The next six visits (Figure 1) involved a counter-balanced, double-blinded design consisting of three exercise modalities: AEE, HIIT, and HIRT, and two nutritional interventions: CHO and PRO. Participants performed each exercise mode twice, each time with a different nutritional intervention. Salivary samples were collected before each exercise session to determine estrogen and cortisol concentrations, and after each exercise session to determine cortisol concentrations. Exercise sessions were performed at the same time of day, with 48-72 hours between each session, and the order was randomly assigned. Subjects were instructed to stay well hydrated, and to be three hours postprandial having refrained from caffeine for five hours prior and strenuous exercise for 24 hours prior to all exercise sessions.

Participants

Twenty-one eumennorheic women, ages 18-35 years, were enrolled to participate in this study; one participant withdrew due to injuries unrelated to this study. Twenty women participated (Table 1), which allowed for an adequate sample size. PS: Power and Sample Size Calculation Version 3.0 (Vanderbilt University, Nashville, TN, USA) was used to calculate sample size using data from Kuo et al. (38), Jamurtas et al. (8), and Paoli et al. (5). All women were recreationally active, as defined by participating in 1-5 hours per week of recreational and/or structured aerobic and/or resistance exercise, excluding competitive athletes. The protocol was approved by the University's Institutional Review Board. All subjects provided written informed consent and completed a health history questionnaire prior to study participation.

Preliminary Testing

Heart Rate Reserve Measurement

Participants reported to the Applied Physiology Laboratory after an eight-hour fast. Participants rested for fifteen minutes and resting heart rate (HR_{rest}) was measured using a Polar heart rate monitor (Polar FT1, Polar USA, Port Washington, NY, USA). Maximal heart rate (HR_{max}) was determined using the age-predicted equation [HR_{max} = 220-age]. Heart rate reserve is the differences between HR_{max} and HR_{rest}. Exercise HR was calculated using the Karvonen equation [THR = ((HR_{max} – HR_{rest}) % intensity) + HR_{max}] (55).

Body Composition Assessment

For baseline data, whole body composition was measured on a Hologic Dual Energy X-ray Absorptiometer (DEXA, Hologic Discovery W, Bedford, MA, USA) using the device's default software (Apex Software Version 3.3, Hologic Discovery W, Bedford, MA, USA). The device uses rectilinear fan beam acquisition to give a three-compartment assessment of body composition, including fat mass (FM), lean mass (LM), percent body fat (% fat), and bone mineral content (BMC). After removing all metal objects from their person, subjects laid supine in the middle of the platform with hands facedown near their sides. Subjects were instructed to remain still and breathe normally for the duration of the scan. All scans were performed by the same, DEXA certified individual. The device was calibrated according to the manufacturer recommendations before testing to ensure valid results.

Maximal Strength Test

After ingesting a standardized snack, each participant performed a 1-repetition maximum (RM) strength test for two exercises, bench press and leg press, using free weights and a spotter. After a 5-minute warm-up and light stretching, participants were familiarized with the equipment and the motion of the movements. Each participant then performed 8-10 repetitions of 50% of their predicted 1-RM. After a 1-minute rest period, each participant performed 4-6 repetitions of 80% of their predicted 1-RM. Following a 1-minute rest period, the weight was increased to an estimated 1-RM load, and participants only lifted the weight one time. After each successful set of one repetition,

the weight was increased until a failed attempt occurs. Two to three minutes of rest was given between each 1-RM attempt.

After the two 1RM tests, participants also performed a multiple-RM strength test for four exercises: alternating stationary lunge, overhead shoulder press, biceps curls, and overhead triceps extension. With free weights and a spotter, participants performed each exercise with a weight that they could lift for one set of no less than three and no more than ten repetitions. The weight for each exercise was set by the lab assistants based on each participant's past strength training experience. If the participant was unable to perform the exercise for three repetitions or was able to perform more than ten repetitions, the weight was decreased or increased, respectively, and the exercise was completed again. A two to three minute rest was given between each exercise.

The multiple-RM obtained from the strength tests was used to estimate 1RM for the lunge, shoulder press, biceps curl, and triceps extension using the following equation (56):

$$1RM = \frac{RepWt}{0.522 + 0.419^{(-0.055 \times RTF)}}$$

Once 1RM was determined for all exercises, 80-85% was calculated for the 6-8RM used for the HIRT sessions.

Energy Intake Assessment

All participants completed a three-day food record prior to exercise testing to assess their regular nutrient intake. Participants were educated on food amounts and were encouraged to eat similar diets the day prior to each exercise session to facilitate similar macronutrient profiles. Nutrition intake was analyzed using a nutrition and fitness

software program (The Food Processor, Version 10.12.0, Esha Research, Salem, OR, USA).

Experimental Protocol

Saliva collection and analysis

In order to account for possible fuel utilization differences between the exercise sessions, estrogen was measured. Estrogen concentrations were determined by a 2.5-5.0 mL saliva sample prior to each exercise bout, using an ELISA Assay for salivary estrogen (Salivary 17β-Estradiol Enzyme Immunoassay Kit**,** Salimetrics, LLC, State College, PA, USA). To ensure valid saliva collection results, participants were asked to avoid drinking alcohol for 12 hours and avoid eating a major meal for 3 hours prior to giving saliva samples. Participants were asked to rinse their mouth with water 10 minutes prior to saliva collection, to remove food residue. To avoid blood in saliva collections, participants were asked to avoid brushing teeth for 45 minutes and obtaining dental work for 48 hours prior to giving saliva samples. All samples were maintained at 4°C no longer than necessary before freezing them at -20°C.

Cortisol was measured to account for the stress response from each modality of exercise. Cortisol concentrations were determined by a 2.5-5.0 mL saliva sample prior to and following each exercise bout, using an ELISA Assay for salivary cortisol (Salivary Cortisol Enzyme Immunoassay Kit**,** Salimetrics, LLC, State College, PA, USA); the aforementioned protocol for collection and storage was used. Once all samples were collected, smaller sub-samples were pooled between the two supplements to produce one

pre- and one post-exercise sample to determine the effect of each modality on cortisol, independent of treatment.

Nutritional Intervention

Immediately prior (34) to the beginning of each exercise session, participants orally ingested 25 grams of CHO (maltodextrin) or PRO (whey isolate) powder (Elite Whey Protein Isolate, Dymatize Nutrition, Farmers Branch, TX, USA) mixed with six ounces of water. Order of ingestion was randomized.

Aerobic Endurance Exercise (AEE)

An AEE bout consisted of a self-selected 5-minute warm-up and a 30-minute treadmill (Q65 Series 90, Quinton Instrument Co., Seattle, WA) jog at 40-55% HRR (1,13). HR was measured using a Polar heart rate monitor.

High-intensity Interval Running (HIIT)

A HIIT bout consisted of a self-selected 5-minute warm-up, ten rounds of a 60 second treadmill run at 85-95% HRR followed by a 60 second passive rest period. The entire exercise bout lasted approximately 20 minutes. HR was measured at the end of each interval using a Polar heart rate monitor.

High-intensity Resistance Training (HIRT)

Data obtained from the strength assessment was used to determine an appropriate weight load for the HIRT session. Exercises were performed in the following order: leg

press and bench press (York Barbell Co., York, PA, USA), lunges, shoulder press, biceps curl, and triceps extension using free weights. Subjects performed a self-selected light warm-up prior to starting each session. A HIRT bout consisted of three sets of each exercise. Each set included 6-8RM followed by a 20-30 second rest. There was a rest period of 2:30 between each exercise (5). HR was measured after each set using a Polar heart rate monitor.

Metabolic Measurements

Resting energy expenditure (REE) was analyzed using oxygen uptake (VO_2), carbon dioxide production (VCO_2), and respiratory exchange ratio (RER) measurements with a metabolic cart (TrueOne 2400, ParvoMedics, Inc., Sandy, UT). The gas analysis was performed via a mouthpiece and hose immediately prior to each exercise session, for 15 minutes to obtain resting measures (base). Immediately after the conclusion of the exercise sessions, participants were seated and reconnected to the metabolic cart for 15 minutes to obtain immediately post (IP) exercise measures. The participants were then disconnected from the cart, and remained quietly seated. Measurements were taken again during minutes 25-35 (30min) and 50-60 (60min); participants were reconnected to the cart three minutes prior to each measure for equilibration.

Statistical Analyses

Data were expressed as mean and standard deviation. A one-way ANOVA was performed to determine baseline differences in salivary estradiol and cortisol levels. A 2 × 3 × 4 mixed-factorial model ANCOVA [treatment (CHO vs. PRO) × modality (AEE

vs. HIIT vs. HIRT) × time (base vs. IP vs. 30min vs. 60min] using baseline salivary estradiol levels as covariates, was performed to reveal any significant interactions in REE (kcal/day) and RER measurements. When necessary, Bonferroni post-hoc tests were run to decompose the model. An ANCOVA was also performed on the change in cortisol, covaried for baseline differences. SPSS Version 20 (IBM; Chicago, IL, USA) was used to perform the statistical analyses. An alpha level of $p \leq 0.05$ was set *a priori*.

CHAPTER IV

MANUSCRIPT

Introduction

More than 60% of women in the United States are overweight, and 1/3 of those women are obese (57). In addition, 75% of normal weight women believe they are overweight and 90% overestimate their body size (58). With such high obesity and body dissatisfaction rates, it is important for women to receive reliable health and weight loss recommendations. In addition, lack of time has shifted the focus on more practical time-efficient strategies for exercise.

For health and weight maintenance, aerobic exercise is usually prescribed (55). However, more recently, higher-intensity exercise modalities have been suggested as more time efficient strategies for improvements in health and energy expenditure (13,21,36). Gore and Withers (13) previously reported an increase in resting energy expenditure (REE) following a 20-minute treadmill run, while Gosselin et al. (4) also demonstrated increases in total caloric energy expenditure in half the time with high-intensity interval training (HIIT). In addition, high-intensity resistance training (HIRT) with short rest intervals has been shown to enhance REE above that seen with more commonly prescribed resistance training (5) of lesser intensity and longer rest periods. While these three common exercise modes seem to be successful at burning calories,

previous research has indicated that high-intensity exercise stimulates greater increases in REE in comparison to lower intensity exercise in half the time (6,59). Research is still conflicted on whether aerobic or resistance exercise is the most effective for augmenting energy expenditure. Higher intensity exercise has also been linked to higher rates of fat oxidation, measured by respiratory exchange ratio (RER) (11,12,36,37).

The combined effect of varying exercise modalities and pre-workout nutrition on fuel utilization and energy expenditure in women is limited. Pre-exercise ingestion of protein (PRO) has been found to increase post-exercise REE more than that of pre-exercise ingestion of carbohydrate (CHO) (18,19). While women rely heavily on fat for fuel during exercise, previous data has demonstrated the importance of pre-exercise feedings on augmenting lipolysis (60). In addition, Henderson et al. (61) reported that there is a difference in fat oxidation between men and women only in the fasted state, not in the postprandial state. Therefore, nutrient timing and content may be especially important when considering fuel utilization in women.

When evaluating exercise and nutritional responses in women, it is important to consider hormonal variances, such as the sex-specific hormone estradiol that varies throughout the menstrual cycle. Estradiol has been shown to have an impact on REE (22,26–29,50–54). Controlling for estradiol concentrations in metabolic evaluations is crucial for determining the practical effects an intervention may have (22). High intensity aerobic exercise has been shown to stimulate increases in cortisol (62,63), which is a stress hormone that regulates fuel utilization by mobilizing amino acids from skeletal muscle and promoting gluconeogenic activity (31). Elevated cortisol concentrations have

been reported as a primary hormonal factor augmenting exercise adaptations from high intensity exercise (63).

To date, no previous investigations have evaluated the combined effects of varying exercise modalities and acute nutritional supplementation in women. Therefore, the purpose of the current study was to examine the effect of common exercise modalities, aerobic endurance exercise (AEE), HIIT, and HIRT, combined with pre-exercise CHO or PRO ingestion on REE and RER in women. In order to do so, the study evaluated a practical combination of one exercise bout and one time CHO/PRO feeding, which mimics a normal exercise routine.

Methods

Experimental Design

To test the study hypotheses, a counter-balanced, crossover, double-blind design was used. After preliminary resting heart rate, body composition, and strength testing, each participant completed six exercise sessions. The six sessions consisted of three exercise modalities: aerobic endurance exercise (AEE), high-intensity interval running (HIIT), and high-intensity resistance training (HIRT), and two acute nutritional interventions: carbohydrate (CHO) and protein (PRO) (Figure 1). Participants performed each exercise mode twice, each time with a different nutritional intervention. Salivary samples were collected before each exercise session to determine estrogen and cortisol concentrations, and after each exercise session to determine cortisol concentrations. Exercise sessions were performed at the same time of day, with at least 48 hours between

each session, and the order was randomly assigned. Subjects were instructed to stay well hydrated, and to be three hours postprandial having refrained from caffeine for five hours prior and strenuous exercise for 24 hours prior to all exercise sessions.

Participants

Twenty-one eumennorheic, college-aged, recreationally active women were enrolled to participate in this study; one participants withdrew due to injuries unrelated to this study. Twenty women (n=20) completed the study (Table 1) and were used in the statistical analyses. The study protocol was approved by the University's Institutional Review Board. Prior to participation, all participants provided written informed consent and completed a health history questionnaire. To be included in this study, participants had to be a woman between the ages of 18 and 35 and be recreationally active, defined as accumulating 1-5 hours per week of recreation and/or structured aerobic and/or resistance exercise, excluding competitive athletes. Participants were excluded if they were unfit, pregnant, had any health risks, injuries, or had any heart, lung, kidney, or liver disease.

Heart Rate Reserve Measurement

Participants reported to the Applied Physiology Laboratory after an eight-hour fast, where they rested for fifteen minutes. Resting heart rate (HR_{rest}) was measured using a Polar heart rate monitor (Polar FT1, Polar USA, Port Washington, NY, USA), while age-predicted maximal heart rate (HR_{max}) was determined. Heart rate reserve is the differences between HR_{max} and HR_{rest}. Exercise HR was calculated using the Karvonen equation [$THR = ((HR_{max} - HR_{rest})\ \%\ intensity) + HR_{max}$] (55).

Body Composition Assessment

For baseline data, whole body composition was measured using a Hologic Dual Energy X-ray Absorptiometer (DEXA, Hologic Discovery W, Bedford, MA, USA) using the device's default software (Apex Software Version 3.3). The device uses rectilinear fan beam acquisition to give a three-compartment assessment of body composition, including fat mass (FM), lean mass (LM), percent body fat (% fat), and bone mineral content (BMC). After removing all metal objects from their person, subjects laid supine in the middle of the platform with hands facedown near their sides. Subjects were instructed to remain still and breathe normally for the duration of the scan. All scans were performed by the same DEXA certified individual. The device was calibrated according to the manufacturer recommendations before testing to ensure valid results.

Maximal Strength Test

In a standard post-absorptive state, each participant performed a 1-repetition maximum (RM) strength test for bench press and leg press, using free weights and a spotter, according to previous guidelines. After a 5-minute warm-up and light stretching, participants were familiarized with the equipment and the motion of the movements. Each participant then performed 8-10 repetitions of 50% of their predicted 1RM. After a 1-minute rest period, each participant performed 4-6 repetitions of 80% of their predicted 1RM. Following a 1-minute rest period, the weight was then increased to an estimated 1RM load, and participants lifted the weight one time. After each successful set of one repetition, the weight was increased until a failed attempt occurred, within four attempts. Two to three minutes of rest was given between each 1-RM attempt.

After the two 1RM tests, participants also performed a multiple-RM strength test for four exercises: alternating stationary lunge, overhead shoulder press, biceps curls, and overhead triceps extension. With free weights and a spotter, participants performed each exercise with a weight that they could lift for one set of no less than three, and no more than ten repetitions. The weight for each exercise, set by the lab assistants, was based on each participant's past strength training experience. If the participant was unable to perform the exercise for three repetitions or was able to perform more than ten repetitions, the weight was decreased or increased, respectively, and the exercise was completed again. A two to three minute rest was given between each exercise.

The multiple-RM obtained from the strength tests was used to estimate 1RM for the lunge, shoulder press, biceps curl, and triceps extension using the following equation (56):

$$1RM = \frac{RepWt}{0.522 + 0.419^{(-0.055 \times RTF)}}$$

Once the participant's 1RM was determined for all exercises, 80-85% was calculated for the 6-8RM used for the HIRT sessions.

Energy Intake Assessment

All participants completed a three-day food record prior to exercise testing to assess their regular nutrient intake. Participants were educated on food amounts and were asked to eat similar diets the day prior to each exercise session to facilitate similar macronutrient profiles. Nutrition intake was analyzed using a nutrition and fitness software program (The Food Processor, Version 10.12.0, Esha Research, Salem, OR, USA). On average, subjects ingested 2078.72 ± 679.92 kilocalories, 253.73 ± 97.61

grams of carbohydrate (~48.82 %CHO), 84.31 ± 29.95 grams of protein (~16.22 %PRO), and 80.92 ± 36.69 grams of fat (~35.04 %fat) per day.

Saliva collection and analysis

In order to account for possible fuel utilization differences between the exercise sessions, estrogen was measured. Estrogen concentrations were determined by a 2.5-5.0 mL saliva sample prior to each exercise bout, using an ELISA Assay for salivary estrogen (Salivary 17β-Estradiol Enzyme Immunoassay Kit**,** Salimetrics, LLC, State College, PA, USA). To ensure valid saliva collection results, participants were asked to avoid drinking alcohol for 12 hours and avoid eating a major meal for 3 hours prior to giving saliva samples. Participants were asked to rinse their mouth with water 10 minutes prior to saliva collection, to remove food residue. To avoid blood in saliva collections, participants were asked to avoid brushing teeth for 45 minutes and obtaining dental work for 48 hours prior to giving saliva samples. All samples were maintained at 4°C no longer than necessary before freezing them at -20°C. Intra-assay precision coefficient of variation (CV) for estrogen was 8.66-18.64%; inter-assay precision CV was 3.86%.

Cortisol was measured to account for the stress response from each modality of exercise. Cortisol concentrations were determined by a 2.5-5.0 mL saliva sample prior to and following each exercise bout, using an ELISA Assay for salivary cortisol (Salivary Cortisol Enzyme Immunoassay Kit**,** Salimetrics, LLC, State College, PA, USA); the aforementioned protocol for collection and storage was used. Once all samples were collected, smaller sub-samples were pooled between the two supplements to produce one pre- and one post-exercise sample to determine the effect of each modality on cortisol,

independent of treatment. Intra-assay precision CV for cortisol was 12.00-17.11%; inter-assay precision CV was 4.85%.

Nutritional Intervention

After providing a saliva sample and immediately prior to beginning each exercise session, in a double-blind fashion, participants orally ingested 25 grams of CHO (maltodextrin) or PRO (whey isolate; Elite Whey Protein Isolate, Dymatize Nutrition, Farmers Branch, TX, USA) mixed with six ounces of water. Order of ingestion was randomized.

Aerobic Endurance Exercise (AEE)

An AEE bout consisted of a self-selected 5-minute warm-up, followed by a 30-minute treadmill (Q65 Series 90, Quinton Instrument Co., Seattle, WA) jog at 45-55% HRR (13). HR was measured using a Polar heart rate monitor, and was averaged over the 30-minute exercise period.

High-intensity Interval Running (HIIT)

A HIIT bout consisted of a self-selected 5-minute warm-up, followed by ten rounds of a 60 second treadmill run at 85-95% HRR with a 60 second passive rest period. The entire exercise bout lasted approximately 20 minutes. HR was measured at the end of each interval using a Polar heart rate monitor, and an average of the ten 60 second exercise intervals was used for statistical analyses.

High-intensity Resistance Training (HIRT)

Data obtained from the strength assessment were used to determine an appropriate weight load for the HIRT session. Exercises were performed in the following order: leg press and bench press (York Barbell Co., York, PA, USA), lunges, shoulder press, biceps curl, and triceps extension using free weights. Subjects performed a self-selected light warm-up prior to starting each session. A HIRT bout consisted of three sets of each exercise. Each set included 6-8RM followed by a 20-30 second rest. There was a rest period of 2:30 between each exercise (5). HR was measured at the end of each set using a Polar heart rate monitor; the average of each of the six exercises was used in the statistical analyses.

Metabolic Measurements

Resting energy expenditure (REE) and respiratory exchange ratio (RER) were analyzed using a metabolic cart and internal software (TrueOne 2400, ParvoMedics, Inc., Sandy, UT). Indirect assessments of oxygen uptake (VO_2) and carbon dioxide production (VCO_2) were measured and used in the following equations to calculate REE (32) and RER (64), respectively:

$$REE\ (kcal/min) = [(3.9 * (VO_2\ (L * min^{-1}))) + (1.1 * (VCO_2\ (L * min^{-1})))] * 1440\ min$$

$$RER\ = \frac{VCO_2\ (L * min^{-1})}{VO_2\ (L * min^{-1})}$$

The gas analysis was performed via a mouthpiece and hose immediately prior to each exercise session, for 15 minutes, to obtain resting measures (base). Immediately after the conclusion of the exercise sessions, participants were seated and reconnected to the metabolic cart for 15 minutes to obtain immediately post (IP) exercise measures. The

participants were then disconnected from the cart, and remained quietly seated. Measurements were taken again during minutes 25-35 (30min) and 50-60 (60min).

Statistical Analyses

Data were expressed as mean and standard deviation. A one-way ANOVA was performed to determine baseline differences in salivary estradiol and cortisol levels. An analysis of covariance (ANCOVA) mixed effect model [treatment (CHO vs. PRO) × modality (AEE vs. HIIT vs. HIRT) × time (base vs. IP vs. 30min vs. 60min)] using baseline salivary estradiol levels as covariates, was performed to reveal any significant interactions in REE (kcal/day) and RER measurements. When necessary, Bonferroni post-hoc tests were run to decompose the model. An ANCOVA was also performed on the change in cortisol, covaried for baseline differences. An ANOVA was performed on HR and RPE differences in modalities. SPSS Version 20 (IBM; Chicago, IL, USA) was used to perform the statistical analyses. An alpha level of $p \leq 0.05$ was set *a priori*.

Results

Estradiol

There was no significant difference (p=0.636; ES=0.035) between baseline estradiol concentrations for each exercise session. While this p-value was not significant, the concentrations obtained were physiologically different between and within subjects. Concentrations spanned from 0.00 to 5.04 pg/mL between subjects, with one subject reporting a range of 0-4.70 pg/mL between testing days. Due to the physiological role of

estrogen on fuel utilization in women (23,26–29), baseline estradiol levels were used as covariates in the REE and RER analyses.

REE (kcal/day)

There was no three-way interaction (p=0.6336) for REE. Significant two-way interactions were found between modality and time (p<0.0001) and time and treatment (p=0.0078). The interaction between modality and treatment was not significant (p=0.0603); (Table 2).

With the decomposition of the modality and time interaction, significant modality differences were found between HIIT and AEE (p<0.0001), and HIIT and HIRT (p<0.0001) over time, but not between AEE and HIRT (p=0.1331). REE was significantly higher as a result of HIIT than AEE IP exercise (p<0.0001), at 30min post (p=0.0022), and at 60min post (p=0.0017) (Figure 2A). REE was significantly higher for HIIT when compared to HIRT IP exercise (p<0.0001). REE was not significantly different at 30min (p=0.3345) or 60min (p=0.1428) post HIIT vs. HIRT.

Decomposition of the time and treatment interaction indicated REE was significantly higher for PRO than for CHO IP exercise (p=0.0074), 30min post (p=0.0098), and 60min post exercise (p=0.0017) (Figure 2B).

RER

An RER over 1.0 is invalid and therefore cannot indicate fuel utilization. Out of 120 IP measurements, fifteen presented an RER between 1.0-1.21a.u. These measurements were taken into account when discussing fuel utilization, assuming all values were equivalent to 1.0.

There was no three-way interaction (p=0.1610) for RER. Significant two-way interactions were found between modality and time (p<0.0001) and time and treatment (p<0.0001). The interaction between modality and treatment was not significant (p=0.6496); (Table 3).

With the decomposition of the modality and time interaction, significant modality differences were found between HIIT and AEE (p<0.0001), HIIT and HIRT (p<0.0001), and AEE and HIRT (p=0.0024). As a result of HIIT, RER was significantly higher than AEE and HIRT IP exercise (p<0.0001) (Figure 4A). RER was significantly lower at 30min (p<0.0001) and 60min post exercise (p=0.0020) for HIIT compared to AEE. RER was significantly lower at 30min post exercise (p=0.0169), but not significantly different from at 60min post (p=0.3603) HIIT vs. HIRT. When comparing HIRT and AEE, there were no significant differences IP exercise (p=0.3370); RER was significantly lower for HIRT at 30min (p=0.0004) and 60min post exercise (p=0.0265).

Evaluation of the time and treatment interaction indicated RER was not significantly different between CHO and PRO immediately post exercise (p=0.1150). However, RER was significantly lower for PRO compared to CHO at 30min post (p=0.0012) and 60min post (p<0.0001) (Figure 4B).

Cortisol

The ANOVA revealed no significant difference (p=0.159; ES=0.092) in baseline cortisol concentrations for each exercise modality. The ANCOVA change scores demonstrated no significant effect of exercise modality on cortisol values (p=0.168; ES=0.015) (Table 4).

HR and RPE

There was a modality effect on average HR (p=0.000) and RPE (p=0.000). AEE produced a significantly lower HR (Δ=-58bpm; p=0.000) and RPE (Δ=-6; p=0.000) compared to HIIT. There was no significant difference in HR (Δ=-3bpm; p=0.834) between AEE and HIRT. RPE however, was significantly lower for AEE compared to HIRT (Δ=-5; p=0.000). HIIT resulted in significantly higher HR (Δ=54bpm; p=0.000) and RPE (Δ=1; p=0.001) compared to HIRT.

Discussion

The purpose of the current study was to determine the effect of acute exercise and nutrition manipulations on REE and RER in women. Previous research evaluating the effect of AEE, HIIT, and HIRT individually on REE and fuel utilization have failed to provide direct comparisons between exercise mode; as well as failing to evaluate similar effects in women. Several studies have examined the difference between CHO and PRO intake on REE and RER with exercise (18,19,43). The current study is the first to compare common exercise modalities, with acute nutritional intake, on REE and RER in women. Results of this study indicate that HIIT produces a significantly higher REE than AEE up to 60min post exercise, and a significantly higher REE than HIRT IP exercise, while AEE and HIRT produced similar REE responses. HIIT and HIRT both also stimulated greater fat utilization via RER, compared to AEE. Previous data has shown that HIIT (6) and HIRT (59) stimulate greater increases in REE, compared to AEE. Ingesting PRO prior to exercise produced an increased caloric expenditure, compared to

CHO consumption. PRO ingestion also resulted in significantly augmented fat utilization 30min and 60min post exercise. Previous research supports these findings reporting higher post-exercise REE with pre-exercise PRO ingestion compared to CHO ingestion (18), and a lower RER with exercise performed in a post-absorptive state in men (20).

Estradiol

It is important to consider hormonal variances when examining the effect of exercise and nutrition on women (22). Estrogen has been shown to have a large impact on energy expenditure (26–29,50–54), as well as fuel utilization (49) In the present study, there were no statistically significant differences in baseline estradiol levels ($p=0.636$). However, due to the physiological implications estradiol has on the primary variables in this study, estradiol levels were accounted for in all analyses to control for potential effects on REE and RER.

REE

As hypothesized, a single bout of HIIT produced a significantly higher REE than AEE through 60min post-exercise ($\Delta=189.2$ kcals/day). Similar to the current study, Sedlock et al. (6) also reported a significantly higher post-exercise total net caloric expenditure with high intensity-short duration cycling (75% VO_{2max}) compared to shorter and longer durations at a low intensity (50% VO_{2max}) in men, indicating that exercise intensity affects the magnitude and duration of excess post-exercise oxygen consumption (EPOC). Gibala and McGee contribute HIIT's metabolic inefficiency to rapid changes in skeletal muscle oxidative capacity due to the high level of muscle fiber recruitment, particularly in type II fibers (3). In contrast to the current study, Gosselin et al. (4) found

bouts of 60 seconds of work with 60 seconds rest, at 90% $VO_{2max,}$ to have the same total net caloric EE as 20 minute bouts at 70% VO_{2max}. A potential explanation for this non-significant finding is that the difference in exercise intensities for the two sessions was not substantial enough to elicit significant changes, such as those found in the current study. Additionally, it is unclear if Gosselin et al. (4) controlled for caloric differences between exercise intensities. Subjects in the present study completed 30 min, instead of 20 min, of AEE in order to closely match caloric expenditure during HIIT (determined during pilot testing, unpublished data).

In contrast to our hypothesis, when compared to HIIT, REE from HIRT was significantly lower IP exercise (Δ=-169.3kcals/day), with no differences extending to 30min and 60min post-exercise. Additionally, there was no modality difference between AEE and HIRT (Δ=19.8kcals/day). Research that has examined REE differences between AEE or HIIT and HIRT is limited. However, similar to the current study, Jamurtas et al. (8) found that single bouts of resistance training and running performed at the same intensity both resulted in elevated REE up to 10 hours post-exercise. The current study's results are contrary to that of Braun et al. (10), which found that total body circuit weight training (3 sets of 15 repetitions at 65% 1-RM) elicited a higher post-exercise REE in women than a treadmill run matched for aerobic energy cost. The current study also matched EE demands of AEE and HIRT during pilot testing, with HIRT being slightly lower, yet at a higher intensity than that of Braun et al. Also in contrast to the results of the present study, two studies have reported a higher REE with HIRT up to 22 hours post-exercise in men (5), and a higher EPOC with HIRT up to two hours post-exercise in women (14), when compared to a traditional resistance training bout, which has

previously been reported to have a higher REE than an aerobic endurance bout (7). REE data from the current study eludes to exercise intensity, instead of duration, being the primary determinant of EPOC variance, similar to the work of Gore and Withers (13), Smith and McNaughton (21), and Warren et al. (36). Collectively, it seems as if HIIT is the most effective and time-efficient exercise modality for increasing energy expenditure in women.

When combining acute PRO feedings with exercise, there was a significantly higher REE for PRO than for CHO up to 60 min post exercise (Δ=59.3kcals/day). This is similar to findings by Hackney et al. (18) and Patterson and Potteiger (19) that demonstrated greater post-exercise REE and fat oxidation following a pre-exercise meal or snack with higher PRO content in men and women. Several studies (65–67) contribute the increase in REE with the PRO treatment to PRO's thermic effect of food (TEF), which is higher and more prolonged than that of CHO and fat. In women, higher PRO diets have been found to elicit an increased effect on diet-induced energy expenditure, in comparison to a lower PRO diet (66–68), which may be explained by amino acid absorption and disposal costs (68). Consuming an additional 100 kcals of PRO prior to exercise, as done in the current study, may maximize energy expenditure up to an hour after exercise.

RER

Exercise intensity has previously been shown to be a driving factor for fuel utilization during and after exercise. Immediately after HIIT exercise, RER was significantly higher than AEE, indicating a higher utilization of CHO during and immediately after. Within 25 minutes afterward, RER was significantly lower than AEE

(Δ=0.079a.u.; $p<0.0001$), demonstrating higher fat utilization, which was maintained up to 60 min post. Similar to the current study, Goto et al. (11,12) reported a significantly lower RER post-exercise with repeated bouts of moderate intensity cycling compared to a single bout in men. Chad and Quigley (37) found a lower RER with a lower intensity cycle bout (50% VO_{2max}) than a higher intensity cycle bout (70% VO_{2max}) through three hours post-exercise, indicating increased fat utilization. Warren et al. (36) presented mixed results regarding exercise duration, intensity, and modality in men and women. Post-exercise RER was lower in long duration (90 minutes) vs. short duration cycling (30 minutes) when matched for intensity, and also lower with high-intensity (85% VO_{2max}) cycling in comparison to low-intensity cycling (50% VO_{2max}). Lastly, continuous vs. interval cycling (matched for EE and intensity) resulted in no difference in RER (36). Previous data demonstrates differing exercise intensity can augment post-exercise REE and fat oxidation, but intervals do not produce differences compared to continuous exercise if total EE, duration, and intensity between the two remain the same throughout the exercise bout. In contrast to the current study, Kuo et al. (38) demonstrated that exercise bouts at 45% VO_{2max} and 65% VO_{2max} resulted in similar RER values when EE of exercise was matched. Although constant diets were kept, the similar RER values could potentially be caused by residual effects of pre-exercise meals, since a fasting period of only 2 hours was implemented prior to exercise.

During the post-exercise period, oxygen consumption is elevated for a period of time as a result of homeostasis disruption caused by exercise. Physiological changes, such as those in cellular ion concentrations, tissue temperatures, and metabolite and hormone levels, take place into recovery, disabling oxygen consumption to lower back to

resting levels (69). This process is referred to as excess post-oxygen consumption (EPOC), and is related to exercise duration and intensity. EPOC can also be influence by sex-based hormones (estrogen) and macronutrient availability, among other things (69). It has been shown that CHO is the primary fuel used during moderate to high-intensity exercise, but during the post-exercise period, the body shifts from CHO to lipid energy sources, resulting in the lowering of RER (38). Some studies have not indicated this shift (37,38), perhaps as a result of exercise intensities being too low. However, the current study demonstrates the fuel shift within 25 minutes post-exercise measurement, indicating a greater fat utilization post-exercise with HIIT. When comparing HIIT and HIRT in the current study, RER was still significantly higher IP, and significantly lower at 30 minutes post exercise. However, RER values were similar (Δ=0.012a.u.; p=0.3603) between HIIT and HIRT 60 minutes after exercise. HIRT and AEE were not different IP exercise, but RER was lower for HIRT at 30min (Δ=0.048a.u.; p=0.0004) and 60min post exercise (Δ=0.029a.u.; p=0.0265). Little research has compared RER between aerobic and resistance training, but the investigations that have been done present conflicting results. In contrast to the present study, Braun et al. (10) demonstrated a higher post-exercise RER with circuit weight training in women (3 sets of 15 repetitions at 65% 1-RM), compared to a treadmill bout that was matched for EE and duration. Also in contrast, Melanson et al. (9) found no post-exercise differences in RER between a 60-minute circuit weight training bout (4 sets of 10 repetitions at 70% 1-RM), and a cycling bout (70% VO_{2max}) in men. Similar to the present study, Haddock and Wilkin (40) reported a lower RER with higher volume resistance training bouts (1 vs. 3 sets of 9 repetitions at 8-RM) in women. In addition, Paoli et al. (5) demonstrated a lower RER 22

hours after a HIRT bout compared to a more traditional resistance training bout (4 sets of 8-12 repetitions with 1-2 minutes rest). In relation to sex, Ortego et al. (70) reported no differences in post-circuit weight training RER between men and women, when menstrual cycle was not controlled for. It is important to examine menstrual cycle due to the effects estrogen may have on fuel utilization. The mid-luteal phase of the menstrual cycle is characterized by high estrogen levels, which have been reported by multiple studies to enhance lipid utilization and reduced CHO oxidation with exercise in women (49,51,53).

In the present study no differences were found in RER between CHO and PRO IP exercise, but in line with our hypothesis, RER was significantly lower for PRO compared to CHO at 30min post (Δ=0.036a.u.; p=0.0012) and 60min post exercise (Δ=0.052a.u.; p<0.0001). Similarly, Patterson and Potteiger (19) reported a higher fat oxidation with a low-CHO diet compared to a moderate-CHO diet at 1-hour post-exercise. Substrate oxidation can be influenced by substrate availability as a result of dietary intake and physical activity. While this study only evaluated acute feedings, low-CHO diets have been shown to decrease circulating insulin levels, which promotes fatty acid utilization in skeletal muscle (19), perhaps supporting the lower RER for PRO ingestion in the current study.

Dietary Intake

Chronic dietary manipulations are known to have an impact on substrate utilization. The purpose of the current study was to include a practical one-time feeding of common pre-workout macronutrients, in order to evaluate the effects on REE and RER. Based on three-day diet logs the women consumed an average of 48.8% CHO,

16.8% PRO, and 34.2% fat daily. Although intakes were within the Acceptable Macronutrient Distribution Ranges (AMDR) for Americans, average baseline PRO intake was 1.3 $g \cdot kg^{-1} day^{-1}$, which is 0.5 $g \cdot kg^{-1} day^{-1}$higher than the Recommended Dietary Allowance (RDA) (71). Interestingly, while average PRO intake was relatively high, an additional acute feeding of 25 grams of PRO significantly augmented REE in the current study. This may be a result of recent data suggesting higher protein needs (1.63 $g \cdot kg^{-1} day^{-1}$) may be necessary for women to maintain nitrogen balance (72). While the importance of chronic dietary manipulations is apparent, the current study supports the benefit of acute PRO feedings on energy expenditure, in healthy women eating within dietary guidelines. Additionally, Henderson et al. (61) demonstrated a positive influence of acute pre-exercise feeding on fat oxidation, in comparison to a fasted state, which blunted fat oxidation. Furthermore, pre-exercise feedings had a larger impact on women, versus men, demonstrating the importance of sex-based evaluations. While not examined against a fasted state in this study, a pre-exercise supplement of PRO resulted in a greater fat oxidation, indicating that feeding PRO prior to exercise, rather than feeding CHO or fasting, can enhance fat oxidation in women. Future long-term studies identifying whether these acute effects extend to weight loss and body composition improvements in women, would be valuable.

Cortisol

Released by the adrenal cortex, cortisol is a regulator of many physiological systems, including skeletal muscle and adipose tissue (30,31). During exercise, cortisol is an important regulator of fuel utilization mobilizing amino acids from skeletal muscle and promoting gluconeogenic activity (31). Previous research has demonstrated

significant increases in cortisol with exercise as a result of increased intensity (30). Sprint and endurance exercise have significantly augmented cortisol, whereas resistance exercise has had less of an effect (62,63), and duration has not been found to have an effect (63). Cortisol levels in the current study were not significantly altered as a result of any modality. However, HIIT resulted in the largest increase (Δ=0.15 μg/dL), compared to HIRT (Δ=0.05 μg/dL) and AEE (Δ=0.00 μg/dL). The participants in the current study were chronically active, and potentially better adapted to the stress caused by exercise, therefore not eliciting a significant increase in cortisol (30).

HR and RPE

While HR and perceived exertion were not primarily variables, previous research has shown that they are valid indicators of perceived exertion (55). Furthermore, HR represents the physiological load of an exercise regime. Previous literature has purported some of the benefits from HIIT occur as a result of the large cardiovascular stress that it requires (73). In line with previous data, the current study demonstrated that HIIT elicited the largest HR response (184 ± 29 bpm), while AEE (126 ± 7 bpm) and HIRT (129 ± 17 bpm) were not different from each other. Intensity of AEE and HIIT was quantified by heart rate reserve (HRR), so it was expected that HIIT would have a higher HR than AEE. However, in contrast to the current study, previous research has found high intensity resistance training to elicit a higher HR response than an endurance bout (10). A potential reason similar responses resulted for AEE and HIRT may be due to the averaged HR across all exercises from HIRT. The smaller muscle group exercises produced lower HR (112 ± 18 bpm with triceps extension) while multi-joint exercises produced a high HR (160 ± 15 bpm with lunges). The Borg RPE scale was created to

subjectively rate feelings during exercise, and is typically correlated with exercise HR (55). In the current study, HIIT resulted in the highest RPE and the lowest with AEE. Chronically training at a high intensity, which results in higher HR and RPE, may enhance the aforementioned benefits of HIIT.

CHAPTER V

CONCLUSION

The results of this study indicate that HIIT elicited the largest increase in post-exercise energy expenditure, as well as augmented RER, compared to AEE and HIRT. Immediately post exercise there was a heightened caloric effect from the HIIT bout stimulating ~800 kcal/day more than HIRT and AEE. Beginning around 30min post exercise, HIIT and HIRT both had a higher fat utilization than AEE; at 60min post, HIIT and HIRT resulted in similar fuel utilization in comparison to each other. In combination with varying exercise modalities, PRO intake elevated REE and fat oxidation to a greater extent than CHO. PRO ingestion prior to exercise may help further maximize the caloric effect, with an additional ~90 kcals/day expended compared to CHO. Around 30min post exercise, PRO increased fat utilization compared to CHO. A limitation in the current study was the use of indirect calorimetry was used to measure REE and RER, rather than whole body direct calorimetry, which is considered the gold standard. Also, excluding the acute PRO and CHO supplementation, no major dietary modifications were made, which could have a potential effect on the acute fuel utilization measurement. However, because no modifications were made, the study results may be more practical for a woman who only wants to change her pre-workout nutrition rather than her whole diet. In addition, since this was an acute intervention, and post-exercise measurements were only conducted for 60 minutes, it is difficult to predict longer term energy expenditure and fuel utilization. Future research should focus on determining the effects of chronic

exercise and nutrition modifications in women using whole body calorimetry. Collectively, these findings suggest a potential benefit of integrating HIIT and pre-exercise PRO intake into exercise routines. Specifically in women, this strategy may have positive implications on their health, weight, and body composition.

TABLES

Table 1. Descriptive statistics for all subjects (n=20) at baseline.

	Mean ± SD
Age (years)	24.6 ± 3.9
Height (cm)	164.4 ± 6.6
Weight (kg)	62.7 ± 6.6
Fat mass (kg)	17.6 ± 4.0
Lean mass (kg)	42.5 ± 4.4
Percent body fat (%)	28.2 ± 4.8

Table 2. REE (kcals/day) for each modality and time. Treatment values were collapsed due to a non-significant interaction (p=0.0603). (Mean ± SD)

Modality	Treatment	Base	IP	30min	60min
AEE	CHO	1746 ± 214	2026 ± 240	1649 ± 173	1573 ± 190
	PRO	1704 ± 247	2149 ± 230	1806 ± 193	1763 ± 175
	Average	1725 ± 231	2088 ± 235	1728 ± 183	1668 ± 183
HIIT	CHO	1681 ± 312	2805 ± 469	1840 ± 279	1750 ± 281
	PRO	1546 ± 301	2828 ± 599	1750 ± 265	1728 ± 267
	Average	1614 ± 307	2817 ± 534*♯	1795 ± 272*	1739 ± 274*
HIRT	CHO	1638 ± 281	2073 ± 340	1699 ± 311	1623 ± 294
	PRO	1688 ± 305	2189 ± 292	1879 ± 198	1786 ± 188
	Average	1663 ± 293	2131 ± 316	1789 ± 255	1705 ± 241

* Indicates significant difference between AEE and HIIT (p<0.0001-p=0.0022)
♯ Indicates significant difference between HIIT and HIRT (p<0.0001)

Table 3. RER (a.u.) for each modality, treatment, and time. (Mean ± SD) Treatment values were collapsed due to a non-significant interaction (p=0.6496).

Modality	Treatment	Base	IP	30min	60min
AEE	CHO	0.82 ± 0.05	0.88 ± 0.06	0.83 ± 0.05	0.83 ± 0.05
	PRO	0.86 ± 0.08	0.87 ± 0.06	0.80 ± 0.04	0.80 ± 0.04
	Average	0.84 ± 0.07	0.88 ± 0.06	0.82 ± 0.05	0.82 ± 0.05
HIIT	CHO	0.83 ± 0.06	0.96 ± 0.07	0.74 ± 0.05	0.80 ± 0.05
	PRO	0.84 ± 0.04	0.97 ± 0.10	0.73 ± 0.05	0.75 ± 0.03
	Collapsed	0.84 ± 0.05	0.97 ± 0.09*♯	0.74 ± 0.05*♯	0.78 ± 0.04*
HIRT	CHO	0.85 ± 0.06	0.88 ± 0.05	0.78 ± 0.05	0.81 ± 0.04
	PRO	0.84 ± 0.05	0.86 ± 0.06	0.76 ± 0.07	0.77 ± 0.05
	Collapsed	0.85 ± 0.06	0.87 ± 0.06	0.77 ± 0.06§	0.79 ± 0.05§

* Indicates significant difference between AEE and HIIT (p<0.0001-p=0.0020)
♯ Indicates significant difference between HIIT and HIRT (p<0.0001-p=0.0169)
§ Indicates significant difference between AEE and HIRT (p=0.0004-0.0265)

Table 4: Change in cortisol (μg/dL) as a result of modality (Mean ± SD).

Modality	Base	Post	Δ
AEE	0.39 ± 0.31	0.39 ± 0.33	0.00 ± 0.22
HIIT	0.44 ± 0.40	0.59 ± 0.50	0.15 ± 0.23
HIRT	0.35 ± 0.18	0.40 ± 0.20	0.05 ± 0.14

Table 5: Average HR and RPE during the duration of each modality (Mean ± SD).

	HR (bpm)	RPE
AEE	126 ± 7*	10 ± 1*§
HIIT	184 ± 29#	16 ± 1#
HIRT	129 ± 17	15 ± 1

* Indicates significant difference between AEE and HIIT ($p<0.0001$)
♯ Indicates significant difference between HIIT and HIRT ($p<0.0001$-$p=0.001$)
§ Indicates significant difference between AEE and HIRT ($p<0.0001$)

FIGURES

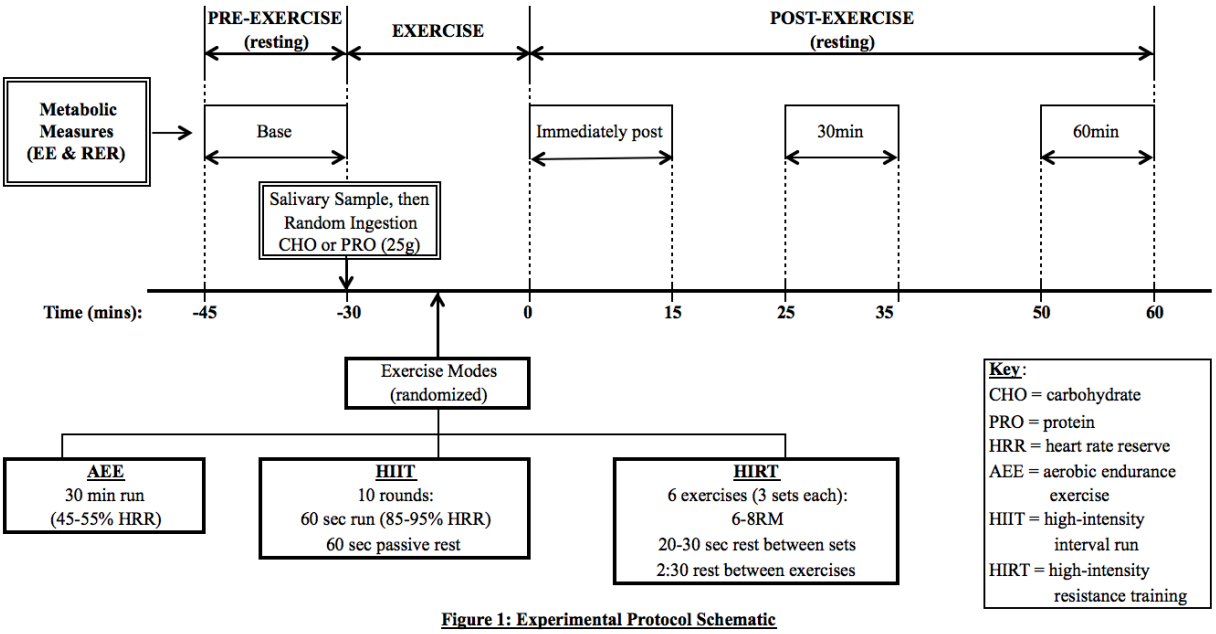
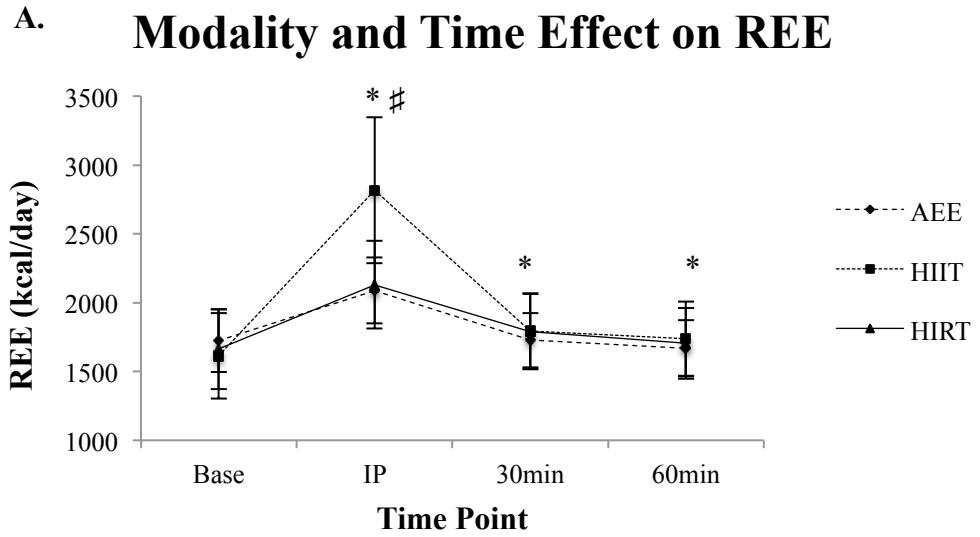


Figure 1: Experimental Protocol Schematic

Figure 1: Experimental Protocol Schematic



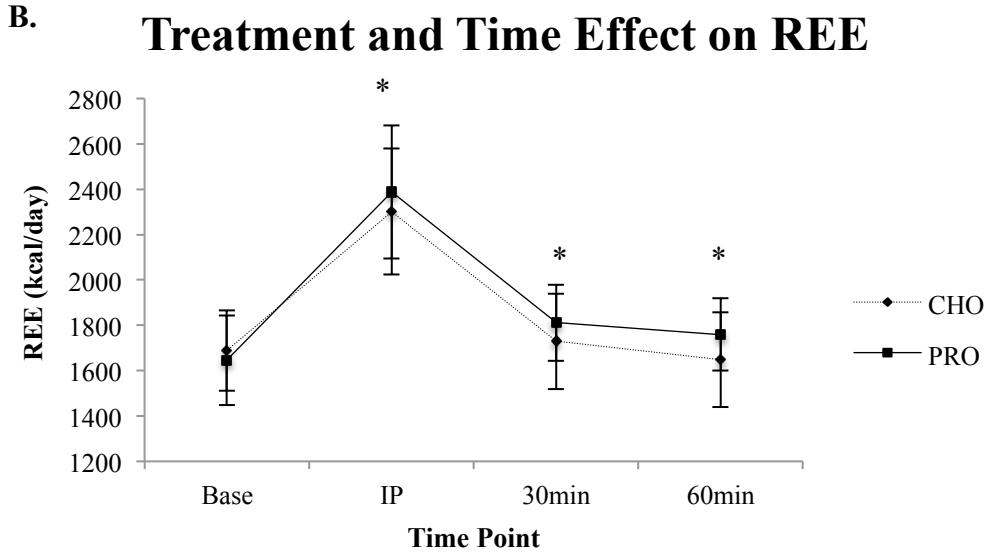


Figure 2: Energy expenditure (REE; kcals/day) as a result of A) modality and time and B) treatment and time. Values expressed as mean ± SD. A) * Indicates significant difference between AEE and HIIT ($p<0.0001$-$p=0.0022$); ♯ Indicates significant difference between HIIT and HIRT ($p<0.0001$). B) * Indicates significant difference between CHO and PRO ($p=0.0017$-$p=0.0098$).

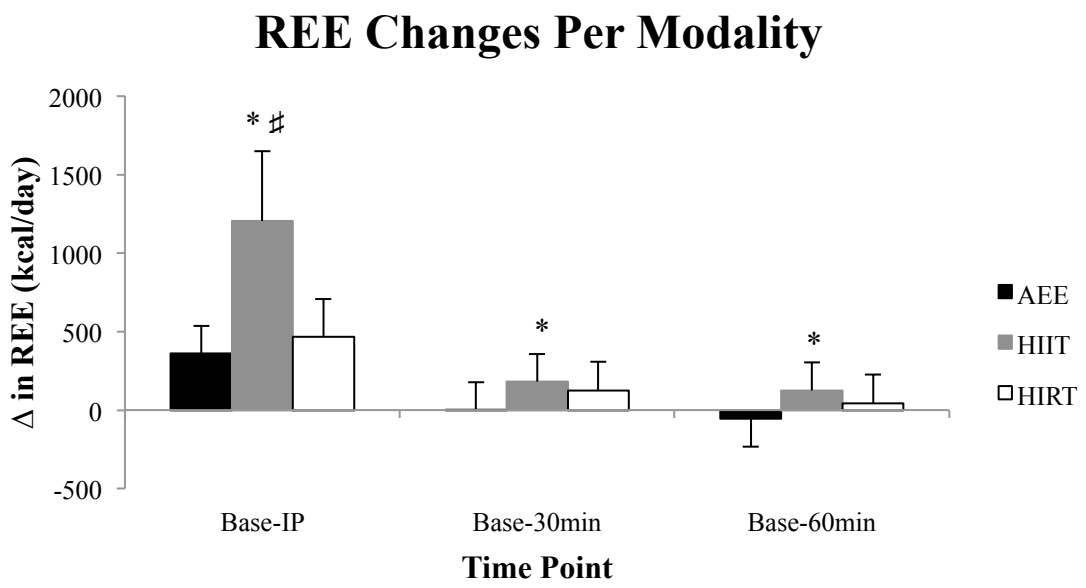


Figure 3: Change in REE (kcal/day) for each modality. Values expressed as mean ± SD.
* Indicates significant difference between AEE and HIIT ($p<0.0001$-$p=0.0022$); ♯ Indicates significant difference between HIIT and HIRT ($p<0.0001$).

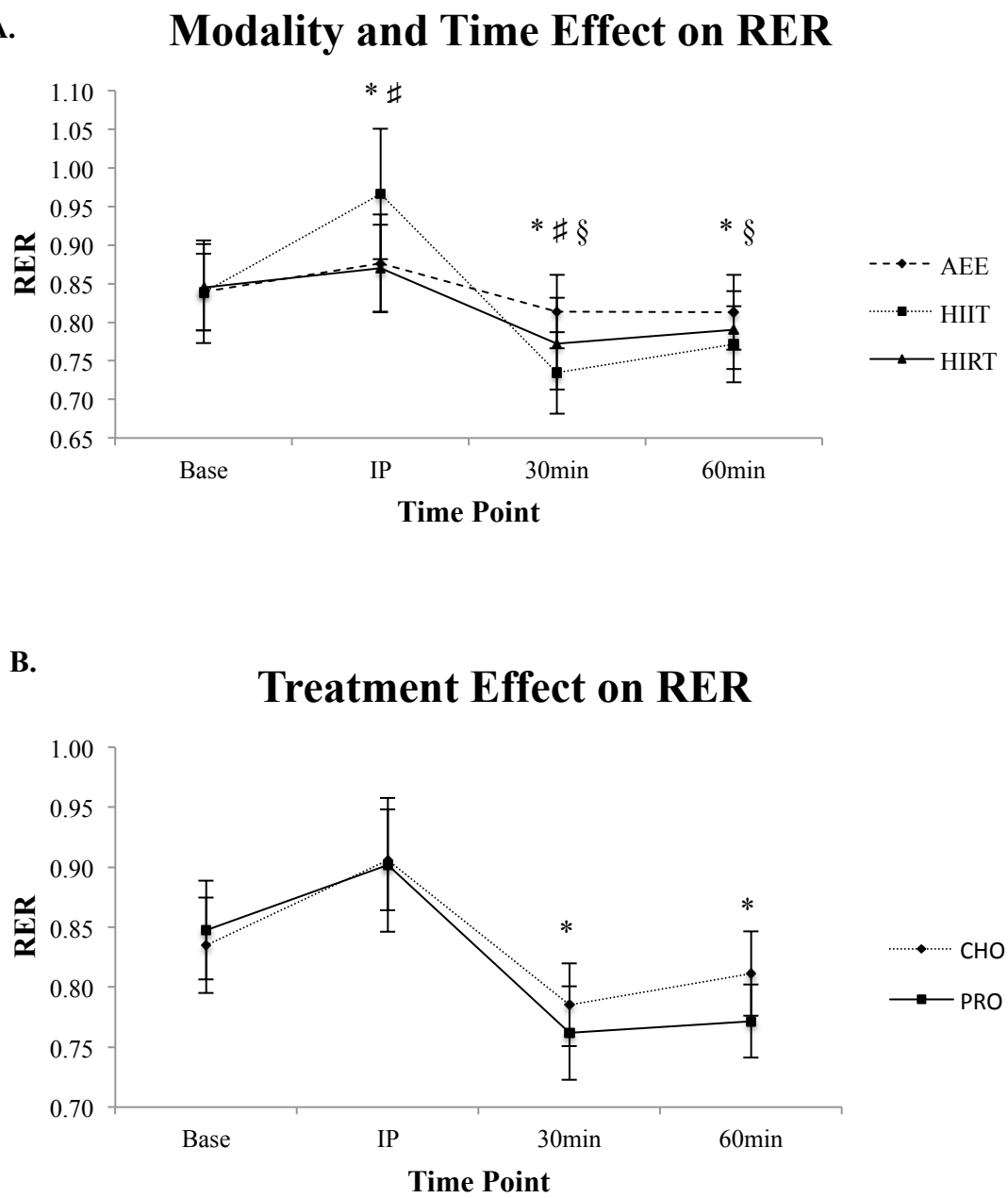


Figure 4: Respiratory Exchange Ratio (a.u.) as a result of A) modality and time and B) treatment and time. Values expressed as mean ± SD. A) * Indicates significant difference between AEE and HIIT ($p<0.0001$-$p=0.0020$); ♯ Indicates significant difference between HIIT and HIRT ($p<0.0001$-$p=0.0169$); § Indicates significant difference between AEE and HIRT ($p=0.0004$-0.0265). B) * Indicates significant difference between CHO and PRO ($p<0.0001$-$p=0.0012$).

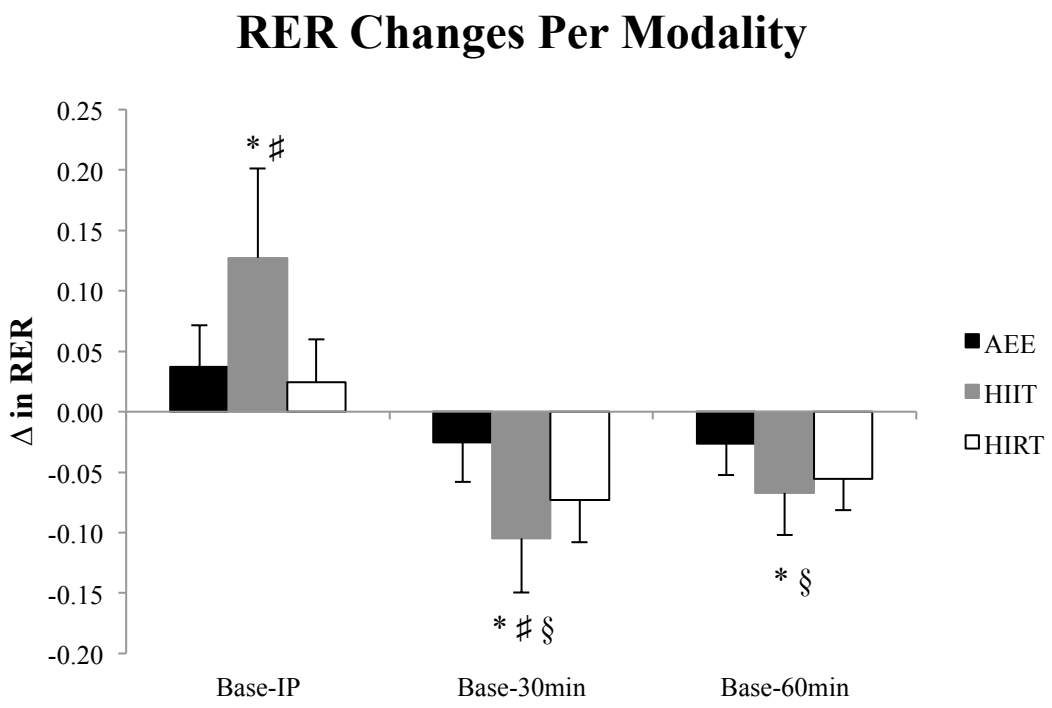


Figure 5: Change in respiratory exchange ratio (a.u.) for each modality. Values are expressed as mean ± SD. * Indicates significant difference between AEE and HIIT ($p<0.0001$-$p=0.0020$); ♯ Indicates significant difference between HIIT and HIRT ($p<0.0001$-$p=0.0169$); § Indicates significant difference between AEE and HIRT ($p=0.0004$-0.0265)

APPENDIX A: SAMPLE SIZE CALCULATIONS

The sample size is necessary to see significant differences between exercise and nutrition interventions, according to the equation listed below, using a standard power level of .80. Mean and standard deviation data were pulled from three articles (5,8,38) that closely resembled the current study protocols and outcome variables. The proposed subject sample size of 25, as listed previously, was used to allow for a dropout of 8 individuals, and allowing for the most powerful statistical approach (ANOVA) to be used.

$$N = \frac{2SD^2\ (Z_\alpha + Z_\beta)^2}{\Delta^2}$$

Z_α = level set by researcher to protect against type I errors; Z_α (.05) = 1.96

Z_β = percent of area on experimental curve between $\bar{X}_2$ (mean of the experimental group) and Z_β

Δ = difference between two mean values being compared

SD = standard deviations of two groups which determine the spread of the curves

N = sample size of group

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