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**THE EFFECT OF FASTED VS FED HIGH-INTENSITY INTERVAL EXERCISE ON  
METABOLISM AND DIET**

By

William Joseph Perez  
B.S. May 2013, Old Dominion University

A Thesis Submitted to the Faculty of  
Old Dominion University in Partial Fulfillment of the  
Requirements for the Degree of

MASTER OF SCIENCE IN EDUCATION

PHYSICAL EDUCATION – EXERCISE SCIENCE AND WELLNESS

OLD DOMINION UNIVERSITY  
August 2016

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## Abstract

### THE EFFECT OF FASTED VS FED HIGH-INTENSITY INTERVAL EXERCISE ON METABOLISM AND DIET

William Joseph Perez  
Old Dominion University, 2016  
Chair: Dr. David P. Swain

The purpose of this study was to investigate immediate and delayed metabolic and dietary responses to a single bout of cycling high-intensity interval exercise (HIIE) performed in the fasted and fed state. Baseline values of 11 subjects (6 female, 5 male) for resting energy expenditure (REE), respiratory exchange ratio (RER), resting  $\text{VO}_2$ ,  $\text{VO}_{2\text{max}}$  and appetite score (VAS) were measured on the first visit. Energy balance (EB) was determined using diet tracking and activity energy expenditure (paEE). Trials followed a day at net energy balance and began in a fasted state. A 240-kcal energy bar was consumed prior to (FED) or after (FST) a high-intensity interval exercise (HIIE) bout. Post-exercise  $\text{VO}_2$  was recorded for one hour (60 of 70 minutes) immediately following HIIE. Metabolic variables were measured before and 12 hours after exercise. Energy intake was not different between conditions the day before trials (mean  $\pm$  SD: 2060  $\pm$  613 kcal FED, 2154  $\pm$  666 kcal FST) or the 12 hours after exercise (1695  $\pm$  484 kcal FED, 1892  $\pm$  822 kcal FST). Post-exercise VAS was greater than 12 hours later ( $p < 0.01$ ). Post-exercise EE (97.0  $\pm$  15.2 kcal $\cdot$ hr $^{-1}$  FED, 89.9  $\pm$  17.2 kcal $\cdot$ hr $^{-1}$  FST) was elevated from pre-exercise (70.8  $\pm$  10.7 kcal $\cdot$ hr $^{-1}$  FED;  $p < 0.01$ , 67.9  $\pm$  10.2 kcal $\cdot$ hr $^{-1}$  FST;  $p = 0.01$ ). Post-exercise  $\text{VO}_2$  (272.2  $\pm$  25.8 mL $\cdot$ hr $^{-1}\cdot$ kg $^{-1}$  FED, 254.2  $\pm$  33.5 mL $\cdot$ hr $^{-1}\cdot$ kg $^{-1}$  FST) was elevated from pre-exercise (203.4  $\pm$  25.4 mL $\cdot$ hr $^{-1}\cdot$ kg $^{-1}$  FED;  $p < 0.01$ , 195.6  $\pm$  12.5 mL $\cdot$ hr $^{-1}\cdot$ kg $^{-1}$  FST;  $p < 0.01$ ). Excess EE (26.2  $\pm$  10.6 kcal FED, 22.0  $\pm$  11.8 kcal FST) and EPOC (68.8  $\pm$  28.6 mL $\cdot$ kg $^{-1}$  FED, 58.6  $\pm$  32.5 mL $\cdot$ kg $^{-1}$  FST) over 60 minutes did not differ between conditions. Appetite was blunted in the hours following HIIE supporting

the transient effect of exercise on appetite. Excess EE and EPOC did occur in the hour following exercise. However, whether a 240-kcal meal replacement bar was consumed immediately before or shortly after morning HIIE did not significantly impact resulting EPOC, RER, EI or appetite.

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## CHAPTER I

### INTRODUCTION

#### **Problem**

Fasting is defined as a prolonged period of time during which energy is no longer being derived from the last meal and no new energy is absorbed. This can also be thought of as an extension of the post-absorptive state following a meal where all consumed nutrients have been either utilized, stored or processed for excretion. Understanding the fundamental physiology behind fasting is necessary to comprehensively examine this concept as a dietary strategy. Medical applications have supplied much of the utility of intentional fasting but the use of fasting as a health-based diet strategy is evolving.

A number of research studies have examined the relationship between exercise and metabolism. However, the nutritional state of the exercise (i.e., fasted state exercise) is normally not an independent variable but is sometimes decidedly used as a control variable such as in studies that include various durations of fasting before exercise. Studies that employ measurement of metabolic factors sometimes prescribe a short fasting period before trials to avoid convoluted results but other times this factor is completely neglected. Fasted exercise is infrequently compared directly to fed exercise regarding metabolic response. Few studies have directly compared fasted exercise to fed exercise at all. Considering the rising popularity of acute fasting for health benefits, it is worth investigating whether concurrent exercise will reveal an interaction effect on health outcomes.

#### **Purpose**

Fasting can have an effect on health via its established impact on metabolic pathways. This impact may extend to weight management and disease prevention, two critical factors of

the modern perspective on health. Questions arise when pondering how fasting elicits its metabolic effects and to what degree. How acute fasting affects metabolism has not been fully elucidated and when combined with exercise the pool of knowledge becomes further limited. Reciprocally, the effects of fed exercise on metabolism are obscured despite the common practice of exercise-focused clinicians to recommend consuming a meal before exercise. The present study will seek to determine how fasted exercise following maintenance of a balanced energy state affects the metabolic environment compared to the traditionally recommended method of performing exercise in a fed state.

### *Metabolic Measures*

Multiple avenues exist to investigate the health benefits of diet and exercise programs. Excess post-exercise oxygen consumption (EPOC) refers to elevated oxygen uptake following a bout of exercise. This is a straightforward measure to assess the body's metabolic response to acute exercise. Multiple immediate factors contribute to EPOC, including replenishment of phosphocreatine and ATP stores, oxygen bound to hemoglobin and myoglobin, and elevated ventilation and heart rate. Lactate metabolism is correlated with EPOC (Aguilar et al., 2014) because it fuels these fast component processes. The slow component is not as well understood but includes contributions from longer-lasting effects like elevated body temperature and stress hormones. Oxygen consumption and carbon dioxide production are the key components in calculating resting metabolic rate (RMR) and the respiratory exchange ratio (RER) through RMR testing. Both of these metabolic factors, along with the derived energy expenditure and substrate utilization values, will serve as the primary outcome measures of this investigation on the acute metabolic effect of fasted

exercise. Resting energy expenditure (REE) is a product of RMR testing and is expressed as kilocalories (kcal). The terms for the metabolic test output, REE and RMR, can be used interchangeably but the test is typically referred to as an RMR test. To avoid confusion for the purposes of the proposed study, the general testing process will be referred to as metabolic testing (MT). This will be subdivided into either an "RMR test" when the traditional resting protocol is used or a "recovery  $\text{VO}_2$  test" (RVT) when the measurement occurs immediately after exercise. Energy expenditure output will be referred to as REE, which will be classified as "recovery REE" where appropriate. Thus REE, not RMR, will express the dependent variable of energy expenditure. Multiple factors can affect REE. For example, a sleepless night reduces post-absorptive and postprandial REE by 5% and 20%, respectively (Benedict et al., 2011). In all but the most physically active individuals, REE makes up the largest portion of total daily energy expenditure (TEE). Besides REE, other contributors to TEE are the thermic effect of feeding, which is usually estimated, and energy expenditure from physical activity (paEE), which can be either estimated using a multiplier of REE or estimated with accelerometry. RER is represented as a ratio of carbon dioxide production over oxygen consumption. An RER of 0.7 indicates the sole presence of fat metabolism while a ratio of 1.0 or higher reveals purely carbohydrate metabolism. Alterations in oxygen consumption following exercise will consequently modify both REE and RER. Exercise has various complex effects on EPOC and differing nutritional states surrounding an exercise bout serve to complicate the analysis. This study will be designed with the intention of explicating how this enigmatic factor contributes to EPOC, particularly the fast component of EPOC immediately after exercise.

### *Nervous System*

Metabolic response to exercise is regulated in part by the autonomic nervous system and feeding behavior. The sympathetic division of the autonomic nervous system is closely associated with catecholamine release, especially epinephrine, which has a definite effect on resting metabolism. Epinephrine at the higher end of the physiologically normal range of concentration has been shown to directly increase both REE and RER (Ratheiser et al., 1998). Well-trained individuals display a blunted REE and lipid oxidation rate by blocking beta-adrenergic stimulation whereas sedentary individuals' REE and lipid oxidation are not affected by blocking beta-adrenergic stimulation (Tremblay et al., 1992). Tremblay et al. (1992) reported that beta-adrenergic blockage reduced heart rate and blood pressure equally in trained and untrained subjects. This suggests a dissociation between cardiovascular and metabolic effects of autonomic influence. Catecholamine and autonomic responses to both fasting and exercise are well-established. All relevant findings will be considered in this context.

### *Appetite and Energy Intake*

Appetite and the resultant energy intake (EI) both affect and are affected by metabolism. The impact of appetite on metabolic response to exercise is not as well-established as the role for the autonomic nervous system. Processing food intake requires energy and this thermic effect of food (TEF) contributes approximately 10% of total daily energy expenditure (TEE). However, TEE is distinct from REE and an inflated TEF could artificially elevate REE and RER measurement. Beyond this, meal composition could further influence the measurement. Macronutrient composition of a diet affects substrate utilization and consequently RER

values. For example, a high-carbohydrate meal ingested just before an RMR test would predictably elevate RER above normal resting levels. Energy balance refers to the magnitude of the difference between energy expended and energy consumed. A negative value encourages catabolism while a positive value implies net anabolism. By subtracting TEE from EI a calculated energy balance (CEB) value is attained. Appetite dictates the previously discussed feeding behaviors along with other factors. Understanding appetite, and its relationship to energy intake, is critical to successful metabolic assessment. If diet is controlled, scrutiny placed on subjective appetite could reveal nutrition-specific behavioral responses to exercise without compromising metabolic investigations. Potential behavioral contributions to metabolism can be quantified using appetite perception surveys. These will be given in the form of a visual analogue scale (VAS). VAS data will be collected to ostensibly better explain observed changes in REE and RER as well as resultant energy intake.

### *HIIE*

Exercise at intensities higher than what would be considered moderate have been increasingly examined in the literature. This has expanded to include near-maximal and supramaximal aerobic intensities. While not conclusive, there is some evidence of a differential effect of near-maximal or supramaximal intensity exercise on metabolism compared to moderate or vigorous aerobic intensity. One common method to prescribe high-intensity exercise is to employ intervals. High-intensity interval exercise (HIIE) protocols are performed near or above maximum aerobic intensity of exercise at the expense of duration and are divided into sections, or intervals, separated by lower-intensity active recovery

periods. Otherwise, the prescribed intensity would be unsustainable for the total duration desired. The intensity criterion for high-intensity exercise is not rigid but is unofficially agreed upon by various study designs, depending on the population and other factors. HIIE commonly incorporates anaerobic work intervals at intensities that could not be performed continuously due to larger energy requirements. This method necessitates reaching a supramaximal aerobic intensity. The inclusion of anaerobic intervals to HIIE prescription does not, however, preclude the use of aerobic intervals. The American College of Sports Medicine (ACSM; Garber et al., 2011) differentiates vigorous aerobic intensity from moderate intensity across a threshold of 60% heart rate reserve (HRR) or  $VO_2$  reserve ( $VO_{2R}$ ). These reserve methods account for resting values for heart rate or oxygen consumption, respectively. Operational definitions of intensity vary amongst authors but many aerobic HIIE prescriptions exist near the aerobic maximum of a given prescription method, usually expressed as a percent of  $VO_{2R}$  or  $VO_{2max}$ . According to ACSM (Garber et al., 2011), exercise performed at 90% to 100%  $VO_{2R}$  is near-maximal intensity. However, a broad range of intensities have been examined. Both aerobic and anaerobic intervals can grant greater average intensity for an exercise bout or increased volume of work at high intensity. HIIE has shown multiple health benefits, possibly surpassing those of moderate-intensity continuous exercise even in diseased populations (Gibala et al., 2012; Little et al., 2011). A review highlighted multiple HIIE studies showing that catecholamine release is greater after HIIE than after moderate-intensity continuous exercise (Boutcher, 2011). Since catecholamines are so closely related to acute metabolic changes, this mode of exercise may have a more profound impact on metabolic response. Thus HIIE is a logical choice for



exercise mode in this kind of investigation. The prescribed intensity will be determined to ensure the expected physiological response to high-intensity exercise.

### *Basis of Interest*

The study will expand on the results of a previous pilot study where REE and RER were assessed in the hours after fasted or fed HIIE (Perez & Rynders, 2014). This previous study drew inspiration from a few related findings and the discovery that the topic was relatively unexplored. Paoli et al. (2011) found that fasted exercise for 36 minutes at 65% of maximum heart rate did not differentially impact 12 and 24-hour EPOC versus fed exercise and that RER, while initially lower in the fasted state, was lower after fed exercise. However, this study had some notable differences in design, namely the test meal being high in fat content (53% of 673 kcal as fat). Irrespective of the test meal, the question asked by the researchers invited further analysis of the concept under an altered structure. A similar study was imparted by Deighton et al. (2012) with 60 minutes of exercise at 70%  $VO_{2max}$  yielding equivalent manifestations of an energy deficit between fasted and fed exercise conditions. Additionally, Deighton et al. did not observe a compensatory feeding response to this effect. Sevits et al. (2013) conducted two studies investigating metabolic response to HIIE. In one of these studies, Sevits et al. observed an increase in TEE in response to sprint interval exercise. TEE was measured with a whole-room calorimeter. This observation occurred despite the result in the concurrent study that showed no changes in REE measured 23 hours after the same exercise protocol, suggesting that elevated energy expenditure values observed in the first study occurred early in the recovery period. In fact, the only significant difference between REE in exercise and control conditions in the first study when time blocks were

isolated was a four-hour window including the exercise protocol in the first hour. In this study, the REE measurement was performed with a ventilated hood. These findings highlighted the need to more meticulously examine various time points along the EPOC spectrum. Paoli et al. (2011), Deighton et al. (2012) and one of the Sevits et al. (2013) experiments measured EPOC multiple hours after exercise, therefore emphasizing the slow component of EPOC, and did not have strong results. Sevits et al.'s second experiment did show elevated energy expenditure in a time range more proximal to the exercise bout. The pilot study focused on the delayed response by measuring REE 12 and 24 hours after exercise. The conclusion drawn from the pilot data was that fasted exercise increased 12-hour REE whereas fed exercise did not. Since REE was only measured 12 and 24 hours after exercise, this outcome applied only to the slow component of EPOC and deterred speculation on what transpired during the fast component of EPOC.

The role that nutritional state plays in EPOC has been studied but is far from being fully comprehended. This is especially true for the fast component of EPOC. Furthermore, how diet is affected by the nutritional state of exercise is only vaguely understood. Ultimately, this study will seek to answer the question of whether fasted exercise differentially affects acute metabolism following exercise compared to fed exercise when diet is tightly controlled. The degree of the contribution of nutritional behavior factors to these potential effects will also be interpreted.

## **Hypotheses**

The hypotheses are as follows:

H1: Fasted exercise will result in larger immediate post-exercise (0-70 minutes) and 12-hour REE and EPOC values than fed exercise.

H2: Perceived appetite will be lower both one and 12 hours after fed exercise than fasted exercise.

H3: Energy intake will be greater after fasted exercise than fed exercise.

H4: RER immediately after and 12 hours following exercise will be the same in fed and fasted states and not differ from baseline.

### **Delimitations and Variables**

Dependent variables will be: REE, RER and  $VO_2$ . Self-reported energy intake and appetite perception are also dependent variables. They will be used to examine the behavioral response to the nutritional state of exercise. The independent variable will be the nutritional state of exercise.

Nutritional state before MT measurements and the exercise protocol will be kept uniform between subjects. Subjects must have not eaten within two hours prior to the MT tests with the exception of the post-exercise RVT measurements. We will assume the test meal TEF to be minimal and equivalent between conditions. Power setting in watts of the exercise protocol will be adjusted to achieve the given percentage of maximal intensity for each subject. Energy intake is the most crucial control variable. Subjects will consume the number of kilocalories required to maintain energy balance preceding each trial.

### **Limitations and Validity**

More extraneous variables are being controlled in the current design than in the previous pilot study; however, there are some remaining limitations. Tighter control of energy intake

preceding trials will be advantageous for direct REE comparison but eliminates the assessment of the frequent fluctuations of real world eating behavior. This will be partially countered through the use of the appetite survey. Several interesting questions are not able to be answered using this design, the most obvious of which is how fasted exercise training impacts metabolic pathways from a long-term outlook. Monitoring of metabolic variables will only be done while the subject is in the laboratory so there can be no speculation of their continuous input throughout the day following exercise.

### **Operational Definitions**

1. Fed - having recently consumed a cluster of kilocalories (as a meal or snack) large enough to offset the effects of acute fasting; from postprandial state to onset of post absorptive state
  - trial: consumed test meal before exercise
2. Fasted - opposite of fed; no recent energy intake (with multiple associated physiological responses); onset of post absorptive state extending until next meal
  - trial: consumed test meal after exercise
3. Metabolism - broad term for various physiological processes involved in organic energy regulation that can be simplified to a dualistic paradigm including processes stimulating anabolism or catabolism
4. RMR (resting metabolic rate) – energy expenditure during a period of quiet rest usually extrapolated to a 24-hour value as  $\text{kcal}\cdot\text{day}^{-1}$ ; caloric value of energy expended daily to maintain basic life processes without active involvement of the individual; largest contributor to TEE in sedentary and lightly active individuals

5. REE (resting energy expenditure) - term used interchangeably with RMR; REE will be identified as the primary variable of energy expenditure for the current study
6. MT (metabolic testing) - general term used here to represent use of the metabolic cart for measuring metabolic variables; subdivided into "RMR test" and "RVT"
7. RMR test - estimation of REE from snapshot 20-minute measurement of oxygen consumption and RER during resting conditions in the post absorptive state; output includes REE, RER and  $VO_2$
8. RVT (recovery  $VO_2$  test) - MT protocol performed after exercise and lasting for 60 minutes with a 10-minute break at the halfway point that does not require the subject be in a supine rested state, otherwise same protocol as an RMR test; output includes REE (cropped to value during test period), RER and  $VO_2$  (EPOC)
9.  $VO_2$  (oxygen consumption) - amount of oxygen consumed by the body during MT that can be expressed in absolute ( $mL \cdot min^{-1}$  or  $L \cdot min^{-1}$ ) and relative ( $mL \cdot min^{-1} \cdot kg^{-1}$ ) terms; subdivided into recovery  $VO_2$  when measured after exercise and resting  $VO_2$  when measured before or multiple hours after exercise.
10. EPOC (excess post-exercise oxygen consumption) - phenomenon where oxygen is consumed beyond true resting values (difference between recovery  $VO_2$  and resting  $VO_2$ ) in the hours following an exercise bout yielding increased REE; EPOC is related to REE but can be analyzed and discussed separately
11. RER (respiratory exchange ratio) - ventilation ratio of carbon dioxide production to oxygen consumption; implies substrate utilization as ratio of carbohydrate to fat under steady-state conditions

12. paEE (physical activity energy expenditure) - caloric value of energy expended due to mechanical work of the musculo-skeletal system during the day; in this study, paEE is energy expended during the food log periods
13. TEE (total energy expenditure) - sum of REE, paEE and TEF (thermic effect of feeding)
14. RQ (respiratory quotient) - cellular representation of RER; sometimes used interchangeably with RER; RQ will not be discussed beyond the literature review
15. Appetite - level of desire to eat (includes hunger [the need to eat]); assessed by survey methods; represented by results of individual appetite component questions and composite appetite score (CAS)
16. VAS (visual analog scale) - survey that includes carefully measured lines that act as a continuous scale to mark answers to questions regarding perceived appetite
17. EI (energy intake) - measurement of kilocalories consumed during any given measurement period
18. CEB (calculated energy balance) - difference between EI and TEE during given measurement period, value of zero indicates theoretical energy homeostasis (no net catabolism or anabolism)
19. HIIE (high-intensity interval exercise) - exercise protocol implemented to induce intermittent maximal aerobic stress by exercising at a near-maximal aerobic intensity for brief periods, followed by periods of reduced intensity
20.  $VO_{2max}$  (maximal oxygen consumption) - level of oxygen consumption measured at the point of volitional exhaustion during a maximal exercise test; reveals point of maximal aerobic metabolism

21.  $P_{\max}$  (maximal aerobic power) - highest power output (in watts) reached during maximal exercise test (i.e., power at  $\text{VO}_{2\max}$ ; value pro-rated based on time in final stage)
22. Relativity - when qualified as being in "relative" terms, all metabolic and exercise performance variables will be expressed using kg of lean body mass in the denominator as opposed to the typical meaning of kg in these equations to allude to total body mass
23. Food log (MyFitnessPal) - software that allows recording of energy intake with a detailed output including caloric intake and macronutrient distribution

### **Significance and Application**

Nutritional status and exercise both have noteworthy effects on metabolism. Furthermore, the interaction between them also displays metabolic effects. If fasted or fed exercise differentially impact REE and EPOC then they may be effectively employed in a weight management or health-focused fitness program. Even if there is no difference, any new information on the mechanisms of metabolic changes after exercise would prove valuable. This information could be highly valuable to the growing number of individuals embarking on these programs to improve body composition and prevent or manage metabolic disease. The novelty of the proposed study relates to analyzing metabolic and behavioral effects of fasted versus fed HIIE when previously in energy balance. *Ad libitum* feeding on trial days allows conclusions regarding real-world feeding behavior in response to this type of exercise while the tight control of energy balance maintains a high degree of internal validity.

## CHAPTER II

### LITERATURE REVIEW

#### Part 1 - Background Research

##### *Narrative 1: Metabolism - Exercise*

#### EPOC

Metabolism and exercise are understood to be directly related. This is rendered most obvious when describing the phenomenon of EPOC. EPOC is a metabolic outcome of exercise where oxygen consumption is elevated above normal resting values. The origin of this term has been described by Gaesser and Brooks (Gaesser & Brooks, 1984) as an alternative definition of the phenomenon where specific mechanisms, namely lactate metabolism, are not implied. This is logical because the specific mechanisms have not been fully explicated and there are likely many contributing factors. Gaesser and Brooks (Gaesser & Brooks, 1984) reported that these factors may include catecholamines, glucocorticoids and temperature, among others. They purported that body temperature is likely the most important factor. Although lactate metabolism cannot fully explain EPOC, it is still considered an important factor. EPOC has been shown to be related to lactate removal and training status (Aguilar et al., 2014).

EPOC consists of fast and slow phases. More recent research has rendered a clear division between these two phases less distinguishable. EPOC within close proximity to exercise has been fairly well established but the slow component, and thus entire process, requires more study to achieve a greater understanding. The majority of studies examining EPOC have found elevation within the first two hours following exercise. In fact, a limit of



two hours has been proposed for the window of the faster phase of EPOC (Speakman & Selman, 2003). Fast-component EPOC is transiently observed immediately after exercise cessation and dissipates fairly quickly. The slow component consists of an extended period of usually at least 12 hours (Maehlum et al., 1986), possibly up to 48 hours (Speakman & Selman, 2003), following a bout of exercise where oxygen consumption is elevated, but this effect has not been universally observed. Due to the dearth of time-component establishment, the delineation between fast and slow phases is more descriptive and subjective.

This observation occurs due to compensatory responses to body temperature elevation and increased ventilation rate as well as replenishment of energy stores. There are many proposed factors contributing to the occurrence of the slow component of EPOC. The EPOC effect has been shown to depend on both exercise duration (Bahr et al., 1987) and intensity (Bahr & Sejersted, 1991). Regarding intensity, the relationship is exponential (LaForgia et al., 2006) as opposed to the linear association between EPOC and exercise duration. Børsheim and Bahr (Børsheim & Bahr, 2003) described multiple possibilities including a shift in substrate metabolism as well as up-regulation in triglyceride and free fatty acid cycling.

A potentially critical aspect of analyzing EPOC is described by Henderson (Henderson, 2014). If attempting to quantify metabolic responses, especially if expecting acceleration or deceleration, EPOC alone does not provide ideal descriptive accuracy. Variations of oxygen consumption values equate to different caloric values determined by substrate utilization. While these differences could be considered modest, so too could the magnitude of EPOC in most circumstances. REE as a direct measure of energy output is thus necessary to attach proper perspective to EPOC measures. RER is also valuable for estimating the contributions

of fat and carbohydrate utilization to both REE and EPOC. Fortunately, all three measures can be derived from the same test as long as the most inclusive protocol is employed.

### **Traditional Exercise Protocols**

One study examined the effects of duration, intensity and mode on oxygen consumption, RER and fat oxidation up to 90 minutes following exercise (Warren et al., 2009). Durations of 90 minutes and 30 minutes were compared at 50%  $VO_{2max}$ . Exercise was completed in a fasted state but not compared to a postprandial state. Duration did not affect EPOC ( $41 \pm 8$   $mL \cdot kg^{-1}$  vs  $35 \pm 5$   $mL \cdot kg^{-1}$ ) or RER, which declined to below baseline levels for both conditions. Fat oxidation rate did not differ between conditions but increased above baseline in both. Intensity trials were matched for EE between 85% and 50%  $VO_{2max}$ . EPOC was greater in the high intensity trial for 40 minutes following exercise and ended up being greater in total compared to low intensity ( $79 \pm 9$   $mL \cdot kg^{-1}$  vs  $24 \pm 7$   $mL \cdot kg^{-1}$ ). RER did not change from baseline in the low-intensity condition but was decreased below baseline in the high-intensity condition (13% lower after 90 minutes). RER was lower in the high-intensity condition than low-intensity at most measurement intervals up to 90 minutes following exercise. After high-intensity exercise, fat oxidation rate experienced more than a 100% increase from baseline. It did not change after low-intensity. The difference between conditions persisted for 40 minutes. Exercise mode was split between continuous exercise at 50%  $VO_{2max}$  for 90 minutes and intervals composed of 60 seconds at 85%  $VO_{2max}$  with recovery periods of 120 seconds at 30%  $VO_{2max}$ . Mode did not affect EPOC ( $38 \pm 8$   $mL \cdot kg^{-1}$  vs  $51 \pm 8$   $mL \cdot kg^{-1}$ ). RER declined below baseline levels for both conditions, of which there was no difference. Fat oxidation rate did not differ between conditions but was elevated in both.

These results display the strong impact of intensity while revealing minimal effects for duration and mode (interval vs continuous).

Knab et al. (Knab et al., 2011) had subjects spend a day in a whole-room calorimeter either without exercise or after cycling for 45 minutes at  $72.8 \pm 5.8\%$   $\text{VO}_{2\text{max}}$  resulting in an exercise energy expenditure of  $519 \pm 60.9$  kcal. On the exercise day, energy expenditure was elevated for 14 hours following exercise resulting in an extra 190 kcal of energy expenditure. This represents an additional 37% of exercise energy expenditure experienced after exercise. Control-day TEE was  $2438 \pm 475$  kcal and exercise-day TEE was  $3188 \pm 559$  kcal. The additional 190 kcal of energy expenditure was 6% of the exercise-day TEE and 8% of the normal TEE.

### **HIIE**

High-intensity exercise, especially with an interval-based protocol, may have a differential impact on metabolic response compared to moderate-intensity exercise. This could be explained by several observations. Aguiar (Aguiar et al., 2014) proposed that anaerobic metabolites yield a greater EPOC due to their findings regarding lactate production and removal during maximal exercise. HIIE increases mobilization of both visceral (Heydari et al., 2012) and subcutaneous (Trapp et al., 2008) fat as well as increasing mitochondrial capacity (Little et al., 2011). Gibala et al. (Gibala et al., 2012) summarized multiple notable studies on HIIE concluding that it offers several advantages over continuous exercise. In one study, HIIE was found to be impactful on various physiological fronts including AMPK activation and PGC-1 alpha expression (Gibala et al., 2009).

Claims that HIIE increases the rate of fat oxidation (Talanian et al., 2007) have been backed up by the finding that HIIE (90%  $\text{VO}_{2\text{peak}}$ ) counters postprandial triglyceride elevation more effectively than continuous (50%  $\text{VO}_{2\text{peak}}$ ) exercise (Trombold et al., 2013). In the Trombold study (Trombold et al., 2013), postprandial fat oxidation 24 hours after HIIE was higher than after continuous exercise.

In general, high-intensity exercise seemingly results in increased energy expenditure and decreased RER. Melby (Melby et al., 1993) conducted two experiments that displayed this outcome. The exercise performed was resistance training which, by nature, is structured by including brief work intervals, or sets. The first experiment included an RMR test for two hours following exercise and another 24 hours later. The results were compared to pre-exercise values. The second experiment used the same procedure except included a control condition and RER analysis. REE and  $\text{VO}_2$  remained elevated for the entire two-hour post-exercise window in both experiments. By the end of the two-hour period, EPOC was still 11-12% above resting levels. REE remained elevated 24 hours after exercise (9.4% and 4.7% increase). RER was reduced 24 hours after exercise. This effect was partially confirmed in a study comparing metabolic response between 60 minutes at 50%  $\text{VO}_{2\text{max}}$  and 30 minutes at 100%  $\text{VO}_{2\text{max}}$  over 60 minutes divided into 2:2 work to rest intervals in a room calorimeter (Treuth et al., 1996). Values for the HIIE condition were greater than the continuous condition for exercise EE ( $349.9 \pm 56.9$  kcal vs  $286.1 \pm 39.0$  kcal), 2-hour post-exercise EE ( $170.4 \pm 33.0$  kcal vs  $148.9 \pm 22.9$  kcal) and 24-hour EE ( $2043.9 \pm 200.0$  kcal vs  $1884.0 \pm 184$  kcal). RER was higher during HIIE but did not differ from continuous exercise in any other time interval. While results for RER in response to HIIE are inconclusive, the elevated energy expenditure response is apparent.

Kelly (Kelly et al., 2013) determined that, within a 60-minute window after HIIE, energy expenditure was elevated above rest while a simultaneously decreased RER was observed. Subjects performed an HIIE protocol of ten one-minute stages (90% of peak HR from a maximal exercise test) or a no-exercise control with REE recording for 12 hours with a focus on the first 60 minutes after exercise. There was no difference between REE or RER from 75 minutes and beyond, referred to as the slow phase of EPOC. REE during the first 60 minutes was higher after exercise than during control (93 kcal vs 79 kcal). RER was also lower after 60 minutes (0.78 vs 0.87). Although significant, the results were admittedly small and there was no effect on the slow component of EPOC. After HIIE (4x1 min, 85-95% MHR) compared to continuous exercise (47 min, 70% MHR) in subjects with metabolic syndrome, Larsen (Larsen et al., 2014) observed differences in EPOC duration and volume. EPOC lasted for  $70.4 \pm 24.8$  minutes, totaling  $2.9 \pm 1.7$  L, after HIIE and  $45.6 \pm 17.6$  minutes, totaling  $1.4 \pm 1.1$  L, after continuous exercise. Interestingly, a concurrent protocol of only one four-minute stage at 85-95% MHR resulted in less total EPOC over a shorter duration than both HIIE and continuous exercise, suggesting that reaching high intensity alone is not enough to stimulate comparable responses. Paoli et al. (Paoli et al., 2012) found increased energy expenditure after circuit resistance training structured similarly to HIIE compared to standard resistance training. However, findings supporting the notion of HIIE affecting EPOC are contested. Williams (Williams et al., 2013) arrived at the conclusion that HIIE does not impact EPOC or RER any differently from continuous exercise. A recent finding by Skelly (Skelly et al., 2014) bridges the gap to some degree. This study found that HIIE yields similar 24-hour EPOC to traditional continuous exercise. Since oxygen consumption was

lower during exercise in HIIE, the logically derived conclusion is that HIIE elicited a minor increase in EPOC over continuous exercise.

An important consideration concerning HIIE and metabolism is the type of stress on the energy systems. Intervals can be structured to elicit work at or near the aerobic maximum (100% of  $VO_{2max}$  or  $VO_{2R}$ ). They can also produce exercise intensity that is aerobically supramaximal and can thus be considered anaerobic. Both styles have been examined in this review and both are worthy of further investigation. Presently, near-maximal aerobic work will be utilized for its more logical comparison to findings regarding continuous moderate-intensity exercise.

### **Sex Differences in Exercise Metabolism**

The causative modulation of exercise on metabolism has been studied extensively but the resulting global conclusions have been tentative. Exercise clearly has some impact on EPOC, REE and RER but the extent and even the direction of the effects continue to be argued. Clarity becomes even more elusive when examining sex differences in metabolic response to exercise.

#### *Sex and Menstrual Phase Have Little Impact on EPOC or REE*

In a study involving only female subjects, EPOC followed the same intensity-dependent relationship as has been established as a general occurrence (Frey et al., 1993). In this study, EPOC was observed in a one-hour window following isocaloric exercise at either high-intensity (HI; 80%  $VO_{2max}$ ) or low-intensity exercise (LI; 65%  $VO_{2max}$ ). Oxygen consumption increased in both conditions but EPOC at HI was greater than EPOC at LI. HI exercise also resulted in higher body temperature, norepinephrine and lactate than with LI

exercise. These are all factors purported to contribute to EPOC. The duration of EPOC in trained subjects was 50 minutes after LI exercise and 40 minutes after HI exercise. EPOC duration could not be determined in untrained subjects due to continuance through the entire 60-minute window. Irrespective of duration, EPOC was elevated with HI exercise in both trained and untrained subjects. In the one-hour measurement period following exercise the increased temperature, norepinephrine concentration and lactate persisted for at least 50 minutes after HI exercise in both trained and untrained subjects. These results held true for LI exercise except for temperature in trained subjects (30-minute duration) and norepinephrine in untrained subjects (5-minute duration). The behavior of these factors help validate the EPOC findings but do little to offer an explanation of how much they individually contribute. There are obviously multiple factors at work in this phenomenon but EPOC in females appears to follow the same patterns as it does in males.

One large article examining many metabolic rate studies displayed an absence of effect of menstrual phase on REE (Weststrate, 1993). Since then, more specific measures have expressed agreement with this conclusion. Postprandial fat oxidation is higher in males than females while postprandial fatty acid uptake is greater in females than males (Uranga et al., 2005). Despite these findings, there were menstruation-mediated differences suggesting that the menstrual cycle does not mediate these substrate metabolism responses to meals. Focusing on exercise metabolism, Fukuba (Fukuba et al., 2000) found that EPOC from exercise at 70% of  $VO_{2max}$  is reduced after pre-exercise energy restriction in females. However, the menstrual phase did not affect the magnitude of EPOC. During high-intensity exercise (a maximal effort ramp test), substrate utilization, oxygen consumption, heart rate and lactate metabolism have been shown to not be affected by menstrual phase (Vaiksaar et

al., 2011; Vaiksaar et al., 2011). From these findings on menstrual phase and REE, it would appear that sex differences in exercise metabolism are minimal. However, there are multiple noteworthy distinctions to be made between male and female metabolic response to exercise.

### *Role of Menstrual Cycle in Exercise Metabolism*

The ovarian cycle focus of the menstrual cycle is subdivided into the follicular phase, ovulation and the luteal phase. The distinction between phases is important because the fluctuating levels of circulating hormones throughout the cycle affect carbohydrate and fat metabolism. For example, EPOC after exercise at 60%  $\text{VO}_{2\text{max}}$  has been found to be greater in the luteal phases compared to the follicular phase while RER was found to be lower (Matsuo et al., 1999). Substrate preference during exercise may also be affected by menstrual phase. Glucose turnover and glycogen utilization have been identified as lower during exercise in the luteal phase than in the follicular phase (Devries et al., 2006). Males experience greater RER, glucose turnover and glycogen utilization during exercise but the disparity in glycogen utilization is only significant when compared to females in the luteal phase. In this case, menstrual phase did appear to have at least a marginal effect on exercise metabolism, although this did not fully explain the primary sex difference. A logical conclusion from these, and previous, studies would be that the menstrual phase plays a larger role in exercise metabolism than resting metabolism but may not have a notable impact on EPOC. One strategy that has been employed in assessing metabolic response to exercise in females is to do all testing during the follicular phase 14 days after the onset of menstruation (Treuth et al., 1996).



### *Sex Differences in Substrate Utilization*

During exercise, females use a greater proportion of fat for fuel than men and the inverse is also true; females use proportionally less carbohydrate than men to fuel exercise (Horton et al., 1998). Males release more epinephrine and norepinephrine during exercise but there is no sex-related difference in glycerol suggesting that females are more sensitive to catecholamine-modulated lipolytic effects. This substrate utilization sex difference is dissipated following exercise. This difference in exercise substrate utilization may be explained by differences in estradiol concentrations (Tarnopolsky, 2008). Estradiol administration to exercising males mitigates some of these sex-related metabolic differences. Considering that females oxidize more fat during fasted exercise, Riddell (Riddell et al., 2003) investigated the impact of carbohydrate ingestion during exercise trials in males and females and discovered that females more efficiently utilize the exogenous carbohydrate and thus predictably spare more endogenous carbohydrate stores. Females do rely on fat more to fuel exercise than males do and this is likely driven by hormonal mechanisms but there may be differences between fasted and fed exercise.

Following exercise, both males and females experience similar alterations in metabolic function (Henderson & Alderman, 2014). Fat oxidation is increased in both sexes and equally in a postprandial state. In a post absorptive state, male fat oxidation rate is greater. RER is reduced equally in both sexes while REE increase in both sexes, but more so in males. This reveals that the higher fat oxidation rate in males is driven by the difference in REE rather than in RER. The post-exercise elevation in fat oxidation supports the concurrent increase in energy expenditure. In the postprandial phase, triglyceride levels increase due to the postprandial lipemia response. In males, previous exercise blunt the postprandial lipemia

response by increasing triglyceride clearance rate (Magkos et al., 2006). Triglyceride secretion rate remains unaffected. In females, exercise similarly depresses postprandial lipemia but not by reducing triglyceride clearance rate (Davitt et al., 2013). It instead appears to be supported by reduced free fatty acid levels. While recovering from exercise, free fatty acid mobilization is increased in both sexes over three hours but more in males (Henderson et al., 2007). This supports the previous finding regarding postprandial free fatty acid availability. This exercise recovery study also found elevated three-hour fat oxidation rate in both sexes but more in males. After 24 hours, fat oxidation rate was elevated in males but not in females. This suggests a sex difference in the prolonged metabolic response to exercise.

The relevance of these differences is discussed in a review by Henderson (Henderson, 2014). Exercise increases REE and post absorptive lipolysis (free fatty acid availability) in males but not females. This explains how male fat oxidation is greater after exercise than in females. What this represents is a more refined protection mechanism against the interruption of metabolic homeostasis, especially by exercise, in females. From a health perspective, the nullifying effect of exercise on postprandial lipemia is more clinically relevant for males. Normally, postprandial triglycerides are higher in males and chronically elevated triglycerides are obviously deleterious to cardiovascular health. Combined with prior acquisition of knowledge in metabolic sex differences, exercise can be viewed as more critical to males for the purpose of regulating triglyceride levels. This could even explain why the differences exist.

Ultimately, the differences in metabolic response to exercise between males and females is clear but not necessarily compelling. Furthermore, substrate utilization is the biggest point of differentiation but the primary inclusive metabolic measures of EPOC and REE seem to be

relatively unaffected. There may be a small menstrual cycle effect on metabolic response to exercise but it does not seem to make a meaningful difference. Any potential concerns of confounding resultant data can be mitigated by controlling for menstrual phase in study design and sex in statistical analysis.

### **Narrative 1 Summary**

EPOC research has been rapidly expanding for years due to its status as a representation of global metabolic response to exercise. When combined with REE and RER measurements, EPOC analysis can help determine the early adaptive response of the human body to exercise. EPOC has been continuously associated directly with exercise intensity and HIIE may produce the largest change, even if the difference from continuous moderate-intensity exercise is not overwhelming. Metabolic response to exercise is not uniform between sexes but the impact on the major categories of energy expended and oxygen consumed is minimal. The main point to be drawn from all this is that metabolism is altered by exercise but the magnitude and specific factors of interest have not been conclusively identified.

### *Narrative 2: Exercise - Diet*

#### **Exercise Effects on Energy Intake and Appetite**

It would be reasonable to assume that exercise would result in temporarily increased energy intake. Since homeostasis is the default human state, and the body has many mechanisms to preserve it, additional energy expenditure would seem likely to be compensated for by increasing hunger and consequently increased food consumption. However, this does not appear to be the case. A case could even be made that the opposite is true.

Exercise has repeatedly been shown to have little or no effect on subsequent energy intake or appetite (Blundell & King, 1999; Hubert et al., 1998; King et al., 2010; King et al., 2010; King et al., 1994; King et al., 1997; Lluch et al., 1998), sometimes even dampening energy intake (Hagobian et al., 2013). A large majority of the relevant studies support this concept (Blundell & King, 1999). Briefly reduced hunger, delay of the first post-exercise meal and a transient shift to a negative energy balance have been observed in response to high-workload (intensity and duration) exercise (King et al., 1994). This delay in desire to eat can extend to over half an hour (King et al., 2013). Exercise at a lower workload did not have the same effect. Hunger and energy intake are not affected over a 48-hour window following high-workload exercise in male subjects (King et al., 1997). The circumstances are slightly different for females. Appetite sensations do respond equivalently between sexes. Hunger suppression is less impactful in females and their short-term energy deficit can be mitigated by a high-fat meal (King et al., 1996). The latter would be more likely in females due to differences in the perceptions of palatability of various foods. Despite the dearth of clarity regarding sex differences in appetite response, females experience the same result as males for energy intake after exercise. Exercise effects on appetite have been suggested as an important contributor to the prevention of weight regain in obese individuals who undergo dramatic weight loss (Martins et al., 2008). This becomes even more important considering that exercise cannot reverse the steep decline in REE after dramatic weight loss even when lean mass is preserved (Johannsen et al., 2012). Metabolic deceleration can be exemplified by the fact that during Ramadan intermittent fasting, resting  $\text{VO}_2$  decreases, although  $\text{VO}_2$  during submaximal exercise is unaffected (Sweileh et al., 2012).

### **Exercise Effects on Diet Modification of Energy Intake**

While exercise-induced energy deficits do not appear to persuade compensatory feeding behavior, high-fat meals may result in elevated energy intake (Lluch et al., 1998).

Additionally, suboptimal energy content in the first meal of the day increases hunger and energy intake (Hubert et al., 1998). In the previous case, exercise did not affect this outcome. However, this notion has been disputed. Exercise may actually be able to run interference on the compensatory feeding response to low-energy meals (Martins et al., 2007). Exercise exerts these effects without altering ghrelin levels during the same post-exercise feeding periods where energy intake, appetite and macronutrient composition remain unaffected (King et al., 2010; King et al., 2010). Decreased hunger immediately after exercise has been recorded concurrently with increased peptide YY in addition to decreased ghrelin but these values return to baseline shortly after (Broom et al., 2009).

The previously discussed occurrences are not universally observed. In subjects with diabetes (Types I and II), greater reductions in blood glucose following exercise are associated with elevated hunger and prospective food consumption (Dubé et al., 2013). Hunger proved to be the strongest predictor of *ad libitum* energy intake. This appears to have a limited application to be used in a specific context. Chronic exercise-induced energy deficits are only compensated for with an increase in energy intake to match 30 percent of the exercise energy expenditure (Blundell et al., 2003). This is a limited compensation mechanism and is not even present in all subjects. Energy intake can remain constant in the presence of exercise-induced energy deficits up to  $956 \text{ kcal}\cdot\text{day}^{-1}$  over multiple weeks.

### **HIIE Effects on Energy Intake and Appetite**

The role of exercise intensity in influencing post-exercise feeding behavior has been studied less frequently. HIIE is beginning to amass some interest in this area. Beyond just energy expenditure responses to HIIE, appetite and energy expenditure have also been examined together. In one notable study, 60 minutes of cycling at 68%  $VO_{2max}$  was contrasted with six Wingate sprints over a 30-minute window (Deighton et al., 2013). Energy intake was unaffected but, interestingly, ghrelin levels were diminished following HIIE despite increased perceived appetite. This suggests that physiological (i.e. exercise-induced) decreases in ghrelin dissociate it from appetite regulation. Appetite was suppressed during HIIE to a greater degree than in continuous exercise but there was no compensatory feeding response observed. Deighton (Deighton & Stensel, 2014) followed up by explaining that under typical circumstances, energy intake and appetite are not affected by exercise unless the intensity is aerobically maximal.

High-intensity exercise and HIIE have even been shown to stimulate reduction of energy intake in the hours following. High-intensity exercise (70%  $VO_{2max}$ ) has resulted in a lower 24-hour energy intake than both isocaloric moderate-intensity exercise (40%  $VO_{2max}$ ) and a non-exercise control in obese adolescents (Thivel et al., 2012). This was observed even though moderate-intensity exercise followed the usual trend of having no effect on subsequent energy intake. Appetite and room calorimeter-measured REE were not different between conditions which suggests that HIIE may be capable of effectively combating the powerful homeostatic mechanisms that encourage energy balance. In another example, HIIE at 100%  $VO_{2max}$  led to lower *ad libitum* energy intake in a test meal 70 minutes after exercise than a non-exercise control (Sim et al., 2014). Meanwhile, energy intake in the test meal after

HIIE at 170%  $\text{VO}_{2\text{max}}$  was lower than both the control and moderate-intensity (60%  $\text{VO}_{2\text{max}}$ ) exercise. The high-intensity HIIE caused a lower 38-hour energy intake than moderate-intensity exercise and the control. Ghrelin and glucose concentration were elevated in this HIIE condition compared to the other trials, including the other HIIE condition. There were no appetite differences between trials. These studies support the claim that sufficient intensity of exercise can actually reduce energy intake without affecting appetite or energy expenditure, meaning that creating an energy deficit could possibly be made easier with this high-intensity style. Furthermore, extremely high-intensity exercise seems to catalyze this effect.

### **Narrative 2 Summary**

Exercise has repeatedly proven to have a weak relationship with energy intake, if any. The impact on appetite is not quite as obvious but appetite generally decreases after exercise. Only in chronic energy deficits created by exercise does a small compensatory increase in energy intake occur. Exercise at high intensities may have an even more pronounced inhibitory effect on appetite and energy intake. Further exploring the relationship between high-intensity exercise, appetite and energy intake during free-eating conditions after exercise should provide a solid foundation for understanding the nature of diet-exercise interaction.

### *Narrative 3: Metabolism - Diet*

#### **Metabolic Response to Fasting**

Diet and metabolism are clearly related as energy intake contributes to both anabolic and catabolic processes. A highlighted interest under the umbrella of diet is the metabolic

response to energy deprivation, specifically, short-term fasting. Substrate metabolism under fasting conditions has been extensively scrutinized for decades. Lipid mobilization increases during short-duration fasting. This effect is even more pronounced in obese subjects (Wolfe et al., 1987). Lipid mobilization has been shown to reach its fasted peak after 18 hours and is associated with the decline in insulin activity (Klein et al., 1993). This appears to occur independent of glucose-sparing mechanisms that balance serum glucose fluctuations. However, Groop (Groop et al., 1990) found that the fat oxidation rate was higher at 12 hours of fasting than 18 hours encompassed by the overnight fasting period. Acute fasting initiates modulation of genetic expression by the hypothalamus that shifts the ratio of substrate utilization toward fat at the expense of glucose (Poplawski et al., 2010). This accentuates a necessity to tightly control overnight fasting duration for studies involving fasting protocols. The increase in fat mobilization and decrease in insulin activity persist through three days of fasting (Stannard et al., 2002) and thus appear to be predictable outcomes of a reasonable time period of fasting.

There exists a distinct conflict regarding how metabolic rate responds to fasts of various durations. Long-term energy restriction studies lasting for multiple weeks collectively endorse a well-established theory of a reduction in REE as a compensatory mechanism to achieve energy balance (Shetty, 1990). This could be assumed to apply to intermittent fasting protocols but there is evidence that the actualization of fasting protocols may not follow this line of logic. Irrespective of this possibility, it has been abundantly reported that energy deficits reduce REE.

Antithetically, shorter-duration fasts lasting for multiple days (72 hours) have displayed the outcome of increased energy expenditure (Chan et al., 2003; Zauner et al., 2000). These



findings defy the notion that energy restriction directly downsizes metabolic rate. Both of these studies found concomitant increases in epinephrine concentration, suggesting an association between fasted elevation in catecholamines and the observed increased energy expenditure. Chan (Chan et al., 2003) measured REE and RER after a 72-hour fast to find elevated REE and depressed RER. Free fatty acid concentration and protein utilization also increased but the latter was likely due to the extended fast duration. Insulin levels decreased significantly. Zauner (Zauner et al., 2000) also measured REE after 72 hours of fasting to observe the elevation. In this study, insulin was not affected but glucose levels declined and it was suggested that this was the basis for increased epinephrine production. Both of these studies support metabolic acceleration and shifting substrate utilization with fasting but the duration of the fasts in both conditions was arguably extreme.

Acute short-duration fasting seems to have the same disagreement of action. Maughan (Maughan et al., 2010) made the claim that metabolic rate is not significantly affected by the early phase of fasting (within 24 hours). The down-regulation of glucose metabolism basically cancels out the up-regulation of fat oxidation. However, a seemingly marginal disparity in the duration of an acute fast can have an effect on resting energy expenditure, causing a reduction. This is exemplified in a recent study by Yoshimura (Yoshimura et al., 2014) where, in a hospital setting, patients fasted either 8 or fewer hours or 10 or more hours. In the 10-hour fast group, both REE ( $17.7 \pm 2.3 \text{ kcal} \cdot \text{kg} \cdot \text{day}^{-1}$ ,  $1088 \pm 159 \text{ kcal} \cdot \text{day}^{-1}$ ) and RER ( $0.71 \pm 0.12$ ) were lower than in the 8-hour fast group (REE =  $19.7 \pm 2.3 \text{ kcal} \cdot \text{kg} \cdot \text{day}^{-1}$ ,  $1181 \pm 259 \text{ kcal} \cdot \text{day}^{-1}$ , RER =  $0.81 \pm 0.09$ ). The 10-hour fast group also had a larger disparity between measured REE and basal EE. This disputes previous statements of early-phase fasting not having a meaningful impact on metabolic rate. However, the fact that

directional difference of the absolute values for REE actually reverses compared to the relative values reported may be perceived to be misleading. There was no statistical difference between groups for absolute REE or BMI. Despite this concern and its clinical focus, the conclusion retains its position as an interesting contribution to the debate.

One aspect that has also been scrutinized is that fasting yields a reduced RER value. This is highlighted in multiple studies. After a 22-hour fast, RER has been shown to decrease from 0.77 to 0.72 (Romijn et al., 1990). In a study attempting to verify the reliability of metabolic measures, post absorptive (10-14 hours of fasting) and postprandial data were collected and compared (Gonzalez et al., 2012). There was no significant difference in either REE or RER between nutritional states. RER was lower in the post absorptive state in both reliability trials (0.78 vs 0.84 and 0.77 vs 0.83) but this difference did not reach statistical significance ( $p = 0.12$ ). Pending conciliation of future conclusions, the effects of acute and chronic fasting on both REE and RER remain enigmatic.

### **Fasting and Energy Intake with Consideration for Energy Expenditure**

Implications of a noteworthy relationship between fasting and appetite have been proposed in the literature. A 36-hour fast does not stimulate a compensatory overfeeding response despite causing an elevated appetite leading up to the breakfast meal (Johnstone et al., 2002). Appetite is regulated by REE and fat-free mass (as a determinant of REE) which in turn is surmised to modulate energy intake (Blundell et al., 2012; Caudwell et al., 2013). One particular study compared energy expenditure responses between normal caloric consumption and various diets after 24 hours including multiple compositions of overfeeding diets, fasting and eucaloric consumption as a control (Thearle et al., 2013). Overfeeding diets

resulted in greater energy expenditure, ranging from 8% to 14% over normal eucaloric values, except for diets with low protein content which still resulted in non-significant increases. Fasting decreased energy expenditure by almost 10% which was significantly different from all other conditions. This may point to short term fasting lacking the capability to induce the catecholamine response necessary to increase energy expenditure. The duration of the fast appears to have discordant effects on energy expenditure.

A combined analysis of four appetite studies (MacIntosh et al., 2001; MacIntosh et al., 2001; MacIntosh et al., 2001; Sturm et al., 2003) confirmed the idea that energy intake during a test meal is associated with perceived appetite as measured by VAS (Parker et al., 2004). Older subjects' energy intake was associated with hunger and prospective food consumption whereas younger subject's energy intake was associated with fullness. However, Flint (Flint et al., 2000) found that hunger is a valid predictor of energy intake in young subjects so both factors are likely relevant. The analysis also revealed that some perceptual factors not directly related to appetite influence energy intake. The interesting result of this question was that drowsiness and calmness were positively correlated to energy intake. Thus in an ideal design for an appetite study based on VAS, subjects will be equally well-rested and relaxed during testing.

### **Further VAS Information**

VAS assessments have been used for decades to record and interpret perceptions of appetite and more specifically, the individual aspects of appetite that allow more detailed analyses of appetite (Hill & Blundell, 1982). These factors include the previously discussed scales for hunger, fullness, prospective food consumption and food preferences. Since they

may have differential impacts on energy intake, it is important to analyze these factors separately as extensions of an overall appetite quantification. A more recent review of VAS tactics concluded that VAS appetite assessments possess within-subject reliability and validity in predicting future energy intake (Stubbs et al., 2000). Furthermore, the validity and reliability of the VAS is experimentally sensitive and is therefore well-suited for within-subjects repeated measures designs in laboratory testing. There is no association between VAS composite appetite scores and TEF, potentially dispelling the theory that the appetite is sensitive to influence from diet-induced metabolic alterations (Ravn et al., 2013).

### **Narrative 3 Summary**

On the surface, the collective knowledge supports the lack of an effect for fasting on REE. This is sometimes asserted as a product of elevated fat oxidation compensating for reduced carbohydrate oxidation. The reality, from a caloric perspective, is murky. Several studies have found that REE is either increased or decreased with fasting of varying durations. Fasting may result in elevated perception of appetite without stimulating modification of REE. If there is a paradoxical interaction between appetite and energy intake in metabolic outcomes, this would be supremely valuable information to precipitate understanding of fasting and metabolism.

#### *Narrative 4: Metabolism, Diet and Exercise Interaction*

### **Fasted Training and Metabolism**

There are many unanswered questions about how the nutritional state of exercise affects subsequent metabolic response. One major concern regarding fasted exercise has been assuaged confidently. Fasted exercise does not appear to elicit greater perception of effort as

determined by RPE (Aziz et al., 2011; Nieman et al., 1987). Exercise performance in general seems to be mostly unaffected.

Following fasted exercise, serum glucose is maintained at normal levels despite depletion of liver glycogen (Dohm et al., 1986). This is likely due to an alteration of energy pathways, specifically via reduced pyruvate dehydrogenase activity. Somewhat paradoxically, fasted resistance training better stimulates the anabolic response to a combined carbohydrate and protein recovery meal by increasing p70s6k phosphorylation (Deldicque et al., 2010).

Chronic HIIE training improves body composition and oxidative capacity with both fasted and fed exercise, but not differentially between the two methods (Gillen et al., 2013).

Several studies have investigated the responses of substrate metabolism and energy pathways to fasted exercise; however, most of these were training studies. The applicability to acute fasted exercise is limited as of the current state of the literature. De Bock (De Bock et al., 2005) found that acute fasted exercise increases intramuscular triglyceride breakdown and glycogen resynthesis. De Bock (De Bock et al., 2007) later discovered that fed exercise is glycogen-sparing compared to fasted exercise. A follow-up study by De Bock (De Bock et al., 2008) led to the realization that chronic fasted exercise training does not extend the effect of intramuscular triglyceride breakdown and does not impact maximal oxygen consumption, suggesting no long term impact on fitness. One training study revealed that fasted training is superior to fed training for muscle adaptation, glucose tolerance and insulin sensitivity during a hyper-caloric high-fat diet (Van Proeyen et al., 2010). Two subsequent training studies displayed fasted training's advantageous tendency for muscle oxidative capacity, intramuscular triglyceride breakdown (contradicting the previous finding of DeBock et al. (De Bock et al., 2008)) and intramuscular signaling which may, in turn, increase muscular

protein translation (Van Proeyen et al., 2011; Van Proeyen et al., 2011). Though limited in number, these few studies suggest that chronic fasted exercise training may have a positive impact on glucose metabolism, fat metabolism and even muscle adaptation to exercise.

### **Acute Fasted Exercise and Metabolism**

Unfortunately, there is little knowledge on how acute fasted exercise impacts RER, REE and EPOC. A decrease in RER during fasted exercise is only apparent at low intensities. Although this lower RER during fasted exercise compared to fed exercise has been observed at 22%, 40% and 59%  $VO_{2\text{peak}}$  but not at 75%  $VO_{2\text{peak}}$ , RER was only absolutely reduced at exercise intensities lower than 40%  $VO_{2\text{peak}}$  (Bergman & Brooks, 1999). RER response following fasted exercise is otherwise not well understood. An energy deficit in the exercise recovery period increases lipid mobilization without affecting the exercise-induced effect on insulin sensitivity (Newsom et al., 2010) but this finding does not apply to exercising in a fasted state. Ultimately, RER following fasted exercise remains unexplored. Similar to the issue encountered in interpreting the previous study, fasting after exercise seems to have examined more than fasted-state exercise. In an early fasted exercise study, the impact of fasting after exercise on EPOC was investigated (Bahr & Sejersted, 1991). Subjects performed 80 minutes of exercise at 75% of  $VO_{2\text{max}}$  in addition to a non-exercise control condition. EPOC was measured for seven hours in a fasted state following exercise in the main trial. The two control conditions consumed a test meal of 1075 kcal two hours after exercise. Consumption of the test meal did not affect EPOC. Significant EPOC was observed in both fasted and fed conditions leading to the conclusion that fasted exercise recovery does not yield a different EPOC value than fed exercise recovery.

In a rare example, one study examined metabolic responses during exercise at 70%  $\text{VO}_{2\text{peak}}$  in various time intervals up to 12 hours after a meal consisting of two grams of carbohydrate per kilogram of body mass (Montain et al., 1991). In addition to the 12-hour fasted exercise measurement, post-exercise RER and glucose were assessed two, four, six and eight hours after the test meal. Eight-hour and 12-hour fasted exercise were the only conditions not to experience reduced glucose levels and were 13-15% lower in carbohydrate oxidation. These results suggest that a fasting duration between eight hours and 12 hours is required to elicit metabolic effects different from fasted exercise. The shift towards fat oxidation and propagation of glucose-sparing mechanisms appeared to begin after crossing this eight-hour threshold. This is useful for defining the fasting period required to consider exercise as being performed in a fasted state. Despite its relevance, this study neglected to measure these factors in the hours or minutes after exercise, leaving a very interesting question unanswered.

#### **Narrative 4 Summary**

Chronic fasted exercise training may magnify the individual effects of fasting and exercise. However, the results of the few studies that travelled this investigatory path are somewhat narrow in scope and far from conclusive regarding the topic. Most of the relevant acute exercise studies examined fasting in the post-exercise period. Few studies have delved into the realm of interactions between pre-exercise diet, exercise and resultant metabolism. Even fewer narrow the focus to fasting protocols. The metabolic effects of fasted HIIE have been overlooked in the literature to date. Finding studies that examine acute fasted HIIE

protocols and their associated metabolic effects is an arduous, if possible, task. How metabolism responds to exercise between fed or fasted states is therefore a novel question.

## **Part 2 - Primary Emphasis Studies**

### *Narrative 1: Basis of Hypothesis*

This proposed study design extracted influence from multiple resources covering a diverse array of related topics. Concentration was then isolated to a few studies that provided the backbone for the current hypothesis. The following studies provoked interest in the area of metabolic response to fasted high-intensity exercise and this interest resulted in a pilot study that examined this specific topic. The results of these studies, including the pilot study, provided some insight into the underexplored events this study will isolate. The proposed design can be interpreted as the logical next step in this line of questioning.

### **Paoli et al. (2011)**

#### *Purpose*

One of the earlier attempts to quantify the metabolic impact of fasted exercise was performed by Paoli et al. (Paoli et al., 2011). This study cited previous studies that had collectively suggested that adaptations to fasting and exercise may combine to have a multiplicative effect on metabolic rate that would surpass conventional moderate-intensity exercise. Paoli et al. sought to establish how metabolism responds to fasted exercise and how this would apply to nutritional manipulation for the expressed purpose of weight management.



### *Methods*

Eight healthy, male subjects were recruited to participate. After a 12-hour overnight fast, subjects came in to the laboratory to perform an exercise session consisting of 36 minutes at 65% of their maximum heart rate. They were fed a test meal either 40 minutes before exercise or immediately after exercise. This meal was composed of 673 kcal divided into 22% carbohydrate, 53% fat and 25% protein. The protocol was designed in such a way to directly compare fasted to fed conditions along the same timeline. Two separate RMR testing periods took place upon arrival with a short break in between to allow consumption of the test meal in the fed condition. In the fasted condition, the second measurement was undertaken in identical conditions to the first. The first measurement served as a baseline value where the second measurement alternately served as the pre-exercise value to be used for analyzing changes over time. Following the second RMR test, the exercise protocol was initiated. The subjects were then released to continue their day as normal and subsequent energy intake was assumed to be equivalent between conditions. Follow-up RMR testing was performed in revisits to the laboratory both 12 and 24 hours after exercise.

### *Results*

The primary outcome of the study was that the nutritional state of exercise did not affect metabolic response and no evidence of EPOC was observed. Baseline  $\text{VO}_2$  was  $3.69 \pm 0.21$   $\text{mL}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$  in the fasted condition and  $3.71 \pm 0.24$   $\text{mL}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$  in the fed condition. These were statistically equivalent. The effect of the test meal was displayed with the results of the pre-exercise RMR test with feeding causing elevated  $\text{VO}_2$  and RER compared to fasted pre-exercise values ( $\text{VO}_2 = 4.21 \pm 0.35$   $\text{mL}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$  vs  $3.74 \pm 0.26$   $\text{mL}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$ ; RER =

0.96 ± 0.06 vs 0.84 ± 0.03). Fed VO<sub>2</sub> was 4.47 ± 0.40 mL·kg<sup>-1</sup>·min<sup>-1</sup> after 12 hours and 4.26 ± 0.44 mL·kg<sup>-1</sup>·min<sup>-1</sup> after 24 hours. Both of these values were significantly greater than the fasted condition where VO<sub>2</sub> was 4.12 ± 0.40 mL·kg<sup>-1</sup>·min<sup>-1</sup> after 12 hours and 3.94 ± 0.27 mL·kg<sup>-1</sup>·min<sup>-1</sup> after 24 hours. Similarly, fed RER was lower than fasted RER after both 12 (0.74 ± 0.03 vs 0.79 ± 0.03) and 24 hours (0.78 ± 0.02 vs 0.87 ± 0.02). This was a reversal of the observation in RER immediately following the meal. Time-component differences in VO<sub>2</sub> were not significant with the exception of 12-hour fasted VO<sub>2</sub> manifesting as increased above fasted pre-exercise VO<sub>2</sub>. Fasted RER after 12 hours was lower than after 24 hours and pre-exercise. Fed RER was lower than pre-exercise after both 12 and 24 hours.

#### *Authors' Conclusion*

Results displayed that fasted exercise did not differentially impact 12 and 24-hour VO<sub>2</sub> versus fed exercise and that RER, while initially lower in the fasted state, was lower after fed exercise. Thus, the conclusion was that fasted exercise provides no clear advantage over fed exercise for fat loss and weight management. The authors recommended feeding before exercise based on these findings. Although VO<sub>2</sub> values were slightly elevated after exercise, these differences mostly did not reach significance and EPOC was stated not to have occurred. The explanation offered for this was that the selected intensity of exercise was not sufficient to stimulate the EPOC response.

#### *Interpretation*

Paoli et al. failed to establish a differential impact of fasted exercise on resultant 24-hour metabolism. However, this study had some questions left open by the design. Despite these concerns, this was one of a very select few studies to examine delayed metabolic response to

fasted exercise. Of particular interest is the finding of reduced RER in the hours following fed exercise compared to fasted exercise. Fasting for a duration equivalent to an overnight fast shifts substrate utilization towards fat during exercise (Montain et al., 1991) but the difference between fasted and fed exercise and reduction from pre-exercise value is only apparent at low intensities of exercise (Bergman & Brooks, 1999). Paoli et al. showed the opposite effect in the hours following exercise. In addition to topical interest, the protocol could be viewed as one of high external validity due to the inclusion of a high-fat meal and moderate-intensity exercise. These represent the general acceptance of high-fat diets and steady-state exercise as commonalities. Thus, the study is highly relevant.

There were several properties and limitations of the design that leave important questions unanswered or may even be outright flaws regarding the conclusion, namely the test meal being high in fat content. The ratio of fat to total kilocalories was no representative of a recommended healthy diet. It also likely wouldn't be the typically utilized dietary strategy for weight management. There was also a higher protein concentration than carbohydrate concentration. This also stands out as somewhat odd. A test meal with a higher carbohydrate concentration would have better served as a counterbalance to energy expended during exercise. The energy content of the meal exceeded what would likely be expended during exercise but this is a very minor issue. The most obvious problem with this choice is that higher-fat meals would encourage lipid oxidation and directly affect RER. If this were to occur, then a potential difference between fed and fasted exercise, especially concerning RER, may be mitigated. Analyzing time effects across the trials is questionable due to the divergent nutritional status of the two pre-exercise RMR tests. The post-meal RMR test was used as the starting point for the fed trial whereas the fasted counterpart encountered no

interference from energy intake. The assumption of equal energy intake between conditions was problematic. This is a big assumption to make and monitoring diet during the trial would provide a simple way to eliminate trial-day feeding as a confounding factor. Even with the rationalization that the intensity was not enough to stimulate EPOC, it is somewhat unexpected that it would not be seen at all. Interesting, though, was that EPOC was observed at the 12-hour mark in the fasted condition. This result created a standout question that begged for further investigation and contributed to the decision to undertake the pilot study to be discussed. Metabolic testing employed a CosMed kb42 measurement system (COSMED, Rome, Italy) which is a useful piece of equipment but underestimates  $VCO_2$  and RER at higher-intensity exercise (250 W) compared to the Douglas bag method (Parr et al.). This likely did not materially affect the results of the Paoli et al. study but may be of limited application if subjects are impacted differentially due to fitness status. This study intentionally examined only moderate-intensity exercise and clearly points toward the need to examine the metabolic environment following higher-intensity exercise.

Metabolic response to moderate-intensity exercise is favorable when performed after a fat-dominant meal compared to being performed in a fasted state continued from the overnight period. This is the only apparent conclusion considering the intentional design choices. Irrespective of the test meal discrepancy and other issues, the question asked by the researchers invited further analysis of the concept under an altered structure.

## **Deighton et al. (2012)**

### *Purpose*

Following the Paoli (Paoli et al., 2011) investigation, Deighton (Deighton et al., 2012) conducted an experiment to expand upon previous findings. Fasted exercise ramps up fat oxidation as a fuel source but there is evidence that postprandial exercise is better for fat loss and weight management due to effects on metabolism and appetite. This had already been displayed by the results of Paoli et al. It had also been suggested that energy intake may be lower after postprandial exercise than fasted but *ad libitum* energy intake had not been assessed. Working from the assumption that acute exercise does not normally precipitate a compensatory feeding response, this study sought to discover if this holds true for fasted exercise along with fed, or postprandial, exercise. Thus the primary aim was to compare fasted and fed exercise for their effects on metabolism, energy intake and appetite. This knowledge would greatly expand the current understanding of acute responses to fasted exercise and how it fits in to the larger picture of energy state following exercise.

### *Methods*

Like the previous study, twelve healthy males comprised the subject pool. Trials began at 8 am after an overnight fast of at least 10 hours and ran until 6 pm. Energy intake before trials was recorded and kept uniform between conditions, though not necessarily at the value required for energy balance. In both experimental conditions, subjects exercised for 60 minutes at 70%  $\text{VO}_{2\text{max}}$ . Exercise took place in the first hour of the session for the fasted condition and from hours four to five in the postprandial condition. There was also a no exercise control condition. In all conditions, a test meal was consumed 90 minutes into the

session. This meal represented 30% of the estimated daily energy requirement and consisted of 73% carbohydrate, 17.5% fat and 9.5% protein. Scheduled 30-minute *ad libitum* meals were provided 5.5 hours into the trials and again at 9.5 hours. Energy intake was also assessed after subjects left the laboratory through the rest of the day to provide a 24-hour diet window. Appetite was assessed with a VAS upon arrival and every 30 minutes throughout the trial. RMR testing was performed at baseline, after 2.25 hours, 3.25 hours, 6.25 hours, 7.25 hours, 8.25 hours, 9.25 hours and at the end of the trial at 10 hours. Each variable was compared between conditions at each time point of measurement.

### *Results*

Baseline VAS, REE and RER were not different between conditions. Exercise energy expenditure ( $776 \pm 101$  kcal fasted;  $773 \pm 104$  kcal fed) and RPE were also unaffected by condition. However, RER during exercise was  $0.91 \pm 0.03$  (31% energy from fat) which was lower than the fed exercise RER of  $0.93 \pm 0.03$  (22% energy from fat). Initial analysis revealed that fasted condition EE was greater than control after 4.25 hours, 4.5 hours, 6.25 hours, 7.25 hours and 8.25 hours along with during exercise. Fasted EE was also greater than fed EE at 2.25 hours (before fed exercise) and at 9.25 hours. Fed EE was only greater than fasted or control EE during fed exercise. After a Bonferroni correction, EE was only elevated between conditions while subjects were exercising. Area under the curve analysis showed that RER in both fasted and fed conditions was lower than the control from 5-10 hours. Furthermore, fasted RER was lower than the control RER for the full 10-hour trial. After a Bonferroni correction, fasted RER remained lower than the control for the full 10-hour trial but the difference between fed and control RER was mitigated.

Energy intake over 24 hours was  $3215 \pm 641$  kcal in the control condition,  $3236 \pm 570$  kcal in the fasted condition and  $3090 \pm 701$  kcal in the fed condition. The macronutrient ratios (carbohydrate/fat/protein) were 55/33/12 in the control, 55/33/12 in the fasted condition and 56/32/12 in the fed condition over 24 hours. There were no significant differences in these values. Energy intake was reduced during the 10-hour trial in both experimental conditions compared to baseline (22.6% lower in fasted, 27.9% in fed when EI adjusted for exercise EE; 33.7% lower in fasted, 38.4% lower in fed when IE adjusted for trial EE) but was not different between conditions. Seven subjects had higher energy intakes in the fasted condition than in the fed condition and the opposite was true for the remaining five subjects. When fasted energy intake exceeded fed energy intake there was a positive correlation with BMI and body mass. During fasted exercise, prospective food consumption was lower than in the control while satiety was higher. At 3.5 hours, satiety was lower in the fasted condition than fed condition while prospective food consumption was higher. From 4-5.5 hours, all measures of appetite significantly differed from both control and fasted conditions culminating in the realization that fed exercise more drastically suppressed appetite during this time. After the Bonferroni correction, 3.5-hour fed satiety was greater than fasted satiety (between meal and exercise in fed) and 4.5-hour hunger was lower in the fed condition than fasted (during fed exercise). Both conditions resulted in an energy deficit compared to control and this can be attributed to the reduced energy intake in the hours following exercise combined with the bonus energy expenditure from exercise.

### *Authors' Conclusion*

Deighton et al. found that overall 10-hour energy expenditure responses to 60 minutes of running at 70%  $VO_{2max}$  did not differ between fasted or fed conditions. Thus, the authors concluded that fed and fasted exercise are equally effective for creating an energy deficit due to the absence of a compensatory feeding response irrespective of appetite fluctuations. Appetite was comparatively lower in the fed condition but this difference did not coincide with any alteration in energy intake, macronutrient ratios or the magnitude of the induced energy deficit. This was speculated to be a byproduct of the short-lived nature of appetite modulation by exercise that would have expired before *ad libitum* feeding ensued. This also confirmed prior affirmations of exercise not dramatically affecting energy intake with a compensatory response to the extra energy expended.

### *Interpretation*

In accordance with several other studies, fasting did not increase subjective exertion or energy expenditure during exercise but did result in a lower RER. This is notable because of the disparate findings regarding RER in the hours following fasted exercise. Reduced RER during fasted exercise is not as highly contested as the existence of reduced RER after fasted exercise. The lower RER area under the curve value for the fasted condition over the full 10-hour trial was the most visible and clear result of the study. This information in isolation does not translate to anything meaningful for exercise prescription and thus depends on a better understanding of how fasted exercise affects REE. The inclusion of appetite and diet measures instantly elevate the descriptive validity of the study. Diet was controlled preceding trials to reduce obstruction of true metabolic modifications. More importantly, the



assessment of energy intake and appetite allowed the authors' conclusions to envelop the duality of the metabolic environment, specifically energy balance. Since there has been difficulty in establishing a change in REE following related interventions, the positive aspect of the energy balance equation is critically important to understand the entire metabolic process following fasted exercise. The conflict regarding REE is highlighted by this study in comparison to the study by Paoli et al. (Paoli et al., 2011). The outcome in this case was that REE initially appeared to be elevated after fasted exercise compared to after fed exercise which is antagonistic to the outcome of the other study. With a more stringent analysis, REE values became statistically indistinguishable. The absence of elevated REE after fed exercise was suggested as possibly being caused by the disparate duration between test meal and exercise from Paoli et al. Deighton et al. allowed 95 minutes more between the meal and exercise in the postprandial condition than did Paoli et al. Ultimately, disagreement in results has left a void in the knowledge of how REE responds to fasted exercise as opposed to fed exercise.

Despite a broader scope, Deighton et al. still left many intriguing potential questions unanswered. Though appetite was claimed to be reduced after fed exercise, the data does not appear to offer strong enough support to make this conclusion a certainty. All appetite factors were different between conditions for 90 minutes beginning at hour four, although this time period included the 60-minute fed exercise session. The only condition differences after correction were satiety just before fed exercise and hunger during fed exercise. This finding is not quite as compelling as it originally appears. The proper inference is that fed exercise results in transiently reduced appetite for a very brief period compared to fasted exercise. In this study, subjects did not have access to food during this time. Additionally, the first *ad*

*libitum* meal following exercise was 30 minutes after fasted exercise but 4.5 hours after fed exercise. If the fed condition had access to food within thirty minutes of exercise, perhaps the result would have been different.

The caloric content of the test meal was based on a calculated estimate of TEE which likely did not dramatically alter metabolic response but leaves questions regarding accuracy of the estimations of physical activity. The macronutrient ratio of the test meal seems more practical than the one used by Paoli et al. but the caloric value (30% of estimated TEE) wound up being similar. No definitive appraisal of the appropriateness of the test meal could be made without knowing the energy cost of exercise in the previous study but Deighton et al. reported exercise energy expenditure that slightly exceeded the energy value of the meal. It is worth emphasizing that the high energy cost of exercise in this study was probably due to the extended duration at an intensity at the higher end of the range for what is considered moderate. This is exemplified by the RER values exceeding 0.90 in both conditions. Thus, in this specific circumstance, a high-kilocalorie test meal is justified. Despite existing on the higher end of the spectrum of moderate intensity, the results for the selected intensity do not translate to high-intensity exercise or HIIE. More concerning is yet another failure to observe EPOC after exercise. After the Bonferroni correction, energy expenditure appeared to be elevated only during exercise. However, the initial analysis showed elevated REE in the fasted condition over the control suggesting that EPOC did occur. Fasted REE was higher than fed REE at 9.25 hours, near the end of the trial. Bonferroni corrections are relatively conservative so the results of the original analysis are worth examining in tandem. Interestingly, fed exercise did not yield an EPOC response, even in the initial analysis. EPOC would be expected to occur after this type of exercise but the bulk of it was likely missed due

to the first RMR test taking place over an hour after exercise in both conditions. The fact that EPOC was observed in the fasted condition suggests that fasted exercise may have a larger impact on the slower phase of EPOC.

A rational resolution to the presented data and conclusions is that moderate-intensity fed and fasted exercise equally stimulate energy deficits via reduced energy intake and potential EPOC in a 10-hour period following exercise. Appetite may have been altered to some degree but energy intake was not increased to compensate for appetite changes or exercise energy expenditure. These effects are displayed when a partial *ad libitum* diet is utilized. Only moderate-intensity exercise was examined. Consequently, no speculation on how this would all be affected if the intensity was categorized as high is possible. This is the ubiquitous omission of exercise and metabolism studies. The structure of the nutrition-exercise paradigm cannot be presumed until the potential differential impact of HIIE on post-exercise metabolism has been clarified.

### **Sevits et al. (2013)**

#### *Purpose*

While nutritional status of exercise is the clear primary element of interest in the previous two studies, the metabolic response to HIIE also implores study. Since EPOC has displayed an exponential relationship with exercise intensity, it is surprising that more research regarding HIIE and EPOC has not been undertaken. Irrespective of this void, two experiments performed by Sevits (Sevits et al., 2013) combined to elucidate this relationship. The expressed purposes of these studies were to highlight the presence, or absence, of HIIE effects on TEE and REE. Alterations in these variables would theoretically imply the

magnitude of EPOC. Exercise had previously been shown to increase both TEE and REE. HIIE has risen in popularity due to the reduced time commitment for busy potential exercise participants with similar adaptive benefits to continuous aerobic exercise training. Its effects on weight management are not well known but results of related investigations, although admittedly limited in number, suggest that the thermogenic response to HIIE may be of a sufficient degree to prevent weight regain after weight loss. These concepts are what led to the creation of the question and design.

### *Methods*

Sevits et al. conducted two separate experiments within the same conceptual framework. Subjects were 27 healthy males divided into exclusive subject pools. Study 1 examined 15 of the subjects over two trials. Both trials consisted of pairs of RMR tests on consecutive mornings. In one trial, the first morning RMR test was followed by the exercise protocol. The other trial did not include exercise and served as the basis for comparison. The HIIE protocol involved four Wingate sprints each dissected by a four-minute rest interval. The second RMR test was initiated 23 hours after exercise. RMR testing was done with a ventilated hood attachment on a metabolic cart and lasted for 45 minutes with 30 minutes of usable data output. Blood samples were extracted after every RMR test.

In Study 2, the remaining 12 subjects were situated in a room calorimeter for two consecutive days totaling 48 hours. Diet was prescribed for the three-day period preceding the trial. The diet was broken down into a 55/30/15 ratio of carbohydrate to protein to fat with a caloric value of 1.5 times the previously measured baseline REE. Diet during the trial days was the same except for the use of 1.4 as the multiplier for calculating required energy

intake. The same Wingate-derived HIIE protocol, as in Study 1, was performed during the second hour of one of the two days in randomized order. The sole difference in protocol from Study 1 was the addition of a fifth Wingate sprint interval to increase the stimulus due to reservation about the degree of stimulus in Study 1. Activity energy expenditure during the trial was carefully controlled and since energy intake and macronutrient ratios were equivalent between trials, TEF was assumed to not have any impact. In addition to the TEE measured by the calorimeter, urine samples were collected. For the exercise day, the resultant 23-hour data were extrapolated to a 24-hour value for comparison to the control day.

### *Results*

In simple terms, HIIE appeared to alter 24-hour cumulative TEE without affecting next-day REE. In Study 1, the 24-hour post-HIIE RMR test results did not differ from values at the equivalent time point in the control trial for all the main variables. REE was  $1718 \pm 68$  kcal·day<sup>-1</sup> after HIIE as opposed to  $1668 \pm 59$  kcal·day<sup>-1</sup>. RER was  $0.85 \pm 0.01$  for both conditions. Serum glucose after HIIE was  $5.04 \pm 0.10$  mmol·L<sup>-1</sup> compared to  $4.98 \pm 0.12$  mmol·L<sup>-1</sup>. The results from Study 2 were more striking.

HIIE resulted in increased 24-hour TEE above control in every subject in Study 2. The mean difference was  $226 \pm 15$  kcal ( $2415 \pm 62$  kcal -  $2190 \pm 58$  kcal) which represented a 10% increase in TEE after HIIE. Analysis of individual time points revealed that TEE and RER after HIIE were greater than control only from the three-hour 8 am to 12 pm interval. In this interval, TEE was  $441 \pm 37$  kcal in the HIIE condition and  $303 \pm 9$  kcal in the control condition. In the same timeframe, RER was  $0.91 \pm 0.01$  in the HIIE condition and  $0.81 \pm 0.01$  in the control condition. Energy intake was equivalent between trial days ( $2338 \pm 73$

kcal vs  $2347 \pm 69$  kcal). Considering energy intake and energy expenditure, energy balance was calculated and naturally proved to significantly different between conditions with a negative value ( $-78 \pm 44$  kcal) after HIIE and a positive value ( $158 \pm 44$  kcal) during the control day. Macronutrient balance was calculated as the difference between intake and oxidation for all three macronutrients. Protein balance was  $+13.3 \pm 4.1$  g·day<sup>-1</sup> in the HIIE condition and  $+12.4 \pm 4.4$  g·day<sup>-1</sup> in the control. Carbohydrate balance, with fiber intake removed from intake, was  $+38.5 \pm 12.8$  g·day<sup>-1</sup> in the HIIE condition and  $+65.6 \pm 16.8$  g·day<sup>-1</sup> in the control. Fat balance was  $-20.6 \pm 8.2$  g·day<sup>-1</sup> in the HIIE condition and  $-9.7 \pm 7.9$  g·day<sup>-1</sup> in the control. None of these differences reached statistical significance. HIIE did not differentially affect catecholamine concentration.

#### *Authors' Conclusion*

Study 2 observed augmentation of 24-hour TEE by HIIE. This observation occurred despite the result in the concurrent study that showed no changes in REE measured 23 hours after the same exercise protocol. The magnitude of the drop in energy balance after HIIE from the second study sufficiently eclipses the 50-150 kcal deficit threshold to prevent weight gain in most people. The authors consequently concluded that HIIE is a viable method for weight management programs due to the effects on recovery metabolism. Furthermore, they conceded that the metabolic recovery pattern that functions to reclaim energy homeostasis likely culminated in a balanced state within 23 hours and before the REE measurement. HIIE indeed seems to increase daily energy expenditure but most of this effect is witnessed during and within the first few hours of exercise. RER appears to follow a similar pattern but overall substrate oxidation and balance are unaffected by HIIE.

### *Interpretation*

The findings of the two studies by Sevits et al. wound up being somewhat conflicting but not as contradictory as they initially appeared. HIIE clearly affected TEE in Study 2 but had no significant impact on REE in Study 1. However, the nature of the design in Study 1 reduces the validity of the potential claim that HIIE does not affect REE. The authors themselves qualified their conclusive statement by stating that this observation applies to REE measured 23 hours after exercise. Based on the results of Study 2 it would be reasonable to conclude that HIIE would actually elevate REE. Although Study 2 measured TEE, physical activity energy expenditure was tightly controlled and dietary equivalency between conditions theoretically mitigated any potential impact of TEF on TEE. Thus, changes in TEE could be attributed to changes in REE. Both studies ostensibly measured the same thing but with differing labels. Oxygen consumption was not reported but it can be assumed to match the pattern of REE changes, as has often been done throughout the literature. To isolate the effects' relevance to weight management, energy balance was not controlled in Study 2 during the trial while operating under the previously established observation that exercise does not stimulate a compensatory feeding response to elevated energy expenditure. This allowed for interpretation of the impact on energy balance. However, a design including unrestricted feeding would provide a more externally valid conclusion regarding energy balance.

Several design options limit the utility of applying the results universally. Most of these were seen in Study 1 and therefore support the findings of Study 2. Most notably, the 23-hour delay before measuring REE in Study 1 eliminated potential findings within the first few hours of exercise. Not coincidentally, this early post-exercise time period was where elevated

TEE was observed in Study 2. Study 1 also neglected to account for dietary behavior preceding and during the trial. This could possibly have had a drastic impact on the results especially if food was consumed in close proximity to the measurement. Metabolic alterations dissipating within 24 hours was assumed for Study 2 in cases where subjects performed the HIIE condition during the first day. While this is cohesive with the results from the first study, multiple other investigations have observed metabolic effects that last for 24 hours after exercise. The lack of continuity in exercise protocols between the studies could feasibly affect the results but this is a minor limitation. Finally, there was no condition that utilized moderate-intensity continuous exercise. This leaves a knowledge void in how the two methods directly compare which is important because both have displayed the capability of manipulating REE and EPOC in the hours following exercise.

### **Narrative 1 Summary**

Collectively, these three studies created a vector for exploration of the metabolic effects of fasted HIIE. Despite this, they leave many questions left unanswered. The failure of the exercise protocols to elicit meaningful EPOC was distressing and highlights both the limitations and clear future directions sprouting from these interpretations. Posing the question of how fasted exercise differentially affects metabolism from fed exercise is a valuable addition to the nutrition-exercise interaction paradigm. While the results presented here are varying, the relation to other EPOC studies weakens the conclusions. Operating under the assumption of being able to establish a measurable EPOC effect, observing effects of fasted versus fed exercise is a tremendously interesting and potentially enlightening topic of study.



Comparing HIIE to moderate-intensity continuous exercise would be ideal but would complicate the research question in a study of fasted versus fed exercise. The selection of HIIE as the exercise protocol in this type of study would be novel and thus the comparison of intensities would logically be reserved for future study. Energy balance is another interesting factor to consider in tandem with the nutritional environment surrounding exercise. This is why allowing *ad libitum* feeding in the exercise period would yield a better picture of the inclusive metabolic response to exercise. This real world application is a natural progression from the path tread by these studies. More specifically, any food provided during trials should be carefully implemented into a logical dietary protocol. A test meal before or after exercise would ideally approximately equate to exercise energy expenditure. These secondary factors are an important part of the encompassing question and are thus worth including in a related study design. Ultimately, these factors, along with the primary aims and purpose of these studies, should be assessed in combination in a testing environment that is valid on both external and internal levels.

### *Narrative 2: Pilot*

#### **Perez et al., 2014**

##### *Purpose and Inspiration*

The current proposed study will expand on the results of a previous pilot study where REE and RER were assessed in the hours after fasted or fed HIIE (Perez & Rynders, 2014). This previous study drew inspiration from a few related findings and the discovery that the topic was relatively unexplored. The most impactful of these foundational studies were detailed in the previous section. Their results inspired the idea and helped drive the

hypotheses while recognition of their limitations contributed to the pilot study's design. Their findings highlighted the need to more meticulously examine various aspects of fasted exercise, including differences among time points along the EPOC spectrum.

Considering the strong evidence that EPOC is intensity-dependent, HIIE was selected to maximize potential metabolic response to the exercise bout. A dearth of general HIIE-focused research was also revealed so this study could potentially add novel information into the realm. The curiosity regarding fasted exercise arose due to previous findings that fasted exercise favors lipolysis and fat oxidation more so than fed or postprandial exercise (De Bock et al., 2007; De Bock et al., 2005; Van Proeyen et al., 2011). The results of the study by Deighton et al. (Deighton et al., 2012) implied a possible multiple-hour time course for EPOC after fasted exercise that exceeds fed exercise. However, this finding was far from substantial and the ultimate conclusion was that energy expenditure was unaffected by the nutritional state of exercise. The primary basis for the study was the investigation by Paoli et al. (Paoli et al., 2011) that found elevated oxygen consumption and reduced RER 12 hours after fed exercise compared to fasted exercise. Thus, the findings of Paoli et al. became the foundation for the hypothesis that fed exercise would result in higher REE and lower RER compared to fasted exercise. To better scrutinize the effects of fasted exercise, the test meal was lower in energy content and fat percentage than the one used by Paoli et al. The choice of a delayed measurement of REE was intended to encapsulate a full day's metabolic response to exercise and was backed up by multiple studies, previously discussed in this review, that showed an EPOC effect lasting at least 12 hours. HIIE was selected to expand upon the results of Deighton et al. and Paoli et al. who had both used moderate-intensity continuous exercise. The primary aim of the study was to determine if the nutritional state of exercise

had a differential effect on metabolic response throughout the rest of the day following high-intensity exercise.

### *Methods*

Six females and four males who were healthy and recreationally healthy completed both a fasted and fed exercise trial in a repeated measures design with order randomized. One of the male subjects was excluded from the analysis due his status as an improbable outlier with an increase in REE in one condition that was more than 10% greater than the maximum expected increase. Subjects recorded dietary intake for three days preceding each trial before arriving for the trial without breaking the overnight fast. The exercise protocol was completed before consuming the test meal in the fasted condition and after the meal in the fed condition. The protocol consisted of 10 one-minute work intervals at a workload designed to elicit 100%  $\text{VO}_{2\text{max}}$  separated by recovery intervals at one-third of this workload. The test meal was a 240 kcal energy bar whose carbohydrate content was 75% of the total energy provided. Subjects returned 12 and 24 hours after exercise for RMR tests that were compared to a baseline measure recorded before each trial began. Dietary intake was again recorded during this 24-hour period.

### *Results*

Macronutrient breakdown of the pre-trial diet was 46% carbohydrate, 34% fat and 20% protein. Energy intake was not reported due to evident under-reporting. Recorded energy intake was 66-72% lower than estimated requirements. However, subjects were deemed to not have been in energy deficit due to the proximity of measured REE and predicted REE. A ratio of less than 0.90 of measured to predicted REE would imply an energy deficient state

but all subjects fell above this cutoff point. Aspects of exercise performance, including heart rate and perceived exertion, did not differ between conditions. In all nine subjects, fasted exercise resulted in elevated REE at 12 hours compared to baseline. REE was  $1707 \pm 287$  kcal·day<sup>-1</sup> 12 hours after fasted exercise and  $1559 \pm 285$  kcal·day<sup>-1</sup> before fasted exercise which was a 9.8% difference worth 148 kcal. REE 24 hours after fasted exercise was not significantly different from REE before fasted exercise. REE in the fed exercise condition was not altered over time (baseline REE =  $1585 \pm 388$  kcal·day<sup>-1</sup>, 12-hour REE =  $1632 \pm 379$  kcal·day<sup>-1</sup>, 24-hour REE =  $1626 \pm 391$  kcal·day<sup>-1</sup>). There were no differences between RER values from baseline to 12 or 24 hours after exercise in either condition.

#### *Discussion and Conclusions*

The conclusion drawn from the pilot data was that fasted exercise increased 12-hour REE whereas fed exercise did not. This outcome applied only to the slow component of EPOC and deterred speculation on what transpired during the fast component of EPOC. The REE measurement served as a proxy for VO<sub>2</sub> behavior and thus these data were not reported. The elevated REE had dissipated by 24 hours, suggesting the effective window for metabolic alterations after HIIE was contained within a given 24-hour period.

The results aligned with the assertion that HIIE affects factors contributing to the slow component of EPOC such as elevated body temperature and serum catecholamine concentration. While HIIE effects on metabolism have yet to be conclusively distinguished from those of moderate-intensity continuous exercise, it is obvious that there is something worth investigating here and provides a clear question for future studies. How does HIIE directly compare to other exercise strategies regarding metabolic response?

The 12-hour REE in the fasted condition represented an additional 148 kcal of energy expenditure. REE has proven to be elevated in the afternoon compared to the morning by about 90 kcal without the influence of exercise (43). Therefore, the actual REE increase due to HIIE was estimated to be closer to 50 kcal assuming a conservative estimate of a 100 kcal increase due to time of day. This is consistent with findings regarding the energy value of post-exercise REE relative to exercise energy expenditure. This value of 50 kcal is 18% of the 275 estimated caloric cost of exercise. Results were also agreeable with findings that an EPOC effect last for multiple hours (12-14 hours) after exercise. Alternatively, some studies have shown a much shorter EPOC window such as 4 hours following exercise in the study by Sevits (Sevits et al., 2013). These varying results can be attributed to differing methods of metabolic evaluation, variability in diet control and prescription as well as considerations of external validity. The short-term window was not examined in this pilot study.

Some authors have suggested that fasted exercise can enhance weight and fat loss. In this study, REE was indeed altered but without a concomitant alteration of RER. This implies that energy expenditure is a more important determinant of weight loss than RER. The physiological mechanisms of how fasted versus fed exercise affect chronic metabolism and weight control are still unknown. Previously, exercise displayed no effect on fat balance when subjects were in energy balance (Melanson et al., 2009). The reduction in RER after fed exercise in the Paoli et al. study may have been due to the consumption of a high-fat test meal in only the fed exercise condition, artificially reducing RER values.

Interestingly, fed or postprandial exercise may be more advantageous for weight management with a long-term outlook despite the absence of REE effects. This would be due to appetite regulation. Exercise after a high-fat meal suppresses subsequent EI (Cheng et al.,

2009). This is notably similar to the Paoli study (Paoli et al., 2011) and may aid in explaining those results. The fed-exercise appetite reduction was confirmed by Deighton (Deighton et al., 2012) but with no observed difference in EI. Discerning whether the appetite and energy intake effects are material is therefore murky territory. This pilot study was unable to assess this area due to the reporting errors in diet tracking. It seems reasonable to infer that appetite suppression may have occurred in the fed exercise condition and consequently affected the 12-hour REE measurement. Considering the available information and results, nutritional state of exercise was concluded to affect energy flux rather than REE or EI independently. The balance of these two factors must be considered in tandem for future studies.

### **Part 3 - Application and Relevance**

#### *Intermittent Fasting for Health*

Intermittent fasting is a dietary strategy that has experienced a recent spike in popularity. Intermittent fasting utilizes periods of fasting broken up by shorter periods of feeding either within a one-day period or over multiple days with longer fasts. The concept has diffused into the health and fitness cultural paradigm due to diet books and public discourse. Fasting has long been used in medicine and religion, such as during Ramadan, for specific purposes but only recently has garnered attention as a deliberate nutritional strategy. Several books have become top sellers by purporting that fasting-based diets can improve health and body composition. One of the most notable of these works authored by Zinczenko (Zinczenko & Moore, 2013) recommends a daily feeding window restricted to eight hours, which has become standard procedure for an intermittent fasting diet. A review by Martin (Martin et al., 2006) concluded that intermittent fasting mimics feeding patterns of human ancestors that

lived as hunter-gatherers and may result in a modern effect of increasing both lifespan and persistence of good health. This is related to the "thrifty gene" hypothesis originally developed by Neel in 1962 (Neel, 1962) stating that genes that resulted in efficient energy storage during food availability were selected. This gene would allow better survival rates during times of famine (Wendorf & Goldfine, 1991).

Intermittent fasting protocols can improve stress resistance (Mattson, 2008), enhance fat oxidation rate (Heilbronn et al., 2005) and improve metabolic markers of health independent of dietary composition (Klempel et al., 2013). Longo and Mattson (Longo & Mattson, 2014) stated that intermittent fasting antagonizes obesity, hypertension, asthma and rheumatoid arthritis in humans. Harvie (Harvie et al., 2013) recently suggested that intermittent fasting may be a superior dietary manipulation to simple caloric restriction for improving insulin sensitivity even though fat metabolism and weight loss are equal between approaches. This suggests that intermittent fasting may provide a slight, but important, edge for long term health-related goals. Competent implementation of this protocol is likely not harmful and may provide significant health benefits. Results of intermittent fasting studies are promising but collectively inconclusive. This makes the topic worthy of investigation, particularly in the physiological background of fasting itself.

Most diet programs include concurrent exercise recommendations and intermittent fasting is no exception. It is well established that diet and exercise are more effective when combined than when utilized alone, especially regarding metabolic adaptation. A major problem with intermittent fasting is the dearth of research focusing on the physiological effects of acute exercise performed in a fasted state compared to a fed state. This makes prescribing exercise for an individual undertaking an intermittent fasting regimen inherently

precarious. The results would be applicable to exercise prescriptions enveloped by a fasting protocol. In this scenario, the fast-breaking meal is often consumed after exercise meaning that exercise is performed in a fasted state regularly. Assuming intermittent fasting proves to be a useful tool in the global struggle against obesity, knowing how to provide exercise that fits in to the program and causes no harm is an obvious necessity.

#### **Part 4 - Summary of Literature**

##### *Summary of Previous Research*

The phenomenon called EPOC is the primary way of quantifying the metabolic response to exercise in the hours following. It is modulated by exercise duration and, to a greater extent, intensity. This may also extend to HIIE but results regarding differences between HIIE and traditional exercise on EPOC are unclear. The duration of EPOC is contested but has been observed to last up to 12 to 14 hours on multiple occasions. This can be explained by discernible phases of the EPOC cycle. Males and females experience different metabolic responses to exercise but these variations are generally minor and can be controlled fairly simply.

Counter intuitively, exercise does not stimulate compensatory overfeeding. In fact, it may even reduce appetite and inhibit the overfeeding response to low-energy diets. HIIE may have an even more dramatic negative effect on appetite. There is no consensus on how fasting affects metabolism with conflicting results across the board. Some studies have found that fasting does not affect energy expenditure but does impact fat metabolism. Fasting may reduce energy expenditure to maintain energy balance.



Few studies have examined the metabolic response to fasted exercise. Several of these studies turned up no results for EPOC and energy expenditure while others found a catalytic impact on fat metabolism. It has been observed that fed exercise results in elevated energy expenditure and fat metabolism compared to fasted exercise. It has also been concluded that fasted and fed exercise do not differentially impact metabolism but that fed exercise may possess a dampening effect on appetite. There are also findings that reveal that fasted exercise may actually accelerate metabolism without affecting fat metabolism. Even if fasted exercise proves to have a more meaningful impact on energy expenditure, the long-term benefit of appetite control with fed exercise may prove to be more advantageous for weight management. Since results are inconclusive, this is all speculative at this point. The inherent contradiction in the current state of the literature demands further investigation on the differential impact of fasted exercise compared to fed exercise on recovery metabolism.

### *Design Influences*

Limitations in the design of the pilot and hypothesis-founding studies led to many of the design-related decisions of the present study. The pilot study, in particular, had the largest influence on the currently proposed design. Most importantly, the unrestricted pre-trial feeding led to a disparity in kilocalories consumed between conditions which guided the conclusion towards a more inclusive interpretation regarding energy flux rather than absolute effects on REE and energy balance. Energy intake was subsequently deemed to be under-reported due to subjects' REE closely matching predicted values as opposed to reaching the reduced values that would be expected with abnormally low energy intake. The major distinction between this investigation and the pilot study is the impending rigid control of

pre-trial energy intake and diet composition to ensure subjects begin each trial in an energy-balanced state. This alteration will confidently permit a conclusion regarding absolute effects of fasted versus fed exercise on accompanying acute metabolic action and behavioral feeding responses. This was impossible in the pilot study due to energy intake being removed from consideration for the analysis. The pilot study did not employ appetite measures necessitating the addition of these variables, through the VAS, to account for behavioral contributions to the observations. Allowance of *ad libitum* feeding following exercise will be retained to answer a specific question. Menstrual phase was not controlled for in female subjects but the impact was ostensibly minimized by restricting the trial window to a two-week period. This will be more tightly controlled in the proposed study. Since females have somewhat different fat metabolism properties than males, it would be worth including a distinct analysis between sexes or implement sex as a variable to control for in potential analyses.

Relative to the model studies for the pilot work, several changes were made to narrow the focus. The major deviations from the work of Paoli (Paoli et al., 2011) include applying HIIE in addition to a test meal with a higher ratio of carbohydrates to total kcal. The groundwork on metabolic response to HIIE laid down by Sevits (Sevits et al., 2013) was expanded upon by examining causative effects of nutritional state on this metabolic response. The proposed design will aim to build of this foundation by elevating the applicability of the results. This will be done by accounting for, and controlling, energy balance of each subject entering the trial. Additionally, the fast component of EPOC will be observed, which many studies have elected not to do. This will create a broader view of how fasted exercise affects metabolism.

Timing of study events will be more uniform to minimize the consequences of confounding factors. The 24-hour RMR measurement will be replaced by the immediate

post-exercise measurement due, in part, to the fairly well-established principle of EPOC expiring within a 24-hour window. The earlier measurement will allow analysis of the early phase of EPOC which was neglected in the previous study while maintaining the examination of delayed effects. Finally, use of a treadmill for the exercise protocol generated small inconsistencies in interval spacing due to latency in treadmill response to manual command input. The preferential selection of an electronic cycle ergometer resolves this concern.

## **CHAPTER III**

### **METHODOLOGY**

#### **Subjects**

Subjects were recruited from the local college student pool ranging in age from 20-25 years. Subjects were healthy males and females. Females were not excluded despite the possibility of differing phases of the menstrual cycle working as confounding factors for metabolic rate. These sex differences were accounted for in the design of the study. Each female subject performed all laboratory visits in the luteal phase, or all in the follicular phase, of menses. The subjects were stratified as low-risk for cardiovascular events in accordance with ACSM guidelines (ACSM, 2013) and reported no signs of cardiovascular disease or thyroid disorder. Smokers were excluded from the study and users of caffeine and alcohol were asked to refrain from ingesting them for 20 hours before and during the trials. This allowed caffeine users a morning consumption period the day before without extending the maximum end of the half-life range into the trial day. Supplements that have a known effect on metabolism were discontinued before participating. Subjects were excluded if they had undergone a major change in diet or weight within the previous two months. A major diet change was defined as an intentional increase or decrease in energy consumed or large-scale shift in macronutrient consumption ratio for the purpose of altering body mass. A major weight change was determined to be a loss or gain of 5% of the initial body mass.

#### **Variables**

Descriptive data included height (cm), body mass (kg), lean body mass (kg), age (years), sex, and body composition (% fat). Three consecutive log periods preceding the trials provided additional descriptive data. These data were constructed of normal energy intake

(kcal), normal macronutrient ratios (% of total calories), normal accelerometer-derived paEE (kcal), CEB (kcal), absolute discrepancy from CEB in normal diet (kcal), and pre-trial prescribed energy intake (kcal). Two separate trial-day food log periods yielded actual pre-trial energy intake (kcal), actual pre-trial CEB (kcal) and absolute discrepancy from CEB in pre-trial diet (kcal).

The independent variable was the nutritional state of exercise. This two-level variable was divided into fasted-state exercise and fed-state exercise. The primary outcome measures and dependent variables were relative and absolute EPOC ( $\text{mL}\cdot\text{kg}^{-1}$  and L, respectively) immediately following exercise as well as 12 hours after exercise. This was derived from oxygen consumption ( $\text{mL}\cdot\text{min}^{-1}\cdot\text{kg}^{-1}$ ) and RER. MT provided an expanded view on metabolic activity by providing data for the immediate post-exercise recovery energy expenditure (kcal) associated with EPOC, 12-hour recovery resting energy expenditure ( $\text{kcal}\cdot\text{day}^{-1}$ ) and respiratory exchange ratio both immediately after exercise and 12 hours later. Thus, in addition to EPOC, both substrate utilization and extrapolated daily energy expenditure from discrete time points was appraised. Appetite scores from the VAS questions (mm) were also assessed as an outcome of interest with four time points each over two trials. The aspects of appetite documented were perceptions of hunger, satiety, fullness, prospective food consumption (amount of food an individual could consume) and specific food preferences. A composite appetite score (mm) derived from the above factors, excluding food preferences, was also utilized. Data for these variables were acquired at multiple time points including baseline, before exercise, one hour following exercise and 12 hours after exercise. Other dependent variables of interest included *ad libitum* energy intake in the 12 hours following trials, energy cost of HIIE (kcal) and heart rate response to exercise

(beats·min<sup>-1</sup>). Maximal exercise test-specific variables were maximal oxygen consumption (mL·min<sup>-1</sup>·kg<sup>-1</sup>), maximal aerobic power output (W), maximum heart rate (bpm) and the energy cost of maximal exercise (kcal).

## **Materials**

### *Metabolic Measurement*

Maximal oxygen consumption (VO<sub>2max</sub>) measured via a maximal exercise test, utilized a metabolic assessment cart (ParvoMedics TrueOne 2400, Sandy, UT) for open circuit spirometry. This system measures ventilation and gas concentration in the exhaled air (i.e., oxygen and carbon dioxide). The flowmeter unit was calibrated with a 3-L syringe and the gas analyzers were calibrated by measuring from a standard gas canister equal to expected values for ventilated oxygen and carbon dioxide during exercise. Subjects inspired ambient air and expired air through a facemask connected to a hose leading to the cart. The mask contains valves that open to allow passage of external air but close during expiration. After passing through the Rudolph valve, ventilated air enters through a pneumotach to record flow data derived from the observed pressure gradient. Gases then pass through a mixing chamber to retain measurement accuracy with dynamic changes in ventilation. Gases are analyzed with paramagnetic oxygen analyzers and infrared carbon dioxide analyzers. The metabolic software presents primary results as absolute and relative oxygen uptake and carbon dioxide production. Ventilation rate, respiratory exchange ratio and other factors are also displayed.

The metabolic cart used for VO<sub>2max</sub> testing was also used for MT enlisting similar but unique software along with some differing hardware. A different tank is used for standard gas calibration to represent the values of ventilated gas ratios expected in a resting state.

Instead of a mouthpiece, for RMR tests, subjects are fitted with a canopy that covers the head and torso from the sternum up. For the RVT, EPOC can be measured with the same mask used during exercise testing to allow more subject mobility. Both of these implements create an ostensibly airtight seal. The other major difference is the presence of a dilution pump to ensure steady carbon dioxide output. Energy expenditure is calculated by inserting oxygen and carbon dioxide values into the modified Weir equation (Weir, 1949).

### *Exercise Equipment*

Exercise was performed on a Velotron cycle ergometer (RacerMate, Seattle, WA). This cycle allows for strict control of power output with a copper flywheel that auto-regulates resistance in accordance with the user's velocity using a set of internal magnets. Adjustable handlebars and seat allow for a wide array of body frames to use the cycle. The ergometer is electronically powered and is controlled manually by an operator through Velotron software that displays instantaneous velocity, average velocity, rpm and power output. The desired power output was achieved by programming a value for watt step increases; that is, the operator could increase or decrease the wattage instantaneously in either the positive or negative direction by the magnitude of the programmed watt value.

During exercise, heart rate was monitored using Polar heart rate monitors (Polar Electro, Kempele, Finland). The transmitter was attached to a chest strap that went directly over the xiphoid process of the sternum. It gathered electrical frequency data and transmitted the results to an external receiver in the form of a wrist watch.

### *Body Composition*

Body composition can be determined from air plethysmography with reasonable accuracy. This was the method employed for this study. The standard tool for this procedure is the BodPod (COSMED, Rome, Italy). Two distinct chambers comprise the unit. One is larger and contains a seat for the subject to sit in. It contains a valve through which air is pumped in during the measurement. The volume of air displaced by the body passes through another valve into a second chamber in the back of the unit where it can be measured. The computer software extracts the body volume value and combined with a measurement of mass from the unit's scale, can calculate body density. Density values are inserted into the Siri equation (Siri, 1961) for body fat percentage. Thoracic volume is measured through a hose in the chamber. Daily machine calibrations include hardware, volume and scale checks.

### *Appetite Assessment*

Appetite was assessed with a VAS consisting of a series of eight appetite-related questions previously developed by Flint (Flint et al., 2000). VAS questions assess appetite with questions regarding hunger, satiety or fullness, prospective food consumption and specific food preferences. The questions are as follows:

1. How hungry do you feel? (I am not hungry at all - I have never been more hungry)
2. How satisfied do you feel? (I am completely empty - I cannot eat another bite)
3. How full do you feel? (Not full at all - Totally full)
4. How much do you think you can eat? (Nothing at all - A lot)
5. Would you like to eat something sweet? (Yes, very much - No, not at all)
6. Would you like to eat something salty? (Yes, very much - No, not at all)



7. Would you like to eat something savory? (Yes, very much - No, not at all)
8. Would you like to eat something fatty? (Yes, very much - No, not at all)

Each question is accompanied by a line that visually represents a continuous scale of possible responses. Lines are presented horizontally and stretch 100 mm with a start point value of zero mm and bookended by cues to explain that marks closer to the ends represent stronger feelings in either the positive or negative direction since the exact center is considered neutral. Subjects must use a marker to draw a straight line at the point they feel best represents their current state. Responses are measured in millimeters. All eight questions appear on one page in a vertical list.

#### *Activity Energy Expenditure (paEE)*

Hip-worn tri-axial accelerometers (Actigraph wGT3X-BT, Pensacola, FL) recorded three-dimensional kinematic data during each food log period. Each accelerometer is encased in a hard plastic shell and lies in a pouch that keeps it oriented properly. The accelerometer pouch attached to a strap to fit tightly around the hip and was properly oriented to ensure directional accuracy. They were only removed before sleeping and in situations where the device may have come in contact with water. Accelerometers measure movement as accelerations accounting for g-force values. Mechanical displacement is converted into an electrical signal that is stored on a chip in the device. The raw data was ultimately converted into energy expenditure data. This yielded a net value for energy expended due to physical activity alone.

### *Diet Tracking*

Subjects were instructed to keep a detailed log of dietary intake for three days after the initial baseline visit and the day before each trial. This was accomplished through utilization of a diet tracking software program available online and through a Smartphone application (MyFitnessPal, San Francisco, CA). This tool is designed to be conveniently utilized thus making the dietary guidelines of the study more easily adhered to. Diet tracking with an electronic device gives an accurate representation of calories and macronutrients consumed (Beasley et al., 2005). MyFitnessPal, in particular, has been highly rated based on content (Breton et al., 2011). The software allows accurate input of food intake with simple search tools and bar code scanning. Mobile access capability further increases the likelihood of accurate self-reporting. The program then produced a dietary composition report consisting of kilocalories consumed during the day as well as the distribution of energy intake into macronutrient ratios. These data were used in tandem with measured resting and activity energy expenditure to ascertain the subject's proximity to an energy-balanced state. Pre-trial diet was more prescriptive but the same recording mechanics applied.

### *Test Meal*

The test-day meal consisted of one 240-kilocalorie energy bar (Powerbar, Inc. Performance Energy Bar, Glendale, CA). The number of kilocalories consumed was comparable to a typical pre-exercise meal but smaller than a normal breakfast meal. The macronutrient breakdown relative to the caloric content roughly equated the substrate utilization of the HIIE session. The high carbohydrate concentration (75% of total kcal) represented the shift of substrate utilization during intense exercise to primarily

carbohydrates and expectedly countered the hypoglycemic effects of fasting. Protein (13%) and fat (12%) accounted for the remaining 25% of the caloric value.

## **Procedure**

### *Design*

Subjects completed each phase of the study in a repeated measures design. Variables of interest were examined under the two levels of the independent variable (fasted or fed exercise). Condition differences in changes from pre-exercise to post-exercise measurements due to the nutritional state of exercise were analyzed. In the first visit to the laboratory, baseline testing included measures of mass, height, body composition, VAS, RMR testing and  $VO_{2max}$ . Subjects used the MyFitnessPal software and record intake for three consecutive days beginning the day of the preliminary visit. There was another one-day food log period before each trial. For each trial, subjects completed the following (in order): VAS assessment, RMR measurement, exercise, post-exercise RVT, post-exercise VAS, *ad libitum* feeding during the subsequent food log period and a 12-hour post-exercise RMR test. The test meal was consumed either before exercise or after the post-exercise VAS.

### *Baseline Visit (day 1)*

During the first visit to the laboratory, subjects were risk stratified and classified as low-risk according to current ACSM guidelines. Following risk stratification, subjects had the procedures, risks and benefits of the study explained to them and were asked to sign the informed consent document. Procedures outlined on these forms, and used in the study, were approved by the Old Dominion University Institutional Review Board. Baseline measures of body mass, height, body composition, VAS (familiarization), RMR testing and  $VO_{2max}$  were

obtained in a post-absorptive state. Finally, subjects were instructed on how to use the MyFitnessPal software and to record intake for three consecutive days beginning the day after the preliminary visit, if possible. These data were collected upon completion.

### **Body Composition**

Body composition was determined with air plethysmography using the BodPod. After an extended warm-up period and a series of calibrations, descriptive information was entered to include subject ID code, height, age, sex and ethnicity. The selected model for body composition was the Siri equation for most subjects with the exception of using the Ortiz model for those of African descent (Ortiz et al., 1992). Thoracic volume was measured as opposed to estimated. Subjects wore approved clothing including the head cap to cover hair and tight-fitting or compression shorts for males and swimsuits or a sports bra and tight-fitting shorts for females. A quick volume calibration preceded the actual measurement. After the calibration, the subjects stood on the BodPod scale and remained still for a few moments for an accurate measurement of body mass. After stepping off the scale, subjects sat in the airtight chamber and were asked to remain still once the BodPod was sealed. There were two separate body volume measurements. The last step was the thoracic volume measurement. Subjects performed complete, relaxed breaths through a tube in the chamber in cadence with a visual metronome. After three gentle huff breaths at the end to induce a small pressure fluctuation, the software calculated thoracic volume and considered it for the body fat equation. Once body volume was assessed, density could be determined by dividing body mass by the volume result. The density value was then inserted either the Siri or the Ortiz

equation to calculate body fat percentage. Results included body mass, lean mass, fat mass, lean mass percent and fat mass percent. These data points functioned as descriptive markers.

### **Visual Analog Scales**

Familiarization with the VAS scales occurred after the body composition measurement. The meaning of the questions and methods for answering was explained before the subject performed the full protocol for practice. As each question was answered, the answer was covered by a sheet of paper so previous answers did not influence the rest. Once completed, the distance of the mark from the start point of the line was carefully measured and recorded in millimeters for every response. This procedure was repeated for every subsequent VAS test.

### **Resting Metabolic Rate Test**

In the hours before an RMR measurement, there are several potential confounding factors to consider. These are detailed by Compher (Compher et al., 2006). Subjects should avoid ingesting food, caffeine, alcohol and nicotine along with avoiding vigorous physical activity. Subjects were asked to refrain from eating for two hours prior to evening RMR tests. All morning RMR tests followed a fast of much longer than two hours. Caffeine, nicotine and alcohol were not to be consumed within 20 hours of an RMR test or at all the day of the trial. Exercise was not permitted for a full day before trials. A 20 min rest period antecedent to the measurement was assumed be enough to mitigate interference from metabolic activity due to activities of daily living. The duration of meaningful data recording will be sufficient for accuracy in REE determination. These general testing qualifications have been confirmed by Compher et al. Prior to the measurement, the dilution pump was attached to the mixing

chamber, but remained off, and calibrations for the flowmeter and standard gas commenced. Subjects rested in a supine position for 20 minutes without falling asleep. This was assumed to be enough to yield a truly rested state. This period proceeded in a darkened and quiet laboratory. No ambient noise was permitted. These environmental conditions were maintained through the test. The dilution pump was switched on and a clear canopy was then draped and secured over the torso and subjects were reminded to continue resting but to stay awake and avoid closing their eyes. The flow rate of the dilution pump was adjusted by the operator within the first five minutes of the test to achieve 1.10% carbon dioxide. Expired gases were collected for 20 minutes and values were inserted in a modified Weir equation to estimate resting metabolic rate. The first five minutes were excluded from the analysis. Subsequent five-minute intervals could be excluded in series at the discretion of the test operator because only the final 15 minutes are considered for the final estimation. This was possible because the test does not conclude without manual input from the tester. RER values were used in tandem with REE results to determine fat oxidation rate.

### **Maximal Oxygen Consumption**

The maximal exercise test followed the RMR test and utilized the same metabolic cart. To transition, the dilution pump was removed from the mixing chamber. After this, the flowmeter and standard gas calibrations were repeated. The mouthpiece was connected to the hose and fixed securely around each subject's head. The protocol involved cycling on an electronically operated Velotron cycle. Cycling began at 25 watts (W) then was remotely increased in 25 W steps every two minutes. Pedaling speed and resistance varied proportionally to maintain the pre-determined power output. Guidance on pedaling rate

attempted to accommodate individual comfort level with a general recommendation of at least 60 revolutions·min<sup>-1</sup>. Heart rate was recorded in the last ten seconds of each two-minute stage through heart rate monitors (Polar Electro, Kempele, Finland). Subjects were verbally encouraged to continue pedaling to exhaustion. The test terminated when the subject chose to stop or if the subject was unable to maintain a consistent pace. The highest value of oxygen consumption achieved over a 60-sec period served as the VO<sub>2max</sub>. To qualify as a true VO<sub>2max</sub>, at least one of the following criteria must have been met: a plateau in VO<sub>2</sub> (in which the increase in VO<sub>2</sub> from the end of one stage to the next is less than 50% of the expected increase) or achieving RER greater than or equal to 1.10. Maximal aerobic power (P<sub>max</sub>) was defined as the power of the highest stage completed, plus a pro-rated portion of the last stage if the last stage was not completed. P<sub>max</sub> was used to determine workload during HIIE.

### **Expository Food Log Period**

#### *Diet Recording*

Following the maximal exercise test, the food log software was explained to the subject. If the subject had a smart phone, an application to be downloaded for convenience was recommended. If not, the software could be employed through the website. The recording period began the day of the visit, or as soon as possible for the subject, and continued for three full days. Everything the subject consumed was recorded either manually or by scanning a barcode on the product label with the phone application. Subjects were instructed to eat what they would consider a normal diet but to avoid dining out or otherwise introducing any factor that would increase the difficulty of reproducing the diet. The initial three-day food log aimed to encapsulate a normal feeding pattern. Subjects were asked to

abstain from alcohol consumption, caffeine intake and use of supplements that may influence metabolism. Otherwise, food selection was not limited and subjects were free to select what they ate. They were not restricted from consuming their preferred foods. Options from the initial food log were selected to compose the pre-trial dietary composition. Thus, an important consideration emphasized to every subject was that whatever they chose to eat for the initial three-day food log would become the basis for the dietary prescription they would receive for the study. The diet summary report was transferred to the investigator at the appropriate time to be analyzed for potential discrepancies in reporting.

#### *Physical Activity Quantification*

During the three-day food log period, a hip-worn accelerometer received movement data. The normal daily paEE was derived from the resulting raw data. Factoring in the thermal effect of feeding, the accelerometer data were combined with baseline REE to calculate normal daily total energy expenditure. Energy intake during the pre-trial food logs was predetermined to maintain energy balance as close as possible by equating to this baseline calculated TEE.

#### *Energy Balance Calculation*

Energy balance refers to a homeostatic state wherein energy expended equals energy consumed. In a state of net energy balance there is neither a deficit nor surplus over a given time period. In this study, subjects were guided into a state of energy balance preceding the two main trials and their adherence to this procedure was recorded. The absolute caloric values for baseline energy balance were calculated for each subject.



The simple formula for energy balance is that energy in equals energy out. Specifically, calories consumed must equal the number of calories expended in the forms of mechanical and heat energy. Determining energy intake is relatively simplified by utilizing accurate diet tracking software, which in this case was the MyFitnessPal software. Energy expended, or TEE, is a product of REE, TEF and paEE. TEF was assumed to be 10% of energy intake for simplicity. The REE value for this equation was demonstrated by an RMR test. The most challenging component to account for is paEE. This information was derived from the accelerometers. These factors combined into this formula yielded the CEB. This was calculated with the following formula:

$$\text{CEB} = \text{EI} - \text{TEE}$$

Normal TEE reflected energy expended on a typical day where no exercise was performed. Baseline measured REE and paEE from the expository food log period were added to the calculated TEF value to acquire a normal TEE value. The number of calories in the daily diet required to achieve energy balance could then be determined and prescribed. If a truly balanced state occurs, the CEB should be zero. The actual energy balance value reflected the direction and magnitude of the difference from the CEB.

#### *Experimental Trials (days 2 and 3)*

The two main trial days followed a one-day food log period culminating in a dinnertime meal between 12 and 14 hours before the morning appointment. Subjects were instructed to not consume any further calories, and no beverages containing caffeine, after that evening meal, which occurred between 4 and 10 pm. Each trial began with a laboratory visit in the morning (between 6 and 8 am) without breaking the overnight fasting period. Overnight fast

duration was limited to a range of 12 to 14 hours. The final meal of this day was consumed within a two-hour window to achieve the desired fasting duration. The order of events began with pre-exercise measurements followed by the exercise and feeding protocols and culminated in post-exercise measurements.

### **Pre-Trial Diet Prescription**

Specific instructions were given to each subject on how to structure feeding during the one-day pre-trial periods in order to match the energy intake required for normal CEB. Construction of the pre-trial diet protocol exemplified a normal feeding pattern by duplicating, to the most reasonable extent, the food choices and macronutrient ratios of the expository three-day log. The number of kcal consumed was pre-determined. The primary delimitation for the pre-trial diet was that it achieved reasonable accuracy concerning CEB by matching energy intake with the deficit created by TEE. Since fluctuations of REE during sleep are unpredictable, and replicating daily paEE is problematic, the actual CEB could be different from zero. This dietary protocol elicited a state of energy balance entering the trial or at least approached true energy balance. Diet was recorded using the same recording methods used during the previous food log period. Subjects were instructed to consume identical diets between both pre-trial food logs.

### **Pre-Exercise Measurements**

Upon arrival, the food log results were examined to ascertain the adherence to the prescription and whether energy balance was achieved. Next, a fasting VAS was administered. The subject then began the resting period that preceded the RMR test.

Following the 20-minute rest period, the RMR assessment commenced. The protocol was identical to the baseline RMR test.

### **Exercise Protocol**

#### *HIIE Design*

The  $P_{\max}$  value established during the maximal exercise test was used to prescribe intensity for both intervals of the HIIE protocol. If the maximal exercise test was terminated between two stages, the prescribed power output was prorated to a value between the stage delineations. The following formula displays how  $P_{\max}$  was calculated in this situation:

$$P_{\max} = (T_C/T_{\text{tot}}) * W_i + W_C$$

Where:  $T_C$  = time completed in stage (seconds);  $T_{\text{tot}}$  = total time possible in stage (120 seconds);  $W_i$  = wattage increment between stages (25 watts);  $W_C$  = wattage of previously completed stage (watts)

Since  $P_{\max}$  represented  $VO_{2\max}$ , exercise intensity was prescribed as a percentage of  $P_{\max}$  in watts. HIIE was structured to elicit ten minutes of work at 90%  $P_{\max}$  and ten minutes at 60%  $P_{\max}$  for an average intensity of 75%  $P_{\max}$  over a 20-minute duration. Since adjustment of power output was incremental and not analog, not every subject achieved exactly 90%  $P_{\max}$ .

#### *HIIE Protocol*

The HIIE protocol was performed on the Velotron cycle ergometer with the same metabolic equipment used for the maximal exercise test. This protocol consisted of ten identical two-minute stages split between one-minute work intervals and one-minute active recovery intervals after a warm-up period constructed of two minutes at 30%  $P_{\max}$ . The

subject completed all ten stages of the HIIE protocol consecutively while the tester manually adjusted the power output between intervals. The tester also recorded heart rate in the last five seconds of each high-intensity interval. Upon protocol completion, watts were reduced to 30%  $P_{\max}$  for a cooldown period mirroring the warm-up protocol.  $VO_2$  was measured for the duration of the exercise protocol. This allowed for determination of exercise energy expenditure. The exercise protocol was identical between conditions and only performed during the morning visit of the trial day.

### **Condition-Specific Protocol**

#### *Fed Exercise Condition*

In the fed exercise condition, consumption of the test meal occurred after the RMR test. Subjects performed the HIIE protocol 20 minutes after the meal. Immediately after this series, subjects underwent the post-exercise measurements.

#### *Fasted Exercise Condition*

The fasted exercise condition employed an almost identical procedure with a few important order distinctions. It was mandatory that the second condition, whether it was the fasted or fed protocol, be performed at the same time of day as the first trial. The initial VAS and RMR test parameters were retained. The next step, however, diverged with the HIIE protocol, RVT and post-exercise VAS taking place before consumption of the test meal. Commensurate with the fed condition, a post-exercise test commenced immediately after exercise.

### **Post-Exercise Measurements**

Post-exercise measurements began immediately following the conclusion of the exercise protocol. A two-minute cool-down period was allotted after exercise but this was the only event in the timeframe between exercise and the post-exercise measurements. First, subjects immediately started an RVT to measure EPOC and the adjuvant REE and RER. After this, another VAS was administered.

Methods for the RVT EPOC measurement differed somewhat from the RMR tests. The set-up procedure including calibration technique and the dilution method remained identical to the RMR test but the major distinctions were the absence of a truly rested condition and use of a mask to allow posture adjustment. Since this was a measure of recovery  $\text{VO}_2$ , not resting  $\text{VO}_2$ , it was not necessary to orient the subjects horizontally and have them rest before the measurement. Thus, subjects were seated for the duration of the RVT. Subjects remained in this seated position but were restricted to the same degree as during an RMR test.

Additionally, the facemask used for the  $\text{VO}_{2\text{max}}$  test and HIIE protocol was used for the RVT which, in turn, mitigated the necessity to restrict movement. Measurement proceeded for 30 minutes after which a 10-minute break was permitted to allow for non-strenuous tasks such as water breaks. After the 10-minute break, another 30-minute  $\text{VO}_2$  measurement was initiated. This RVT protocol provided 60 total minutes of  $\text{VO}_2$  data over a 70-minute time period.

Oxygen consumption values from the pre-exercise RMR test were subtracted from those during the RVT to determine EPOC over a one-hour post-exercise measurement. For this calculation, total  $\text{VO}_2$  from the 15 minutes of valid data of the pre-exercise RMR test was

multiplied by four to extrapolate a one-hour estimation of resting  $\text{VO}_2$ . All of these resultant values, including EPOC, were also recorded relative to body mass.

### **Trial-Day Feeding Behavior**

The trial-day food log period encompassed an *ad libitum* feeding period. Subjects were expected to record everything they consumed as they did in previous logs. The difference was that no recommendations were made and intake was completely unrestricted. The exception to this is that ingestion of caffeine, nicotine and alcohol was restricted similarly to the pre-trial diet. Exercise was not permitted during this time. Recording of food intake continued through the next laboratory visit 12 hours after exercise. This 12-hour measurement period was not expected to include a complete day's dietary content due to multiple interruptions of the normal feeding pattern. This period could not be assumed to represent typical feeding behavior. The trial day food log instead encompassed a multiple-hour post-exercise feeding window. Thus, the results simply reflected dietary behavior in the hours following a high-intensity exercise bout performed in either a fasted or fed state.

### **Evening Measurements**

Twelve hours after the onset of the initial laboratory visit, subjects returned after fasting for at least two hours for a VAS measurement followed by an RMR test. Energy intake data were recorded from the food log at the conclusion of the session. Oxygen consumption values from the pre-exercise RMR test were extrapolated to a 12-hour period and subtracted from the values of the post-exercise period including the RVT and 12-hour RMR. This rough time-course for EPOC was plotted linearly using  $\text{VO}_2$  values from the RVT and from the evening RMR test. However, due to uncertainty about confounding factors, this 12-hour

EPOC time course was not reported. Instead, 12-hour  $\text{VO}_2$  was compared directly with pre-exercise  $\text{VO}_2$  as discrete singular measurements.

## **Data and Statistics**

### *Data Organization*

One master data sheet contained the results for the primary outcome measures along with descriptive data. All physically recorded data were congregated in one binder specific to the study. This binder was kept in a locked cabinet when not in use. Digitally stored data remained in its original location but all output was only identified by a subject code.

VAS answers were grouped by question ranging from 1-8. Results for each question were assessed individually. Question 1 determined the level of hunger. Questions 2 and 3 related to fullness or satiety. Question 4 inquired about prospective food consumption. Questions 5-8 all examined specific food characteristic preferences. In addition to the constituent parts of appetite, a composite appetite score (CAS) was determined by deriving from hunger, fullness and prospective food consumption results. The scores for the appetite question and prospective food consumption question were considered as recorded. These results proportionally reflect the magnitude of appetite. For the two fullness questions, the scale was inverted. Specifically, the value of the score (in mm) was subtracted from the value for strongest possible answer of 100 mm. This inversion was constrained by the logical inverse relationship between fullness score and appetite. With this adjustment, the CAS score illustrates a proportional relationship with appetite. The CAS was analyzed individually and independently from each VAS question.

Subject compliance to the *ad libitum* trial-day diet was assessed using a difference score from the prescribed value. A difference score was also used to confirm the acquisition of an energy-balanced state in the pre-trial diet.

### *Statistical Analysis*

Variables compared between pre-intervention and post-intervention time points were analyzed with a 2x4 repeated measures ANOVA (fed versus fasted exercise; base versus pre-exercise versus post-exercise versus 12 hours after exercise). Sphericity was assumed and was determined not to have been violated upon analysis. A normal distribution of values for the dependent variables was assumed and thus a parametric model was utilized. Normality was tested with the Shapiro-Wilk test. *Post-hoc* comparisons were made using dependent student's t-tests with Bonferroni adjustments. Pearson-r correlations were made between descriptive and metabolic variables but these results were not noteworthy and thus are not presented in the results section. All analyses utilized the Statistical Package for Social Sciences (IBM SPSS Statistics v22) software (IBM, Armonk, NY). The selected alpha level was 0.05. Results were presented as mean  $\pm$  standard deviation with p-values in parentheses.



## CHAPTER IV

### RESULTS

#### Subjects

There were 14 subjects who began the study and signed the informed consent document. Of these 14, only 11 were able to complete the study. All three subjects who failed to complete the study cited schedule conflicts. Due to some missing data points, not all variables have a sample size of 11. When data was missing for a subject, they were excluded from that particular analysis. Descriptive information for all completed subjects ( $n = 11$ ) is presented in Table 1. The data include performance on the initial maximal exercise test, namely  $\text{VO}_{2\text{max}}$  and maximum power output ( $P_{\text{max}}$ ). Beyond these data, maximal heart rate ( $184 \pm 13 \text{ beats} \cdot \text{min}^{-1}$ ), resting heart rate ( $69 \pm 11 \text{ beats} \cdot \text{min}^{-1}$ ) and HRR ( $115 \pm 13 \text{ beats} \cdot \text{min}^{-1}$ ) were also determined.

**Table 1. Subject Characteristics**

subject	sex	age (yr)	height (cm)	mass (kg)	fat (%)	lean mass (kg)	VO <sub>2max</sub> (mL·min <sup>-1</sup> ·kg <sup>-1</sup> )	P <sub>max</sub> (W)
1	male	25	182	85.0	10.2	76.3	44.5	257.5
2	female	25	165	74.9	31.4	51.4	30.6	164.4
3	male	24	168	84.5	22.4	65.6	33.3	225.0
4	female	20	168	66.0	17.0	54.8	38.1	197.5
5	male	21	174	74.6	14.2	64.0	44.4	216.7
6	male	22	169	55.7	9.0	50.7	23.2	91.5
7	female	25	160	62.0	19.4	50.0	35.7	166.0
8	female	22	160	78.2	20.4	62.2	32.3	167.0
9	female	21	163	61.3	23.4	47.0	37.0	175.0
10	male	22	191	73.0	5.7	68.8	50.7	225.0
11	female	22	172	63.4	25.3	47.4	32.7	150.0
Mean		23	170	70.8	18.0	58.0	36.6	185.1
SD		2	9	9.8	7.7	9.9	7.7	45.6

**Exercise**

Data from the HIIE exercise protocols is summarized in Table 2 (n = 11) as means ± standard deviations. Due to software limitations in power interval programming, the actual power outputs for high-intensity intervals ( $89 \pm 2\%$  P<sub>max</sub>) and recovery intervals ( $59 \pm 2\%$  P<sub>max</sub>) were a percentage point below intended values. Net values were calculated by

extrapolating pre-exercise values for a given variable to the duration of exercise and then subtracting the resulting value from the gross value. Data are presented as means  $\pm$  standard deviations.

**Table 2. High-Intensity Exercise Data**

<b>condition</b>	<b>gross VO<sub>2</sub></b> <b>(L)</b>	<b>net VO<sub>2</sub></b> <b>(L)</b>	<b>gross EE</b> <b>(kcal)</b>	<b>net EE</b> <b>(kcal)</b>	<b>HR</b> <b>(bpm)</b>
fed	42.9 $\pm$ 10.0	38.1 $\pm$ 9.4	221 $\pm$ 52*	200 $\pm$ 49*	187 $\pm$ 9*
fasted	40.3 $\pm$ 10.8	35.7 $\pm$ 10.2	206 $\pm$ 54	185 $\pm$ 51	182 $\pm$ 11

\*Fed value significantly greater ( $p < 0.05$ ) than fasted value.

Heart rate from the final stage of exercise was compared to maximum heart rate (MHR) and heart rate reserve (HRR) determined from the baseline maximal exercise test. Fed-state exercise heart rate was  $187 \pm 9$  beats $\cdot$ min<sup>-1</sup> equating  $102 \pm 6\%$  MHR and  $103 \pm 10\%$  HRR. Fasted exercise heart rate was  $182 \pm 11$  beats $\cdot$ min<sup>-1</sup> equating  $99 \pm 7\%$  MHR and  $99 \pm 10\%$  HRR. Fed exercise heart rate significantly exceeded fasted exercise heart rate ( $p = 0.029$ ) including MHR ( $p = 0.027$ ) and HRR ( $p = 0.028$ ).

Absolute VO<sub>2</sub> in the fed condition was 42.9 L gross and 38.1 L net. Fasted absolute VO<sub>2</sub> was 40.3 L gross and 35.7 L net. Energy expenditure during fed exercise was greater than during fasted exercise ( $p = 0.050$ ).

## Metabolic Response

### *Oxygen Consumption*

Oxygen consumption at each discrete time point throughout the trial is displayed in Table 3 (n = 10) as means  $\pm$  standard deviations. Baseline values are not included due to separation from the trial phase. There was no meaningful distinction between effects on absolute versus relative values; therefore, they are interchangeable for the purpose of analysis.  $\text{VO}_2$  was  $3.33 \pm 0.32 \text{ mL}\cdot\text{min}^{-1}\cdot\text{kg}^{-1}$  at baseline.

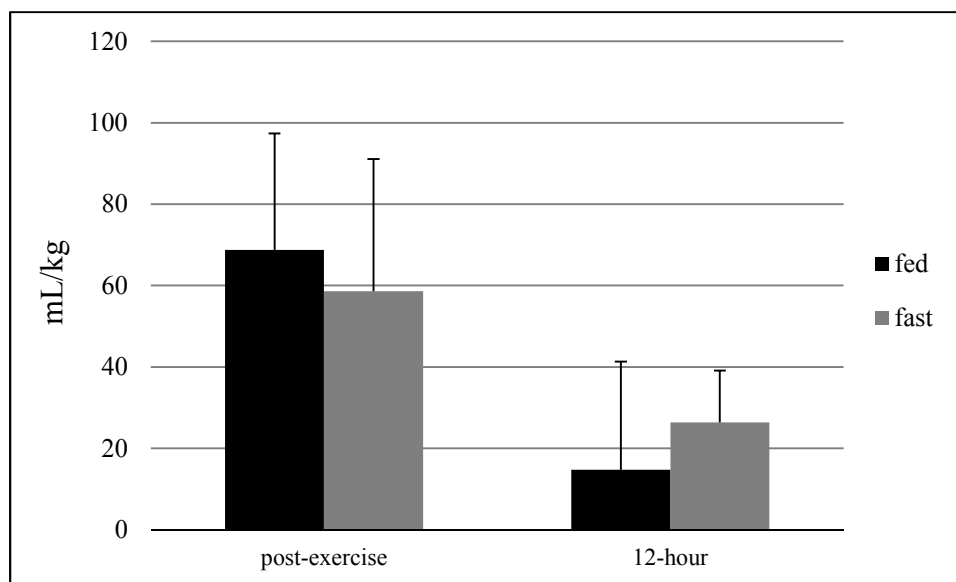
**Table 3. Oxygen Consumption ( $\text{mL}\cdot\text{min}^{-1}\cdot\text{kg}^{-1}$ ) Time Course**

<b>condition</b>	<b>pre-exercise</b>	<b>post-exercise</b>	<b>12-hour</b>
fed	$3.38 \pm 0.42$	$4.53 \pm 0.43$	$3.63 \pm 0.32$
fast	$3.27 \pm 0.27$	$4.23 \pm 0.55$	$3.70 \pm 0.48$

While there was no main effect for condition, there was for time ( $p < 0.001$ ). Fed state  $\text{VO}_2$  was significantly greater post-exercise than at baseline ( $p < 0.001$ ), pre-exercise ( $p = 0.001$ ) and 12 hours after exercise ( $p = 0.013$ ). Fasted state  $\text{VO}_2$  was significantly greater post-exercise than at baseline ( $p = 0.014$ ) or pre-exercise ( $p = 0.006$ ). Twelve hours after exercise, fasted state  $\text{VO}_2$  was higher than pre-exercise ( $p = 0.002$ ) but not different from post-exercise; fed state  $\text{VO}_2$  at 12 hours was not significantly greater than pre-exercise ( $p = 1$ ). The interaction effect was insignificant but revealed a trend ( $p = 0.081$ ) despite the absence of a condition effect.

Excess post-exercise oxygen consumption is illustrated over 60-minute intervals for the immediate post-exercise window and 12 hours after exercise in Figure 1 (n = 10). These values reflect the additional volume after subtracting the pre-exercise measurement. Values from the 12-hour measurement were extrapolated to one hour from a 20-minute measurement.

**Figure 1. Post-exercise and 12-hour EPOC**



EPOC over 60 minutes immediately after exercise was  $68.8 \pm 28.6 \text{ mL}\cdot\text{kg}^{-1}$  in the fed condition and  $58.6 \pm 32.5 \text{ mL}\cdot\text{kg}^{-1}$  in the fasted condition. These represented 34% and 29% increases above rest, respectively.  $\text{VO}_2$  12 hours after exercise was  $14.8 \pm 26.6 \text{ mL}\cdot\text{kg}^{-1}$  above rest in the fed condition, but this was not significantly different from zero.  $\text{VO}_2$  12

hours after exercise was  $26.4 \pm 12.7 \text{ mL}\cdot\text{kg}^{-1}$  in the fasted condition. Neither set of values differed between conditions.  $\text{VO}_2$  was significantly higher than pre-exercise and baseline immediately after exercise in both conditions signaling an immediate EPOC effect irrespective of condition. Elevated  $\text{VO}_2$  after 12 hours compared to pre-exercise was only significant in the fasted condition.

### *Energy Expenditure*

Running parallel to oxygen consumption, the time course for energy expenditure is shown in Table 4 ( $n = 10$ ) as means  $\pm$  standard deviations. All EE data shown are presented as  $\text{kcal}\cdot\text{hr}^{-1}$ . For measurements that did not equal one hour, the data were extrapolated to fit this qualifier. Baseline EE was  $69.6 \pm 8.6 \text{ kcal}\cdot\text{hr}^{-1}$ .

**Table 4. Energy Expenditure ( $\text{kcal}\cdot\text{hr}^{-1}$ ) Time Course**

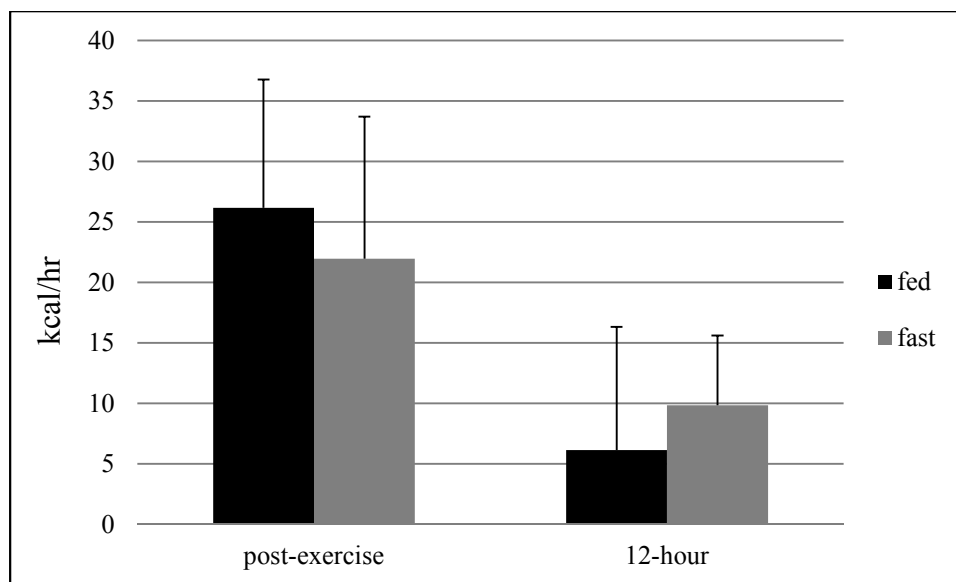
<b>condition</b>	<b>pre-exercise</b>	<b>post-exercise</b>	<b>12-hour</b>
fed	$70.8 \pm 10.7$	$97.0 \pm 15.6$	$76.9 \pm 13.1$
fast	$67.9 \pm 10.2$	$89.9 \pm 17.2$	$77.8 \pm 15.2$

Similar to  $\text{VO}_2$ , there was no main effect for condition, but there was for time ( $p < 0.001$ ). Post-exercise EE in the fed condition was greater than EE at baseline ( $p < 0.001$ ), pre-exercise ( $p = 0.001$ ) and 12 hours later ( $p = 0.011$ ). Post-exercise EE in the fasted condition was greater than EE at baseline ( $p = 0.013$ ) and pre-exercise ( $p = 0.005$ ). EE 12

hours after exercise was greater than pre-exercise ( $p = 0.009$ ). Again, the interaction effect was not significant but did exhibit a stronger trend than  $\text{VO}_2$  ( $p = 0.067$ ).

Figure 2 reveals excess EE over 60-minute intervals for the immediate post-exercise window and 12 hours after exercise ( $n = 10$ ). To attain these values, pre-exercise energy expenditure was subtracted from the number of kcal expended during the target period. Values from the 12-hour measurement were extrapolated to one hour from a 20-minute measurement.

**Figure 2. Post-exercise and 12-hour Excess Energy Expenditure**



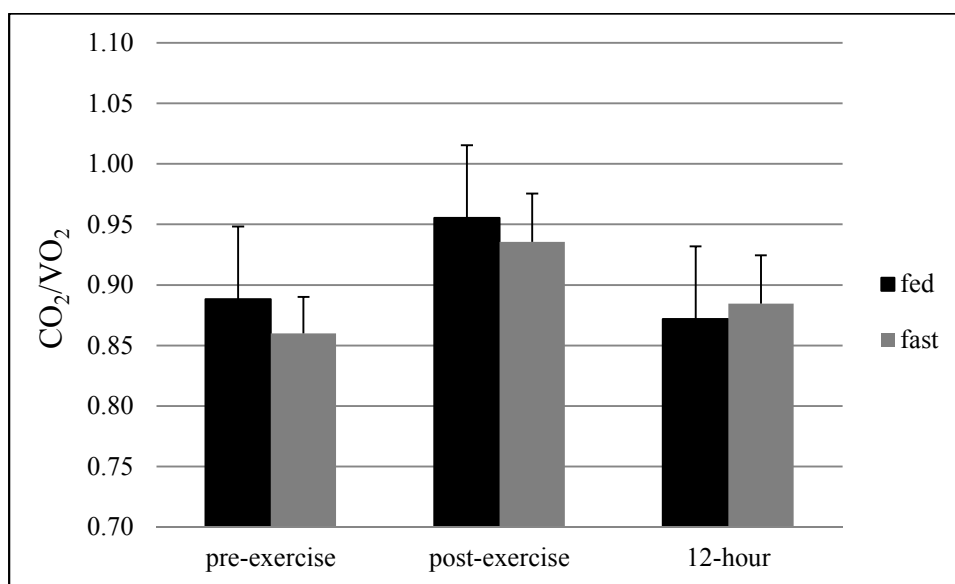
Excess EE over 60 minutes immediately after exercise was  $26.2 \pm 10.6$  kcal in the fed condition and  $22.0 \pm 11.8$  kcal in the fasted condition. EE 12 hours following exercise

exceeded pre-exercise EE by  $5.6 \pm 9.9$  kcal in the fed condition and  $9.0 \pm 6.2$  kcal in the fasted condition. Neither set of values differed between conditions. Like  $\text{VO}_2$ , EE was significantly higher than pre-exercise and baseline immediately after exercise in both conditions. Also mirroring  $\text{VO}_2$ , EE was elevated beyond pre-exercise only in the fasted condition.

### Respiratory Exchange Ratio

Values for respiratory exchange ratio attained through metabolic tests are displayed in Figure 3 ( $n = 10$ ). This time course runs parallel with oxygen consumption and energy expenditure measurements. RER values represent averages over the full measurement period.

**Figure 3. RER Time Course**





Baseline RER was  $0.87 \pm 0.05$ . Pre-exercise RER was  $0.88 \pm 0.06$  in the fed condition and  $0.86 \pm 0.32$  in the fasted condition. Post-exercise RER was  $0.96 \pm 0.06$  in the fed condition and  $0.94 \pm 0.45$  in the fasted condition. RER 12 hours after exercise was  $0.87 \pm 0.06$  in the fed condition and  $0.88 \pm 0.04$  in the fasted condition.

RER displayed no main effect for condition but did for time ( $p < 0.001$ ). Post-exercise RER in the fed condition was higher than at baseline ( $p = 0.027$ ), pre-exercise ( $p = 0.014$ ) and 12 hours after exercise ( $p = 0.004$ ). Post-exercise RER in the fasted condition differed from pre-exercise ( $p = 0.022$ ) but not baseline or 12 hours after. In contrast to  $\text{VO}_2$  and EE, the significance of the interaction effect was not strong enough to qualify as a trend.

## **Energy Balance**

### *Baseline Energy Status*

Constituents of baseline energy balance are contained in Table 5 ( $n = 11$ ). EI is the average energy intake over three days prior to trials. CEB was calculated as the difference between EI and TEE. The units for each variable are kcal.

**Table 5. Baseline Energy Balance (kcal)**

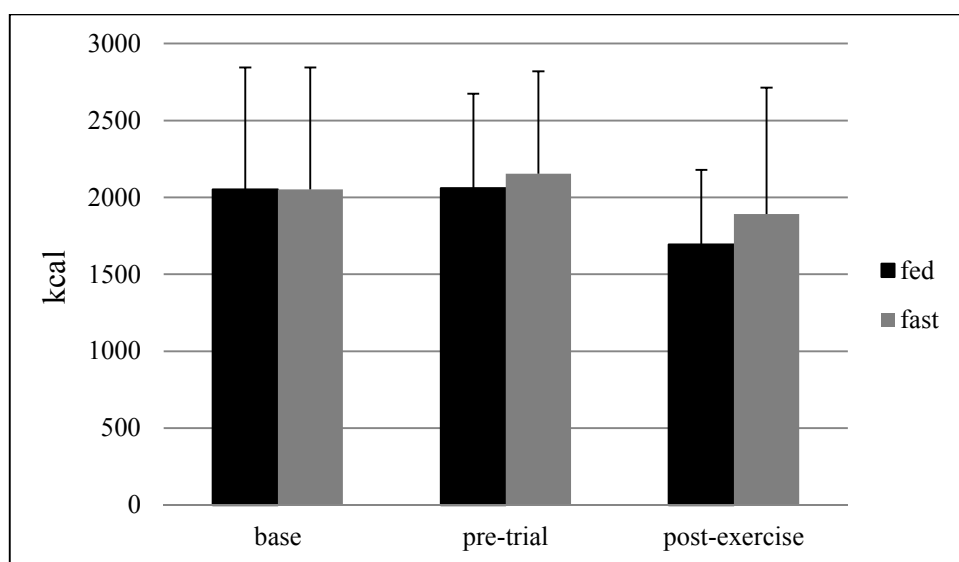
subject	EI	TEF	REE	paEE	TEE	CEB
1	3354	335.4	2112	661.0	3108.4	245.3
2	1086	108.6	1582	431.9	2122.5	-1036.8
3	2605	260.5	1790	640.4	2690.9	-85.9
4	1946	194.6	1488	367.8	2050.5	-104.1
5	2889	288.9	1608	268.6	2165.5	723.2
6	2407	240.7	1385	437.4	2063.1	343.9
7	1592	159.2	1246	348.3	1753.5	-161.8
8	845	84.5	1709	264.8	2058.3	-1213.3
9	1428	142.8	1754	502.0	2398.8	-970.8
10	2651	265.1	1760	174.9	2199.9	450.6
11	1766	176.6	1507	132.0	1815.6	-49.9
Mean	2052	205.2	1631	384.5	2220.6	-169.1
SD	792	79.2	233	172.6	389.4	642.4

### *Energy Intake*

Energy intake throughout the trial, shown in Figure 4 (n = 10), was separated into three time periods including baseline, pre-trial and post-exercise. In this case, pre-trial refers to the day preceding the trial and post-exercise encompasses the 12 hours between laboratory visits during one trial. Considering fast duration at the end of the pre-trial diet, the timeframe

of this pre-trial feeding period was expected to roughly equal the post-exercise feeding period. All values are in kcal.

**Figure 4. Energy Intake Time Course**



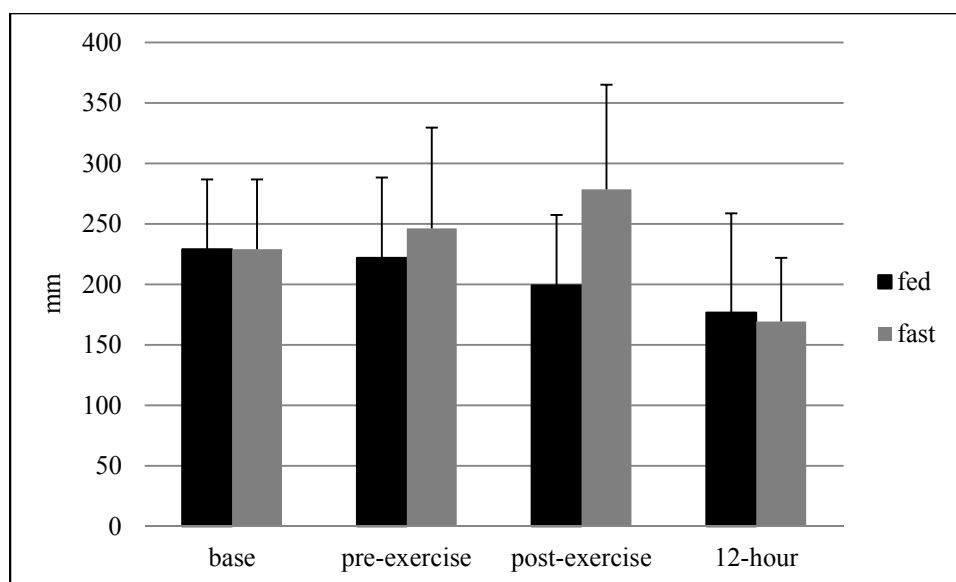
As previously stated, baseline EI was  $1968 \pm 782$  kcal. Pre-trial EI was  $2060 \pm 613$  kcal in the fed condition and  $2154 \pm 666$  kcal in the fasted condition. Post-exercise EI was  $1695 \pm 485$  kcal in the fed condition and  $1892 \pm 822$  kcal in the fasted condition.

There was no condition effect for EI but the time effect was significant ( $p = 0.048$ ); however, there were no significant differences between individual time points. Pre-trial EI tended to be higher than post-exercise EI by way of a weak trend ( $p = 0.091$ ). Pre-trial EI did not differ at all between conditions ( $p = 1$ ).

### Visual Analog Scale

Composite appetite score (CAS) time course (n = 11) is plotted on Figure 5. The values are displayed in millimeters with a maximum theoretical value of 400 mm based on possible scores from the first four questions. CAS represents total appetite on a scale of 0-400 with higher numbers suggesting a greater likelihood to consume more energy following the measurement. Values for the factors that determined CAS are displayed in Table 6. These individual values fell within a range of 0-100 mm.

**Figure 5. Composite Appetite Score**



Composite appetite score (CAS) was  $229 \pm 57$  at baseline. Pre-exercise CAS was  $222 \pm 66$  in the fed condition and  $246 \pm 83$  in the fasted condition. Post-exercise CAS was

199 ± 58 in the fed condition and 279 ± 86 in the fasted condition. CAS 12 hours after exercise was 177 ± 82 in the fed condition and 169 ± 53 in the fasted condition.

The time effect for CAS was significant ( $p = 0.001$ ). There were weak trends for both condition effects ( $p = 0.088$ ) and interaction effects ( $p = 0.094$ ) derived from the disparity between conditions from pre-exercise to post-exercise. The only condition difference was the gap between post-exercise in fed and fasted conditions ( $p = 0.022$ ). The time difference was accounted for by the appetite reduction 12 hours after exercise compared to pre- and post-exercise ( $p = 0.015$  pre-exercise vs 12 hours;  $p = 0.006$  post-exercise vs 12 hours). Appetite was reduced in the fasted condition only 12 hours after exercise compared to baseline ( $p = 0.014$ ), pre-exercise ( $p = 0.005$ ) and post-exercise ( $p = 0.017$ ). These differences were not present in the fed condition.

**Table 6. VAS factors**

<b>time</b>	<b>hunger (mm)</b>	<b>satiety (mm)</b>	<b>fullness (mm)</b>	<b>PFC (mm)</b>
base	45 ± 16	39 ± 18	36 ± 22	60 ± 21
pre-exc FED	48 ± 19	37 ± 21	43 ± 20	53 ± 17
pre-exc FST	56 ± 21	41 ± 26	34 ± 27	66 ± 20
post-exc FED	43 ± 14	48 ± 18	48 ± 21	52 ± 20
post-exc FST	63 ± 24	31 ± 21	24 ± 23	70 ± 25
12-hour FED	36 ± 24	49 ± 23	58 ± 20	48 ± 26
12-hour FST	32 ± 20	50 ± 20	61 ± 19	48 ± 19

Hunger had a significant main effect for condition ( $p = 0.045$ ) and time ( $p = 0.010$ ) without any interaction effect. There were no significant differences revealed by post-hoc analysis. Fullness displayed a time effect ( $p < 0.001$ ) and a weak trend for a condition by time interaction effect ( $p = 0.096$ ). There was a trend for fasted post-exercise fullness to be lower than 12 hours after exercise in the fasted condition ( $p = 0.068$ ). Otherwise, there were no differences for fullness. There was a weak trend for a condition effect regarding prospective food consumption (PFC) ( $p = 0.087$ ). The time factor for PFC was close to violating sphericity ( $p = 0.064$ ) and would not have been even a trend with a corrected test so it was not evaluated. There were no significant differences revealed by post-hoc analysis. There were no main or interaction effects for satiety.

Preference for savory food showed a time effect ( $p = 0.017$ ) with no significant differences between time points. There were no statistical differences in the other three measures of food preference. These were sweet, salty and fatty.

## CHAPTER V

### DISCUSSION

#### Hypotheses

The hypotheses are reiterated here along with the concomitant primary findings.

Hypothesis 1 was that fasted exercise would result in larger immediate post-exercise (0-70 minutes) and 12-hour REE and EPOC values than fed exercise. The finding was that fasting or feeding before exercise had no differential effect on EPOC or excess EE; however, feeding caused elevated heart rate and EE during exercise.

Hypothesis 2 predicted perceived appetite would be lower both one and 12 hours after fed exercise than fasted exercise. Although, appetite was indeed lower right after fed exercise than after fasted exercise, after 12 hours, fasted-condition appetite was lower than all other time points. This was not the case with the fed condition but there were no condition differences.

Hypothesis 3 predicted energy intake would be greater after fasted exercise than fed exercise. Ultimately, there was no condition difference in energy intake following exercise.

Hypothesis 4 was that RER immediately after and 12 hours following exercise would be the same in fed and fasted states and not differ from baseline. Post-exercise RER ended up being significantly greater than before exercise in both conditions with no condition differences.



## Specific Interpretations

### *Oxygen Consumption and EPOC*

#### **Oxygen Consumption Time Course**

The time course for oxygen consumption throughout the trials followed a predictable plot. There was no difference between baseline  $\text{VO}_2$  and pre-exercise fasted  $\text{VO}_2$ . Post-exercise  $\text{VO}_2$  was elevated compared to pre-exercise in both conditions.  $\text{VO}_2$  12 hours after exercise was greater than pre-exercise in the fasted condition and was not statistically different from post-exercise  $\text{VO}_2$ . Fed 12-hour  $\text{VO}_2$  was not different from pre-exercise and was significantly lower than post-exercise.

There were no direct condition differences, supporting an early finding that there is no difference in  $\text{VO}_2$  response between exercise in fasted or fed states (Pacy et al., 1985). However, there is less clarity when considering other findings, specifically the response 12 hours after exercise. The elevation in  $\text{VO}_2$  from pre-trial to 12 hours after exercise was present only in the fasted condition, supporting a finding from the pilot study preceding this one (Perez & Rynders, 2014). Despite this statistical difference, the values were fairly similar (218  $\text{mL}\cdot\text{hr}^{-1}\cdot\text{kg}^{-1}$  fed 12-hour vs 203  $\text{mL}\cdot\text{hr}^{-1}\cdot\text{kg}^{-1}$  fed pre-exercise; 222  $\text{mL}\cdot\text{hr}^{-1}\cdot\text{kg}^{-1}$  fast 12-hour vs 196  $\text{mL}\cdot\text{hr}^{-1}\cdot\text{kg}^{-1}$  fast pre-exercise) from a practical perspective. This makes a definitive conclusion more problematic. This contrasts with the finding of (Paoli et al., 2011) that 12 hours after exercise,  $\text{VO}_2$  was greater after fed exercise than fasted. The problem with these aforementioned studies, as well as the current one, is the absence of a control measurement for the 12-hour metabolic measurement. Thus it is possible that the status of various metabolic factors at that time is determined entirely by normal circadian fluctuations.

Though the time course of REE has not been determined to consistently follow a singular circadian pattern (Zurlo et al., 1986), at minimum there are certainly small fluctuations throughout the day. The difference between morning and evening may even be up to 100 kcal per day (Haugen et al., 2003). Most notably, subjects ate during the intervening 12 hours (approximately 1700-1900 kcal), which would have resulted in a thermogenic effect in metabolizing that food. An attempt to partially mitigate this outcome was made by asking subjects to refrain from eating for 2 hours prior to the measurement which placed the previous meal around a normal dinner time for most subjects. However, there was no way to completely remove this factor due to the nature of the question regarding *ad libitum* energy intake following exercise. Considering this confounding concept, the only reliable conclusions to be drawn concern the immediate EPOC period, for which there was no difference between fasted and fed conditions.

While the results seem to support the original discovery that fasting before exercise does not impact resulting metabolism, the underlying truth is more equivocal. There was a trend for significance in the interaction of condition and time. It would be speculative to suggest a larger sample size might elucidate this potential interaction. As it is presented, the trend for interaction is interesting but not definitive. If expanded studies revealed the trend to be something more significant, this could further support the claim of (Paoli et al., 2011) that feeding enhances the metabolic response to exercise. This, of course, assumes that the values would again be greater for fed-state exercise rather than for fasted-state.

## **EPOC**

EPOC was determined as the difference between actual  $\text{VO}_2$  and expected  $\text{VO}_2$  based on pre-trial measurements. The HIIE protocol clearly initiated an EPOC response in both conditions. This effect was illustrated by the significant increase in  $\text{VO}_2$  in the post-exercise period compared to pre-exercise. The calculated values for EPOC ( $69 \text{ mL}\cdot\text{kg}^{-1}$  FED;  $59 \text{ mL}\cdot\text{kg}^{-1}$  FAST) proved to be comparable to previously established EPOC values ( $49\text{-}79 \text{ mL}\cdot\text{kg}^{-1}$  over 40-60 min) for high-intensity exercise in healthy populations (Skelly et al., 2014; Warren et al., 2009). In a study performed concurrently with the presented research, EPOC was measured after the same HIIE protocol was used (Simmons, 2016). EPOC was 3 L over 41 minutes in that study while EPOC over 60 minutes in the current study was 5 L in the fed condition and 4.2 L fasted. Similar to the previously encountered problem with 12-hour measurements, EPOC in the hours after exercise was difficult to make any firm conclusions about. Total  $\text{VO}_2$  from exercise to 12 hours later was apparently elevated over expected levels. However, without control measurements for these times of the day, this is merely a possibility worth examining with a more detailed study design rather than a conclusion that can be drawn presently.

### *Energy Expenditure and Excess Energy Expenditure*

Energy expenditure mirrored the  $\text{VO}_2$  time course. Also reflecting the  $\text{VO}_2$  data was the lack of condition differences. Excess EE (calculated similarly to EPOC) was not different between conditions. These results unsurprisingly matched the results of  $\text{VO}_2$  once analyzed. Again, there was a trend for an interaction effect. The trend was somewhat stronger

than that of  $\text{VO}_2$ . This suggests that these interactions are worth exploring further with a more rigorous design to determine whether there actually is an effect.

Notably, the absence of condition effects on EE does not necessarily signal equivalent metabolic responses. (Bennard & Doucet, 2006) found that fasted exercise increased fat oxidation both during and after exercise compared to fed exercise without any difference in energy expenditure. A similar effect was recorded by (Shimada et al., 2013) where 24-hour accumulated fat oxidation was greater after fasted exercise despite no differences in EE. While the current study does not contribute to this outcome, it certainly remains possible to reveal with an altered study design. Since EE was not dramatically affected by fasting or feeding before exercise in this study, the substrate ratio of the elevated EE values is the next interesting question.

### *Respiratory Exchange Ratio*

As expected with high-intensity exercise, RER was elevated immediately afterward. There were no condition differences and, unlike the previous two variables, no trend for an interaction effect. RER was not affected by fasting or feeding; however, this may be an incomplete picture of substrate utilization surrounding exercise. The values for RER are relatively homogenous in humans, especially around exercise. Even with small standard deviations, a much larger sample size would be required to highlight any potential changes. This variable is the most difficult to ascertain for this reason. Additionally, RER does not represent the full paradigm of substrate utilization. Since RER is a measurement of expired gases, the true internal respiratory quotient is unaddressed when measuring RER. Metabolites of fat oxidation provide further useful information on the process. This area is arguably the

one most in need of additional research. Despite the present findings, it appears likely that the fat oxidation environment is mediated by the nutritional state of exercise. As previously mentioned, (Bennard & Doucet, 2006) and (Shimada et al., 2013) found higher fat oxidation rates after fasted exercise whereas (Paoli et al., 2011) observed the opposite, fed exercise resulting in lower RER than fasted exercise later in the day. Even though the nutritional state of exercise may have little or no impact on energy expenditure, it may yet affect the ratio of its substrate components. Understanding this important distinction could create a much clearer illustration of the metabolic effects of fasted exercise.

#### *Physiological Response During Exercise*

In contrast with the metabolic response after exercise, the metabolic environment during exercise was demonstrated to be differentially affected by the nutritional state preceding exercise. Thus it is reasonable to ponder whether one approach to exercise nutrition may be advantageous if the resulting metabolic response to exercise is unaltered. This would extend to performance-based considerations as well as the accumulated metabolic responses over numerous exercise bouts as part of a training program.

#### **Heart Rate**

Heart rate during fed-state exercise exceeded fasted-state exercise as absolute values, percent of maximum heart rate and percent of heart rate reserve. It was unclear whether this difference was related to perceived effort. It is possible that the restored energy reserve provided by pre-exercise feeding allowed subjects to exceed theoretical performance limitations determined from a baseline test that was performed without an immediate pre-exercise meal. This conclusion was derived from the super-maximal heart rate values reached

during fed, but not fasted, exercise. Potential mechanistic factors for this, as well as for  $\text{VO}_2$  and EE, are discussed below.

On several occasions, including some of the means, heart rate during HIIE exceeded previously established maximum heart rate. This reveals that HIIE exhibited the desired maximal intensity and, more importantly, that the baseline maximal exercise test failed to establish a true physiological maximum heart rate for at least some of the subjects. This could have been due to local muscular fatigue or perceived exertion deriving from the relative inexperience with high-intensity cycling possessed by the subject pool. Although this may appear worthy of concern, the actual heart rate values that exceeded the maximum were within a few percentage points and thus likely achieved a desirable level of validity.

### **Oxygen Consumption and Energy Expenditure**

Energy expenditure was significantly greater during fed exercise than fasted exercise. The higher energy cost could be partially generated by the thermic effect of feeding. The total difference of 15.7 kcal over 20 minutes was unlikely to be comprised entirely of this effect but it is unknown if the difference would remain significant if this was accounted for. Interestingly, albeit statistically weaker, was the presence of a trend for higher  $\text{VO}_2$  during fed exercise. These differences contrast with the metabolic results from the post-exercise periods.

### **Potentially Influential Factors**

A fast duration of 24 hours has been suggested to impede performance during high-intensity exercise (Gleeson et al., 1988) despite higher plasma free fatty acid concentration with no difference in muscle glycogen (Loy et al., 1986). Feeding before

exercise has also shown to increase time to fatigue again despite the higher free fatty acid levels during fasted exercise (Schabert et al., 1999). The fast used in the current study lasted 12-14 hours and it is likely this duration did not cross the potential threshold of performance mitigation. There are admittedly fewer comparison studies of fasting periods of this length due to the prevalence of examinations of 24-hour fasts coinciding with Ramadan fasting.

Fasted exercise yields similar lactate concentrations (Dohm et al., 1986; de Lima et al., 2015) to fed exercise with reduced glucose levels and higher concentrations of triglycerides (de Lima et al., 2015). Type I muscle fibers, the predominantly active fibers in endurance exercise, rely heavily on intra-muscular triglycerides for energy during fasted exercise (Loon et al., 2003; De Bock et al., 2005). Their utilization is prevented by ingesting carbohydrate (De Bock et al., 2005). In these Type I fibers, glycogen content is reduced in both fasted and fed exercise. This glycogen reduction is present in Type IIa fibers during fasted exercise only (De Bock et al., 2007). These findings point toward a differential effect on substrate utilization during fasted exercise. Changes in metabolic behavior would logically concatenate.

Exercising in a fasted state can result in higher rates of fat utilization and lower RER (Dohm et al., 1986). However, RER may only be reduced during fasted exercise at intensities less than 80%  $VO_{2peak}$  (Bergman & Brooks, 1999). This discovery leads to a deduction that protocols like HIIE may differ from moderate-intensity protocols in metabolic effects when comparing fasted to fed states. Fat oxidation both during and after fasted exercise has been shown to be greater than fed exercise with no difference in energy expenditure (Bennard & Doucet, 2006). The presently observed difference in energy expenditure may possibly be a product of the high-intensity exercise protocol interacting with

nutritional state. The various relevant findings are difficult to consolidate considering discrepancies in exercise intensity. If high-intensity exercise causes differentiation of energy expenditure between fasted and fed exercise, it may result in mitigation of the fat oxidation effects. A direct comparison of exercise intensities in both nutritional states is thus warranted.

### *Dietary Results*

#### **Calculated Energy Balance**

Energy balance was planned to achieve a value of zero, or net energy balance, preceding trials. While the actual value was a non-zero number, it was not statistically different from zero and there was no difference between conditions. It was therefore determined that subjects were indeed in an energy-balanced state preceding trials. There were no differences in CEB by condition.

Interestingly, the mean CEB at baseline was negative. This could have been due to the sample being taken from an active population with interests in exercise. The standard deviation of energy expenditure from physical activity was greater than the absolute value of CEB.

#### **Energy Intake**

Energy Intake did not significantly differ between conditions. There was no difference whatsoever in pre-trial energy intake between conditions, suggesting subjects complied with the diet prescription. Energy intake at baseline was enough to maintain a positive CEB if only REE and the thermic effect of feeding were accounted for. Physical activity variation appeared to explain whether caloric intake pushed CEB into positive or



negative territories. Thus, subjects' self-regulated energy intake ostensibly placed them at or near and energy-balanced state. Post-exercise energy intake was less than pre-trial energy intake via a weak trend. These time periods covered approximately the same length of time but due to inherent circumstance it would be a stretch to make a conclusion based on a direct comparison.

### **Appetite**

In the fasted condition, appetite was lower after 12 hours than pre- or post-exercise. This did not occur in the fed condition. The difference from pre-exercise is more interesting due to pre-exercise measurements being identically performed in each condition. However, there was no statistical difference between conditions for pre-exercise or 12 hours after exercise. This limits the viability of this datum.

Appetite did not differ greatly between conditions with one notable exception. Post-exercise appetite was not significantly greater than pre-exercise in either condition but was greater after fasted exercise than fed exercise. Post-exercise appetite was measured before breaking the fast in the fasted condition. While this was expected, the effect of HIIE on appetite in a fasted state is not fully established. Fasted exercise has been shown to reduce appetite, but not to the same degree as fed exercise (Deighton et al., 2012). Furthermore, HIIE may not reduce appetite to the same degree as traditional endurance protocols (Deighton et al., 2013). In both of these cases, resulting energy intake was not impacted. How nutritional state and exercise protocol interact remains an unknown quality. Another consideration is that energy deficits manifested through exercise do not increase hunger in contrast with energy deficits derived from restriction of energy intake (Deighton & Stensel,

2014). Fasting necessitates acute periods of energy restriction that may differentially affect appetite from exercise. How these two factors interact is an intriguing question.

### **Limitations**

In addition to the limitations outlined in Chapter 1, several issues became clear during and after data collection. Upon further review, many similar studies utilized a test meal with a caloric content around 100 kcal greater than the 240-kcal meal presently utilized. The assessment of the 12-hour measurements of EE, excess EE,  $VO_2$  and EPOC were constrained by the lack of a control measurement for evening values of these data points. Thus, there was no way to account for diurnal variation in metabolism. It is possible that the circadian rhythm of REE was not a major factor but without a control measurement in the current study, it is not prudent to make conclusions. Diet prescription before trials, intended to achieve a neutral energy balance, was generally adhered to. However, not every subject followed the prescription absolutely. This did not result in a significant difference in energy state from that desired but a better method would be to keep subjects overnight preceding trials. This would not have been possible in the present study.

Physical activity energy expenditure was recorded pre-trial and during the trial days in addition to baseline but measurements taken from the former two time points were dismissed due to an unacceptable number of cases of missing data. Due to this circumstance, baseline paEE was inserted into the formula for calculating energy balance. Consequently, alterations in physical activity levels after HIIE could not be assessed. The findings were intriguing enough to warrant an examination of mechanistic factors of metabolic response to

nutritional state of exercise. The absence of these measures in this study renders the findings doorways to more specific research investigating the primary questions.

## **Practical Applications and Significance**

### *EPOC, HIIE and Exercise Nutrition*

The presented findings contribute to the existing literature regarding the magnitude of EPOC. More specifically, they elucidate the effect after high-intensity, near-maximal, exercise. This is an expanding area of research that could have a dramatic impact on exercise prescription in multiple scenarios. The design also provided insight into the entire metabolic environment surrounding high-intensity exercise due to measurements before, during and after exercise. The role of nutritional state in exercise is another burgeoning field, especially how fasted-state exercise compares to post-prandial exercise. The present results suggest a possible difference between the two in metabolism during exercise that could affect how nutrition is implemented in training programs. Beyond acute exercise, fasted exercise training programs may increase  $VO_{2max}$  to a greater degree as well as increasing resting muscle glycogen content (Stannard et al., 2010). Some factors, like fat metabolism, may only reveal differences between fed and fasted training when sex is accounted for; it appears that males may respond better to fasted training than females (Stannard et al., 2010).

### *Health Impact*

The concept of intermittent fasting became popular in the media faster than research could keep up with. A range of studies on intermittent fasting effects on metabolism and exercise have begun to emerge. Their findings have initiated the analysis of mechanisms involved in fasting. Several older examples set in motion research a direction of research that

has recently accelerated. Ketone concentration and clearance rates appear to be dependent on fast duration (Fery & Balasse, 1983). Fasting increases glucose-sparing hormones and this increase remains even with glucose infusion during fasted exercise (Galbo et al., 1980). How fasting interacts with exercise training programs is a critical question to investigate in the coming years.

If fasted exercise differs from fed exercise in metabolic response, it would be important to know. This could improve desirable outcomes or more importantly, avoid causing any harm in diet and exercise prescriptions. It would be of interest to investigate how fasted or fed exercise fits into a dietary strategy that involves unconventional meal timing, such as intermittent fasting. One suggestion involves a modified fasting strategy that includes one small meal to intersect a longer duration daily fast to improve adherence by blunting appetite spikes (Heilbronn et al., 2005).

### **Future Research Direction**

There are multiple specific inclusions that would benefit the scope of future studies in this area. Tighter control of the delayed metabolic environment, in-person diet and physical activity monitoring, hormone and enzyme activity, variations of exercise intensity and mode along with the obvious culmination in a training study of long-term effects would all be tremendously valuable assets for relevant studies. Subjects of varied training and health statuses are needed to extrapolate results to a broader population.

Considering the rapidly expanding interest in intermittent fasting for metabolic benefit, it is crucial going forward that a better understanding of these metabolic effects is attained. Since exercise has a well-established impact on metabolic health, the union of these

two concepts in research is an obvious path. Studies, like this one, should serve as a first step into a meticulous analysis of fasting as a part of a nutritional protocol. Follow-up studies would ideally include tight control of metabolic factors, especially energy balance and energy intake. Measurement of enzymes, hormones and gene expression would add further layers of detail and help explain the mechanistic side of results that will continue to be revealed. This is an important research question that will be scrutinized and expanded upon greatly in the coming years.

## APPENDIX A

### INFORMED CONSENT DOCUMENT

#### OLD DOMINION UNIVERSITY

#### **PROJECT TITLE:**

The Effect of Fasted vs Fed High-Intensity Interval Exercise on Metabolism and Diet

#### **INTRODUCTION**

The purposes of this form are to give you information that may affect your decision whether to say YES or NO to participation in this research, and to record the consent of those who say YES. This study, The Effect of Fasted vs Fed High-Intensity Interval Exercise on Metabolism and Diet, will take place in the Human Performance Lab (room 2003) of the ODU Student Recreation Center.

#### **RESEARCHERS**

David Swain, PhD, Responsible Project Investigator  
William Perez, Graduate Student

#### **DESCRIPTION OF RESEARCH STUDY**

Several studies have been conducted looking into the subject of fasting and its effects on exercise and metabolism. None of them have explained the effect of fasting and high-intensity interval exercise on the energy expenditure after exercise, or on diet and appetite.

If you decide to participate, then you will join a study involving research of exercising in fasted vs fed conditions. If you say YES, then your participation will last for 6-8 hours over 5 testing sessions at the Student Recreation Center, room 2003. Approximately 20 students will be participating in this study. The following list outlines what will be asked of you.

#### *Visit 1*

- Height, weight, body mass, body composition (sit in chamber for 2 minutes total)
- Resting metabolic rate test (RMR)
  - lie on back with clear canopy over head for 20 minutes to measure metabolism
- Familiarization with the visual analog scale for appetite (VAS)
- Maximal exercise test on stationary bicycle
  - increasing intensity every 2 minutes until you cannot continue
  - done while wearing mouthpiece to collect expired gases
- You will given an accelerometer (similar to an advanced step counter) to wear on your hip and directions for using diet recording software

*Baseline Food Log*

- You will record everything consumed for 3 days with the food log during a normal diet
- Accelerometer will be worn around hip during waking hours
- Avoid strenuous exercise and drug use (nicotine, alcohol, drugs with metabolic effects)
- Turn log and accelerometer in to investigator in person

*Pre-trial Food Log*

- The baseline food log will be used to prescribe a calorically-balanced diet for you before each trial (*visits 2a and 3a*)
  - Diet will be recorded and accelerometer will be worn
- Again, avoid exercise and drug use (including caffeine, this time)
- Time last meal of day to be 12-14 hours before morning visit

*Visits 2a and 2b*

- Arrive at laboratory without breaking the fast
- RMR/VAS
- The following events will differ between *visits 2a and 3a*
  - condition 1 order: meal replacement bar, exercise, metabolic measurement, VAS
  - condition 2 order: exercise, metabolic measurement, VAS, meal replacement bar
- Exercise protocol
  - 20 minutes of interval exercise over 10 rounds
  - each round is 1 minute of near-maximal work followed by 1 minute of low-intensity active recovery work
- Metabolic measurement
  - using RMR equipment, you will sit down while wearing a facemask to collect expired gases for a total of 60 minutes with a 10-minute break in the middle

*Visits 2b and 3b*

- You will be asked to return to the laboratory 12 hours later for RMR/VAS
- During first 10 hours between *visits a and b*, you will keep a food log and wear the accelerometer (Do not consume anything within 2 hours of the return visit)
- You will be allowed to consume anything you want as long as it is recorded
  - exercise and drug use, however, will still not be permitted

**EXCLUSIONARY CRITERIA**

You should have completed a risk stratification questionnaire. To the best of your knowledge, you should not have more than one risk factor or any symptoms that would keep you from participating in this study. You must be a non-smoker. If you are female, you may not be pregnant or think you may be pregnant. You must be between 18 and 35

years old.

### **RISKS AND BENEFITS**

**RISKS:** If you decide to participate in this study, then you may face the known risks of cardiovascular exercise including heart attack and stroke. The researcher tried to reduce these risks by monitoring heart rate during exercise and taking necessary precaution in prescribing and administering the exercise test as well as performing a health screening prior to testing. And, as with any research, there is some possibility that you may be subject to risks that have not yet been identified.

**BENEFITS:** There are no direct benefits for participating in this study. A potential benefit to you for participating in this study is learning how your body responds to exercise in different nutritional conditions. Some people may also benefit by knowing how exercise for metabolic benefit can be made most efficient.

### **COSTS AND PAYMENTS**

The researchers are unable to give you any payment for participating in this study.

### **NEW INFORMATION**

If the researchers find new information during this study that would reasonably change your decision about participating, then they will give it to you.

### **CONFIDENTIALITY**

The researchers will take reasonable steps to keep private information confidential. ID numbers will be used to identify subjects on all forms beyond this consent form. The results of this study may be used in reports, presentations, and publications; but the researcher will not identify you. Of course, your records may be subpoenaed by court order or inspected by government bodies with oversight authority.

### **WITHDRAWAL PRIVILEGE**

It is OK for you to say NO. Even if you say YES now, you are free to say NO later, and walk away or withdraw from the study at any time. Your decision will not affect your relationship with Old Dominion University, or otherwise cause a loss of benefits to which you might otherwise be entitled.

### **COMPENSATION FOR ILLNESS AND INJURY**

If you say YES, then your consent in this document does not waive any of your legal rights. However, in the event of injury arising from this study, neither Old Dominion University nor the researchers are able to give you any money, insurance coverage, free medical care, or any other compensation for such injury. In the event that you suffer injury as a result of participation in any research project, you may contact Dr. David Swain at 757-683-6028, Dr. George Maihafer the current IRB chair at 757-683-4520 at Old Dominion University, or the Old Dominion University Office of Research at 757-683-3460 who will be glad to review the matter with you.



**VOLUNTARY CONSENT**

By signing this form, you are saying several things. You are saying that you have read this form or have had it read to you, that you are satisfied that you understand this form, the research study, and its risks and benefits. The researchers should have answered any questions you may have had about the research. If you have any questions later on, then the researchers should be able to answer them:

Dr. David Swain, 757-683-6028  
William Perez, 757-683-6407

If at any time you feel pressured to participate, or if you have any questions about your rights or this form, then you should call Dr. George Maihafer, the current IRB chair, at 757-683-4520, or the Old Dominion University Office of Research, at 757-683-3460.

And importantly, by signing below, you are telling the researcher YES, that you agree to participate in this study. The researcher should give you a copy of this form for your records.

<b>Subject's Printed Name &amp; Signature</b>	<b>Date</b>
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**INVESTIGATOR'S STATEMENT**

I certify that I have explained to this subject the nature and purpose of this research, including benefits, risks, costs, and any experimental procedures. I have described the rights and protections afforded to human subjects and have done nothing to pressure, coerce, or falsely entice this subject into participating. I am aware of my obligations under state and federal laws, and promise compliance. I have answered the subject's questions and have encouraged him/her to ask additional questions at any time during the course of this study. I have witnessed the above signature(s) on this consent form.

<b>Investigator's Printed Name &amp; Signature</b>	<b>Date</b>
--	-------------



## APPENDIX C

## VAS QUESTIONNAIRE

I am not hungry at all	How hungry do you feel? _____	I have never been more hungry
I am completely empty	How satisfied do you feel? _____	I cannot eat another bite
Not at all full	How full do you feel? _____	Totally full
Nothing at all	How much do you think you can eat? _____	A lot
Yes, very much	Would you like to eat something sweet? _____	No, not at all
Yes, very much	Would you like to eat something salty? _____	No, not at all
Yes, very much	Would you like to eat something savoury? _____	No, not at all
Yes, very much	Would you like to eat something fatty? _____	No, not at all

## REFERENCES

- ACSM. (2013). *ACSM's guidelines for exercise testing and prescription*: Lippincott Williams & Wilkins.
- Aguiar, R. A., Cruz, R. S., Turnes, T., Pereira, K. L., & Caputo, F. (2014). Relationships between  $\dot{V}O_2$  and blood lactate responses after all-out running exercise. *Appl Physiol Nutr Metab*, 1-6. doi:10.1139/apnm-2014-0364
- Aziz, A. R., Chia, M., Singh, R., & Wahid, M. F. (2011). Effects of Ramadan fasting on perceived exercise intensity during high-intensity interval training in elite youth soccer players. *International Journal of Sports Science and Coaching*, 6(1), 87-98.
- Bahr, R., Ingnes, I., Vaage, O., Sejersted, O. M., & Newsholme, E. A. (1987). Effect of duration of exercise on excess postexercise O<sub>2</sub> consumption. *J Appl Physiol (1985)*, 62(2), 485-490.
- Bahr, R., & Sejersted, O. M. (1991a). Effect of feeding and fasting on excess postexercise oxygen consumption. *J Appl Physiol (1985)*, 71(6), 2088-2093.
- Bahr, R., & Sejersted, O. M. (1991b). Effect of intensity of exercise on excess postexercise O<sub>2</sub> consumption. *Metabolism*, 40(8), 836-841.
- Beasley, J., Riley, W. T., & Jean-Mary, J. (2005). Accuracy of a PDA-based dietary assessment program. *Nutrition*, 21(6), 672-677.
- Benedict, C., Hallschmid, M., Lassen, A., Mahnke, C., Schultes, B., Schiöth, H. B., . . . Lange, T. (2011). Acute sleep deprivation reduces energy expenditure in healthy men. *Am J Clin Nutr*, 93(6), 1229-1236. doi:10.3945/ajcn.110.006460
- Bennard, P., & Doucet, E. (2006). Acute effects of exercise timing and breakfast meal glycemic index on exercise-induced fat oxidation. *Applied Physiology, Nutrition, and Metabolism*,

31(5), 502-511.

- Bergman, B. C., & Brooks, G. A. (1999). Respiratory gas-exchange ratios during graded exercise in fed and fasted trained and untrained men. *J Appl Physiol* (1985), 86(2), 479-487.
- Blundell, J. E., Caudwell, P., Gibbons, C., Hopkins, M., Naslund, E., King, N., & Finlayson, G. (2012). Role of resting metabolic rate and energy expenditure in hunger and appetite control: a new formulation. *Dis Model Mech*, 5(5), 608-613. doi:10.1242/dmm.009837
- Blundell, J. E., & King, N. A. (1999). Physical activity and regulation of food intake: current evidence. *Med Sci Sports Exerc*, 31(11 Suppl), S573-583.
- Blundell, J. E., Stubbs, R. J., Hughes, D. A., Whybrow, S., & King, N. A. (2003). Cross talk between physical activity and appetite control: does physical activity stimulate appetite? *Proc Nutr Soc*, 62(3), 651-661. doi:10.1079/PNS2003286
- Boutcher, S. H. (2011). High-intensity intermittent exercise and fat loss. *J Obes*, 2011, 868305. doi:10.1155/2011/868305
- Breton, E. R., Fuemmeler, B. F., & Abrams, L. C. (2011). Weight loss-there is an app for that! But does it adhere to evidence-informed practices? *Transl Behav Med*, 1(4), 523-529. doi:10.1007/s13142-011-0076-5
- Broom, D. R., Batterham, R. L., King, J. A., & Stensel, D. J. (2009). Influence of resistance and aerobic exercise on hunger, circulating levels of acylated ghrelin, and peptide YY in healthy males. *Am J Physiol Regul Integr Comp Physiol*, 296(1), R29-35. doi:10.1152/ajpregu.90706.2008
- Børsheim, E., & Bahr, R. (2003). Effect of exercise intensity, duration and mode on post-exercise oxygen consumption. *Sports Med*, 33(14), 1037-1060.
- Caudwell, P., Finlayson, G., Gibbons, C., Hopkins, M., King, N., Näslund, E., & Blundell, J. E.

- (2013). Resting metabolic rate is associated with hunger, self-determined meal size, and daily energy intake and may represent a marker for appetite. *Am J Clin Nutr*, 97(1), 7-14. doi:10.3945/ajcn.111.029975
- Chan, J. L., Heist, K., DePaoli, A. M., Veldhuis, J. D., & Mantzoros, C. S. (2003). The role of falling leptin levels in the neuroendocrine and metabolic adaptation to short-term starvation in healthy men. *J Clin Invest*, 111(9), 1409-1421. doi:10.1172/JCI17490
- Cheng, M. H., Bushnell, D., Cannon, D. T., & Kern, M. (2009). Appetite regulation via exercise prior or subsequent to high-fat meal consumption. *Appetite*, 52(1), 193-198. doi:10.1016/j.appet.2008.09.015
- Compher, C., Frankenfield, D., Keim, N., & Roth-Yousey, L. (2006). Best practice methods to apply to measurement of resting metabolic rate in adults: a systematic review. *Journal of the American Dietetic Association*, 106(6), 881-903.
- Davitt, P. M., Arent, S. M., Tuazon, M. A., Golem, D. L., & Henderson, G. C. (2013). Postprandial triglyceride and free fatty acid metabolism in obese women after either endurance or resistance exercise. *J Appl Physiol (1985)*, 114(12), 1743-1754. doi:10.1152/jappphysiol.00095.2013
- De Bock, K., Derave, W., Eijnde, B. O., Hesselink, M. K., Koninckx, E., Rose, A. J., . . . Hespel, P. (2008). Effect of training in the fasted state on metabolic responses during exercise with carbohydrate intake. *J Appl Physiol (1985)*, 104(4), 1045-1055. doi:10.1152/jappphysiol.01195.2007
- De Bock, K., Derave, W., Ramaekers, M., Richter, E. A., & Hespel, P. (2007). Fiber type-specific muscle glycogen sparing due to carbohydrate intake before and during exercise. *J Appl Physiol (1985)*, 102(1), 183-188. doi:10.1152/jappphysiol.00799.2006

- De Bock, K., Richter, E. A., Russell, A. P., Eijnde, B. O., Derave, W., Ramaekers, M., . . . Hespel, P. (2005). Exercise in the fasted state facilitates fibre type-specific intramyocellular lipid breakdown and stimulates glycogen resynthesis in humans. *J Physiol*, *564*(Pt 2), 649-660. doi:10.1113/jphysiol.2005.083170
- de Lima, F. D., Correia, A. L. M., da Silva Teixeira, D., da Silva Neto, D. V., Fernandes, Í. S. G., Viana, M. B. X., . . . Alves, S. T. (2015). Acute metabolic response to fasted and postprandial exercise. *International journal of general medicine*, *8*, 255.
- Deighton, K., Barry, R., Connon, C. E., & Stensel, D. J. (2013). Appetite, gut hormone and energy intake responses to low volume sprint interval and traditional endurance exercise. *Eur J Appl Physiol*, *113*(5), 1147-1156. doi:10.1007/s00421-012-2535-1
- Deighton, K., Batterham, R. L., & Stensel, D. J. (2014). Appetite and gut peptide responses to exercise and calorie restriction. The effect of modest energy deficits. *Appetite*, *81*, 52-59.
- Deighton, K., & Stensel, D. J. (2014). Creating an acute energy deficit without stimulating compensatory increases in appetite: is there an optimal exercise protocol? *Proc Nutr Soc*, *73*(2), 352-358. doi:10.1017/S002966511400007X
- Deighton, K., Zahra, J. C., & Stensel, D. J. (2012). Appetite, energy intake and resting metabolic responses to 60 min treadmill running performed in a fasted versus a postprandial state. *Appetite*, *58*(3), 946-954. doi:10.1016/j.appet.2012.02.041
- Deldicque, L., De Bock, K., Maris, M., Ramaekers, M., Nielens, H., Francaux, M., & Hespel, P. (2010). Increased p70s6k phosphorylation during intake of a protein-carbohydrate drink following resistance exercise in the fasted state. *Eur J Appl Physiol*, *108*(4), 791-800.
- Devries, M. C., Hamadeh, M. J., Phillips, S. M., & Tarnopolsky, M. A. (2006). Menstrual cycle phase and sex influence muscle glycogen utilization and glucose turnover during

- moderate-intensity endurance exercise. *Am J Physiol Regul Integr Comp Physiol*, 291(4), R1120-1128. doi:10.1152/ajpregu.00700.2005
- Dohm, G. L., Beeker, R. T., Israel, R. G., & Tapscott, E. B. (1986). Metabolic responses to exercise after fasting. *J Appl Physiol* (1985), 61(4), 1363-1368.
- Dubé, M. C., Tremblay, A., Lavoie, C., & John Weisnagel, S. (2013). Effect of exercise on food consumption and appetite sensations in subjects with diabetes. *Appetite*, 71, 403-410. doi:10.1016/j.appet.2013.08.023
- Fery, F., & Balasse, E. (1983). Ketone body turnover during and after exercise in overnight-fasted and starved humans. *American Journal of Physiology-Endocrinology and Metabolism*, 245(4), E318-E325.
- Flint, A., Raben, A., Blundell, J., & Astrup, A. (2000). Reproducibility, power and validity of visual analogue scales in assessment of appetite sensations in single test meal studies. *International journal of obesity and related metabolic disorders: journal of the International Association for the Study of Obesity*, 24(1), 38-48.
- Frey, G. C., Byrnes, W. C., & Mazzeo, R. S. (1993). Factors influencing excess postexercise oxygen consumption in trained and untrained women. *Metabolism*, 42(7), 822-828.
- Fukuba, Y., Yano, Y., Murakami, H., Kan, A., & Miura, A. (2000). The effect of dietary restriction and menstrual cycle on excess post-exercise oxygen consumption (EPOC) in young women. *Clin Physiol*, 20(2), 165-169.
- Gaesser, G. A., & Brooks, G. A. (1984). Metabolic bases of excess post-exercise oxygen consumption: a review. *Med Sci Sports Exerc*, 16(1), 29-43.
- Galbo, H., Christensen, N., Mikines, K., Sonne, B., Hilsted, J., Hagen, C., & Fahrenkrug, J. (1981). The Effect of Fasting on the Hormonal Response to Graded Exercise\*. *The*



- Journal of Clinical Endocrinology & Metabolism*, 52(6), 1106-1112.
- Garber, C. E., Blissmer, B., Deschenes, M. R., Franklin, B. A., Lamonte, M. J., Lee, I.-M., . . . Swain, D. P. (2011). American College of Sports Medicine position stand. Quantity and quality of exercise for developing and maintaining cardiorespiratory, musculoskeletal, and neuromotor fitness in apparently healthy adults: guidance for prescribing exercise. *Medicine and science in sports and exercise*, 43(7), 1334-1359.
- Gibala, M. J., Little, J. P., Macdonald, M. J., & Hawley, J. A. (2012). Physiological adaptations to low-volume, high-intensity interval training in health and disease. *J Physiol*, 590(Pt 5), 1077-1084. doi:10.1113/jphysiol.2011.224725
- Gibala, M. J., McGee, S. L., Garnham, A. P., Howlett, K. F., Snow, R. J., & Hargreaves, M. (2009). Brief intense interval exercise activates AMPK and p38 MAPK signaling and increases the expression of PGC-1alpha in human skeletal muscle. *J Appl Physiol (1985)*, 106(3), 929-934. doi:10.1152/jappphysiol.90880.2008
- Gillen, J. B., Percival, M. E., Ludzki, A., Tarnopolsky, M. A., & Gibala, M. J. (2013). Interval training in the fed or fasted state improves body composition and muscle oxidative capacity in overweight women. *Obesity (Silver Spring)*. doi:10.1002/oby.20379
- Gleeson, M., Greenhaff, P., & Maughan, R. (1988). Influence of a 24 h fast on high intensity cycle exercise performance in man. *European journal of applied physiology and occupational physiology*, 57(6), 653-659.
- Gonzalez, J. T., Veasey, R. C., Rumbold, P. L., & Stevenson, E. J. (2012). Consistency of metabolic responses and appetite sensations under post absorptive and postprandial conditions. *Appetite*, 59(2), 228-233. doi:10.1016/j.appet.2012.02.043
- Groop, L. C., Bonadonna, R., DelPrato, S., Ratheiser, K., & DeFronzo, R. A. (1990). Effect of

- prolonged overnight fasting on energy metabolism in non-insulin-dependent diabetic and non-diabetic subjects. *Acta Endocrinol (Copenh)*, 123(1), 30-36.
- Hagobian, T. A., Yamashiro, M., Hinkel-Lipsker, J., Streder, K., Evero, N., & Hackney, T. (2013). Effects of acute exercise on appetite hormones and ad libitum energy intake in men and women. *Appl Physiol Nutr Metab*, 38(1), 66-72. doi:10.1139/apnm-2012-0104
- Harvie, M., Wright, C., Pegington, M., McMullan, D., Mitchell, E., Martin, B., . . . Howell, A. (2013). The effect of intermittent energy and carbohydrate restriction v. daily energy restriction on weight loss and metabolic disease risk markers in overweight women. *Br J Nutr*, 110(8), 1534-1547. doi:10.1017/S0007114513000792
- Haugen, H. A., Melanson, E. L., Tran, Z. V., Kearney, J. T., & Hill, J. O. (2003). Variability of measured resting metabolic rate. *Am J Clin Nutr*, 78(6), 1141-1145.
- Heilbronn, L. K., Smith, S. R., Martin, C. K., Anton, S. D., & Ravussin, E. (2005). Alternate-day fasting in nonobese subjects: effects on body weight, body composition, and energy metabolism. *Am J Clin Nutr*, 81(1), 69-73.
- Henderson, G. C. (2014). Sexual dimorphism in the effects of exercise on metabolism of lipids to support resting metabolism. *Front Endocrinol (Lausanne)*, 5, 162. doi:10.3389/fendo.2014.00162
- Henderson, G. C., & Alderman, B. L. (2014). Determinants of resting lipid oxidation in response to a prior bout of endurance exercise. *J Appl Physiol (1985)*, 116(1), 95-103. doi:10.1152/jappphysiol.00956.2013
- Henderson, G. C., Fattor, J. A., Horning, M. A., Faghihnia, N., Johnson, M. L., Mau, T. L., . . . Brooks, G. A. (2007). Lipolysis and fatty acid metabolism in men and women during the postexercise recovery period. *J Physiol*, 584(Pt 3), 963-981.

doi:10.1113/jphysiol.2007.137331

Heydari, M., Freund, J., & Boutcher, S. H. (2012). The effect of high-intensity intermittent exercise on body composition of overweight young males. *J Obes*, 2012, 480467.

doi:10.1155/2012/480467

Hill, A. J., & Blundell, J. E. (1982). Nutrients and behaviour: research strategies for the investigation of taste characteristics, food preferences, hunger sensations and eating patterns in man. *J Psychiatr Res*, 17(2), 203-212.

Horton, T. J., Pagliassotti, M. J., Hobbs, K., & Hill, J. O. (1998). Fuel metabolism in men and women during and after long-duration exercise. *J Appl Physiol* (1985), 85(5), 1823-1832.

Hubert, P., King, N. A., & Blundell, J. E. (1998). Uncoupling the effects of energy expenditure and energy intake: appetite response to short-term energy deficit induced by meal omission and physical activity. *Appetite*, 31(1), 9-19. doi:10.1006/appe.1997.0148

Johannsen, D. L., Knuth, N. D., Huizenga, R., Rood, J. C., Ravussin, E., & Hall, K. D. (2012). Metabolic slowing with massive weight loss despite preservation of fat-free mass. *J Clin Endocrinol Metab*, 97(7), 2489-2496. doi:10.1210/jc.2012-1444

Johnstone, A. M., Faber, P., Gibney, E. R., Elia, M., Horgan, G., Golden, B. E., & Stubbs, R. J. (2002). Effect of an acute fast on energy compensation and feeding behaviour in lean men and women. *Int J Obes Relat Metab Disord*, 26(12), 1623-1628.

doi:10.1038/sj.ijo.0802151

Kelly, B., King, J. A., Goerlach, J., & Nimmo, M. A. (2013). The impact of high-intensity intermittent exercise on resting metabolic rate in healthy males. *Eur J Appl Physiol*.

doi:10.1007/s00421-013-2741-5

King, J. A., Miyashita, M., Wasse, L. K., & Stensel, D. J. (2010). Influence of prolonged

- treadmill running on appetite, energy intake and circulating concentrations of acylated ghrelin. *Appetite*, 54(3), 492-498. doi:10.1016/j.appet.2010.02.002
- King, J. A., Wasse, L. K., Broom, D. R., & Stensel, D. J. (2010). Influence of brisk walking on appetite, energy intake, and plasma acylated ghrelin. *Med Sci Sports Exerc*, 42(3), 485-492. doi:10.1249/MSS.0b013e3181ba10c4
- King, J. A., Wasse, L. K., & Stensel, D. J. (2013). Acute exercise increases feeding latency in healthy normal weight young males but does not alter energy intake. *Appetite*, 61(1), 45-51. doi:10.1016/j.appet.2012.10.018
- King, N. A., Burley, V. J., & Blundell, J. E. (1994). Exercise-induced suppression of appetite: effects on food intake and implications for energy balance. *Eur J Clin Nutr*, 48(10), 715-724.
- King, N. A., Lluch, A., Stubbs, R. J., & Blundell, J. E. (1997). High dose exercise does not increase hunger or energy intake in free living males. *Eur J Clin Nutr*, 51(7), 478-483.
- King, N. A., Snell, L., Smith, R. D., & Blundell, J. E. (1996). Effects of short-term exercise on appetite responses in unrestrained females. *Eur J Clin Nutr*, 50(10), 663-667.
- Klein, S., Sakurai, Y., Romijn, J. A., & Carroll, R. M. (1993). Progressive alterations in lipid and glucose metabolism during short-term fasting in young adult men. *Am J Physiol*, 265(5 Pt 1), E801-806.
- Klempel, M. C., Kroeger, C. M., & Varady, K. A. (2013). Alternate day fasting (ADF) with a high-fat diet produces similar weight loss and cardio-protection as ADF with a low-fat diet. *Metabolism*, 62(1), 137-143. doi:10.1016/j.metabol.2012.07.002
- Knab, A. M., Shanely, R. A., Corbin, K. D., Jin, F., Sha, W., & Nieman, D. C. (2011). A 45-minute vigorous exercise bout increases metabolic rate for 14 hours. *Med Sci Sports*

- Exerc*, 43(9), 1643-1648. doi:10.1249/MSS.0b013e3182118891
- LaForgia, J., Withers, R. T., & Gore, C. J. (2006). Effects of exercise intensity and duration on the excess post-exercise oxygen consumption. *J Sports Sci*, 24(12), 1247-1264. doi:10.1080/02640410600552064
- Larsen, I., Welde, B., Martins, C., & Tjønnå, A. E. (2014). High- and moderate-intensity aerobic exercise and excess post-exercise oxygen consumption in men with metabolic syndrome. *Scand J Med Sci Sports*, 24(3), e174-179. doi:10.1111/sms.12132
- Little, J. P., Gillen, J. B., Percival, M. E., Safdar, A., Tarnopolsky, M. A., Punthakee, Z., . . . Gibala, M. J. (2011). Low-volume high-intensity interval training reduces hyperglycemia and increases muscle mitochondrial capacity in patients with type 2 diabetes. *J Appl Physiol (1985)*, 111(6), 1554-1560. doi:10.1152/jappphysiol.00921.2011
- Lluch, A., King, N. A., & Blundell, J. E. (1998). Exercise in dietary restrained women: no effect on energy intake but change in hedonic ratings. *Eur J Clin Nutr*, 52(4), 300-307.
- Longo, V. D., & Mattson, M. P. (2014). Fasting: molecular mechanisms and clinical applications. *Cell Metab*, 19(2), 181-192. doi:10.1016/j.cmet.2013.12.008
- Loon, L. J., Koopman, R., Stegen, J. H., Wagenmakers, A. J., Keizer, H. A., & Saris, W. H. (2003). Intramyocellular lipids form an important substrate source during moderate intensity exercise in endurance-trained males in a fasted state. *The Journal of physiology*, 553(2), 611-625.
- Loy, S. F., Conlee, R. K., Winder, W. W., Nelson, A. G., Arnall, D. A., & Fisher, A. G. (1986). Effects of 24-hour fast on cycling endurance time at two different intensities. *Journal of Applied Physiology*, 61(2), 654-659.
- MacIntosh, C. G., Horowitz, M., Verhagen, M. A., Smout, A. J., Wishart, J., Morris, H., . . .

- Chapman, I. M. (2001). Effect of small intestinal nutrient infusion on appetite, gastrointestinal hormone release, and gastric myoelectrical activity in young and older men. *Am J Gastroenterol*, *96*(4), 997-1007. doi:10.1111/j.1572-0241.2001.03684.x
- MacIntosh, C. G., Morley, J. E., Wishart, J., Morris, H., Jansen, J. B., Horowitz, M., & Chapman, I. M. (2001). Effect of exogenous cholecystokinin (CCK)-8 on food intake and plasma CCK, leptin, and insulin concentrations in older and young adults: evidence for increased CCK activity as a cause of the anorexia of aging. *J Clin Endocrinol Metab*, *86*(12), 5830-5837. doi:10.1210/jcem.86.12.8107
- MacIntosh, C. G., Sheehan, J., Davani, N., Morley, J. E., Horowitz, M., & Chapman, I. M. (2001). Effects of aging on the opioid modulation of feeding in humans. *J Am Geriatr Soc*, *49*(11), 1518-1524.
- Maehlum, S., Grandmontagne, M., Newsholme, E. A., & Sejersted, O. M. (1986). Magnitude and duration of excess postexercise oxygen consumption in healthy young subjects. *Metabolism*, *35*(5), 425-429.
- Magkos, F., Wright, D. C., Patterson, B. W., Mohammed, B. S., & Mittendorfer, B. (2006). Lipid metabolism response to a single, prolonged bout of endurance exercise in healthy young men. *Am J Physiol Endocrinol Metab*, *290*(2), E355-362. doi:10.1152/ajpendo.00259.2005
- Martin, B., Mattson, M. P., & Maudsley, S. (2006). Caloric restriction and intermittent fasting: two potential diets for successful brain aging. *Ageing Res Rev*, *5*(3), 332-353. doi:10.1016/j.arr.2006.04.002
- Martins, C., Morgan, L., & Truby, H. (2008). A review of the effects of exercise on appetite regulation: an obesity perspective. *Int J Obes (Lond)*, *32*(9), 1337-1347.

doi:10.1038/ijo.2008.98

Martins, C., Truby, H., & Morgan, L. M. (2007). Short-term appetite control in response to a 6-week exercise programme in sedentary volunteers. *Br J Nutr*, *98*(4), 834-842.

doi:10.1017/S000711450774922X

Matsuo, T., Saitoh, S., & Suzuki, M. (1999). Effects of the menstrual cycle on excess postexercise oxygen consumption in healthy young women. *Metabolism*, *48*(3), 275-277.

Mattson, M. P. (2008). Dietary factors, hormesis and health. *Ageing Res Rev*, *7*(1), 43-48.

doi:10.1016/j.arr.2007.08.004

Maughan, R. J., Fallah, J., & Coyle, E. F. (2010). The effects of fasting on metabolism and performance. *Br J Sports Med*, *44*(7), 490-494. doi:10.1136/bjism.2010.072181

Melanson, E. L., MacLean, P. S., & Hill, J. O. (2009). Exercise improves fat metabolism in muscle but does not increase 24-h fat oxidation. *Exerc Sport Sci Rev*, *37*(2), 93-101.

doi:10.1097/JES.0b013e31819c2f0b

Melby, C., Scholl, C., Edwards, G., & Bullough, R. (1993). Effect of acute resistance exercise on postexercise energy expenditure and resting metabolic rate. *J Appl Physiol (1985)*, *75*(4), 1847-1853.

Montain, S. J., Hopper, M. K., Coggan, A. R., & Coyle, E. F. (1991). Exercise metabolism at different time intervals after a meal. *J Appl Physiol (1985)*, *70*(2), 882-888.

Neel, J. V. (1962). Diabetes mellitus: a "thrifty" genotype rendered detrimental by "progress"? *American journal of human genetics*, *14*(4), 353.

Newsom, S. A., Schenk, S., Thomas, K. M., Harber, M. P., Knuth, N. D., Goldenberg, N., & Horowitz, J. F. (2010). Energy deficit after exercise augments lipid mobilization but does not contribute to the exercise-induced increase in insulin sensitivity. *J Appl Physiol*

- (1985), 108(3), 554-560. doi:10.1152/jappphysiol.01106.2009
- Nieman, D. C., Carlson, K. A., Brandstater, M. E., Naegele, R. T., & Blankenship, J. W. (1987). Running endurance in 27-h-fasted humans. *J Appl Physiol* (1985), 63(6), 2502-2509.
- Ortiz, O., Russell, M., Daley, T. L., Baumgartner, R. N., Waki, M., Lichtman, S., . . . Heymsfield, S. B. (1992). Differences in skeletal muscle and bone mineral mass between black and white females and their relevance to estimates of body composition. *The American journal of clinical nutrition*, 55(1), 8-13.
- Pacy, P. J., Barton, N., Webster, J. D., & Garrow, J. S. (1985). The energy cost of aerobic exercise in fed and fasted normal subjects. *The American journal of clinical nutrition*, 42(5), 764-768.
- Paoli, A., Marcolin, G., Zonin, F., Neri, M., Sivieri, A., & Pacelli, Q. F. (2011). Exercising fasting or fed to enhance fat loss? Influence of food intake on respiratory ratio and excess postexercise oxygen consumption after a bout of endurance training. *Int J Sport Nutr Exerc Metab*, 21(1), 48-54.
- Paoli, A., Moro, T., Marcolin, G., Neri, M., Bianco, A., Palma, A., & Grimaldi, K. (2012). High-Intensity Interval Resistance Training (HIRT) influences resting energy expenditure and respiratory ratio in non-dieting individuals. *J Transl Med*, 10, 237. doi:10.1186/1479-5876-10-237
- Parker, B. A., Sturm, K., MacIntosh, C. G., Feinle, C., Horowitz, M., & Chapman, I. M. (2004). Relation between food intake and visual analogue scale ratings of appetite and other sensations in healthy older and young subjects. *Eur J Clin Nutr*, 58(2), 212-218. doi:10.1038/sj.ejcn.1601768
- Parr, B. B., Strath, S. J., & Howley, E. T. Validation of the COSMED K4b2 portable metabolic



measurement system.

Perez, W. J., & Rynders, C. A. (2014). Low-volume high intensity interval exercise performed in the fasted, not postprandial state increases post-exercise metabolic rate. Manuscript submitted for publication.

Poplawski, M. M., Mastaitis, J. W., Yang, X. J., & Mobbs, C. V. (2010). Hypothalamic responses to fasting indicate metabolic reprogramming away from glycolysis toward lipid oxidation. *Endocrinology*, *151*(11), 5206-5217. doi:10.1210/en.2010-0702

Ratheiser, K. M., Brillon, D. J., Campbell, R. G., & Matthews, D. E. (1998). Epinephrine produces a prolonged elevation in metabolic rate in humans. *Am J Clin Nutr*, *68*(5), 1046-1052.

Ravn, A. M., Gregersen, N. T., Christensen, R., Rasmussen, L. G., Hels, O., Belza, A., . . . Astrup, A. (2013). Thermic effect of a meal and appetite in adults: an individual participant data meta-analysis of meal-test trials. *Food Nutr Res*, *57*. doi:10.3402/fnr.v57i0.19676

Riddell, M. C., Partington, S. L., Stupka, N., Armstrong, D., Rennie, C., & Tarnopolsky, M. A. (2003). Substrate utilization during exercise performed with and without glucose ingestion in female and male endurance trained athletes. *Int J Sport Nutr Exerc Metab*, *13*(4), 407-421.

Romijn, J. A., Godfried, M. H., Hommes, M. J., Endert, E., & Sauerwein, H. P. (1990). Decreased glucose oxidation during short-term starvation. *Metabolism*, *39*(5), 525-530.

Schabort, E. J., Bosch, A. N., Weltan, S. M., & Noakes, T. D. (1999). The effect of a preexercise meal on time to fatigue during prolonged cycling exercise. *Medicine and science in sports and exercise*, *31*(3), 464-471.

- Sevits, K. J., Melanson, E. L., Swibas, T., Binns, S. E., Klochak, A. L., Lonac, M. C., . . . Bell, C. (2013). Total daily energy expenditure is increased following a single bout of sprint interval training. *Physiol Rep*, *1*(5), e00131. doi:10.1002/phy2.131
- Shetty, P. S. (1990). Physiological mechanisms in the adaptive response of metabolic rates to energy restriction. *Nutr Res Rev*, *3*(1), 49-74. doi:10.1079/NRR19900006
- Shimada, K., Yamamoto, Y., Iwayama, K., Nakamura, K., Yamaguchi, S., Hibi, M., . . . Tokuyama, K. (2013). Effects of post-absorptive and postprandial exercise on 24h fat oxidation. *Metabolism*, *62*(6), 793-800.
- Sim, A. Y., Wallman, K. E., Fairchild, T. J., & Guelfi, K. J. (2014). High-intensity intermittent exercise attenuates ad-libitum energy intake. *Int J Obes (Lond)*, *38*(3), 417-422. doi:10.1038/ijo.2013.102
- Simmons, R. L. "The Effects of Fitness Level and Sex on Epoc Following High Intensity Interval and Moderate Intensity Aerobic Exercise" (master's thesis, Old Dominion University, 2016), 28-29.
- Siri, W. E. (1961). Body composition from fluid spaces and density: analysis of methods. *Techniques for measuring body composition*, *61*, 223-244.
- Skelly, L. E., Andrews, P. C., Gillen, J. B., Martin, B. J., Percival, M. E., & Gibala, M. J. (2014). High-intensity interval exercise induces 24-h energy expenditure similar to traditional endurance exercise despite reduced time commitment. *Appl Physiol Nutr Metab*. doi:10.1139/apnm-2013-0562
- Speakman, J. R., & Selman, C. (2003). Physical activity and resting metabolic rate. *Proc Nutr Soc*, *62*(3), 621-634. doi:10.1079/PNS2003282
- Stannard, S. R., Buckley, A. J., Edge, J. A., & Thompson, M. W. (2010). Adaptations to skeletal

- muscle with endurance exercise training in the acutely fed versus overnight-fasted state. *Journal of Science and Medicine in Sport*, 13(4), 465-469.
- Stannard, S. R., Thompson, M. W., Fairbairn, K., Huard, B., Sachinwalla, T., & Thompson, C. H. (2002). Fasting for 72 h increases intramyocellular lipid content in nondiabetic, physically fit men. *Am J Physiol Endocrinol Metab*, 283(6), E1185-1191. doi:10.1152/ajpendo.00108.2002
- Stubbs, R. J., Hughes, D. A., Johnstone, A. M., Rowley, E., Reid, C., Elia, M., . . . Blundell, J. E. (2000). The use of visual analogue scales to assess motivation to eat in human subjects: a review of their reliability and validity with an evaluation of new hand-held computerized systems for temporal tracking of appetite ratings. *Br J Nutr*, 84(4), 405-415.
- Sturm, K., MacIntosh, C. G., Parker, B. A., Wishart, J., Horowitz, M., & Chapman, I. M. (2003). Appetite, food intake, and plasma concentrations of cholecystokinin, ghrelin, and other gastrointestinal hormones in undernourished older women and well-nourished young and older women. *J Clin Endocrinol Metab*, 88(8), 3747-3755. doi:10.1210/jc.2002-021656
- Sweileh, N., Schnitzler, A., Hunter, G. R., & Davis, B. (1992). Body composition and energy metabolism in resting and exercising muslims during Ramadan fast. *J Sports Med Phys Fitness*, 32(2), 156-163.
- Talanian, J. L., Galloway, S. D., Heigenhauser, G. J., Bonen, A., & Spriet, L. L. (2007). Two weeks of high-intensity aerobic interval training increases the capacity for fat oxidation during exercise in women. *J Appl Physiol (1985)*, 102(4), 1439-1447. doi:10.1152/jappphysiol.01098.2006
- Tarnopolsky, M. A. (2008). Sex differences in exercise metabolism and the role of 17-beta estradiol. *Med Sci Sports Exerc*, 40(4), 648-654. doi:10.1249/MSS.0b013e31816212ff

- Thearle, M. S., Pannacciulli, N., Bonfiglio, S., Pacak, K., & Krakoff, J. (2013). Extent and determinants of thermogenic responses to 24 hours of fasting, energy balance, and five different overfeeding diets in humans. *J Clin Endocrinol Metab*, *98*(7), 2791-2799. doi:10.1210/jc.2013-1289
- Thivel, D., Isacco, L., Montaurier, C., Boirie, Y., Duché, P., & Morio, B. (2012). The 24-h energy intake of obese adolescents is spontaneously reduced after intensive exercise: a randomized controlled trial in calorimetric chambers. *PLoS One*, *7*(1), e29840. doi:10.1371/journal.pone.0029840
- Trapp, E. G., Chisholm, D. J., Freund, J., & Boutcher, S. H. (2008). The effects of high-intensity intermittent exercise training on fat loss and fasting insulin levels of young women. *Int J Obes (Lond)*, *32*(4), 684-691. doi:10.1038/sj.ijo.0803781
- Tremblay, A., Coveney, S., Després, J. P., Nadeau, A., & Prud'homme, D. (1992). Increased resting metabolic rate and lipid oxidation in exercise-trained individuals: evidence for a role of beta-adrenergic stimulation. *Can J Physiol Pharmacol*, *70*(10), 1342-1347.
- Treuth, M. S., Hunter, G. R., & Williams, M. (1996). Effects of exercise intensity on 24-h energy expenditure and substrate oxidation. *Med Sci Sports Exerc*, *28*(9), 1138-1143.
- Trombold, J. R., Christmas, K. M., Machin, D. R., Kim, I. Y., & Coyle, E. F. (2013). Acute high-intensity endurance exercise is more effective than moderate-intensity exercise for attenuation of postprandial triglyceride elevation. *J Appl Physiol (1985)*, *114*(6), 792-800. doi:10.1152/jappphysiol.01028.2012
- Uranga, A. P., Levine, J., & Jensen, M. (2005). Isotope tracer measures of meal fatty acid metabolism: reproducibility and effects of the menstrual cycle. *Am J Physiol Endocrinol Metab*, *288*(3), E547-555. doi:10.1152/ajpendo.00340.2004

- Vaiksaar, S., Jürimäe, J., Mäestu, J., Purge, P., Kalytka, S., Shakhlina, L., & Jürimäe, T. (2011a). No effect of menstrual cycle phase and oral contraceptive use on endurance performance in rowers. *J Strength Cond Res*, 25(6), 1571-1578. doi:10.1519/JSC.0b013e3181df7fd2
- Vaiksaar, S., Jürimäe, J., Mäestu, J., Purge, P., Kalytka, S., Shakhlina, L., & Jürimäe, T. (2011b). No effect of menstrual cycle phase on fuel oxidation during exercise in rowers. *Eur J Appl Physiol*, 111(6), 1027-1034. doi:10.1007/s00421-010-1730-1
- Van Proeyen, K., De Bock, K., & Hespel, P. (2011). Training in the fasted state facilitates re-activation of eEF2 activity during recovery from endurance exercise. *Eur J Appl Physiol*, 111(7), 1297-1305. doi:10.1007/s00421-010-1753-7
- Van Proeyen, K., Szlufcik, K., Nielens, H., Pelgrim, K., Deldicque, L., Hesselink, M., . . . Hespel, P. (2010). Training in the fasted state improves glucose tolerance during fat-rich diet. *J Physiol*, 588(Pt 21), 4289-4302. doi:10.1113/jphysiol.2010.196493
- Van Proeyen, K., Szlufcik, K., Nielens, H., Ramaekers, M., & Hespel, P. (2011). Beneficial metabolic adaptations due to endurance exercise training in the fasted state. *J Appl Physiol (1985)*, 110(1), 236-245. doi:10.1152/jappphysiol.00907.2010
- Warren, A., Howden, E. J., Williams, A. D., Fell, J. W., & Johnson, N. A. (2009). Postexercise fat oxidation: effect of exercise duration, intensity, and modality. *Int J Sport Nutr Exerc Metab*, 19(6), 607-623.
- Weir, J. d. V. (1949). New methods for calculating metabolic rate with special reference to protein metabolism. *The Journal of physiology*, 109(1-2), 1.
- Wendorf, M., & Goldfine, I. D. (1991). Archaeology of NIDDM. Excavation of the "thrifty" genotype. *Diabetes*, 40(2), 161-165.
- Weststrate, J. A. (1993). Resting metabolic rate and diet-induced thermogenesis: a

- methodological reappraisal. *Am J Clin Nutr*, 58(5), 592-601.
- Williams, C. B., Zelt, J. G., Castellani, L. N., Little, J. P., Jung, M. E., Wright, D. C., . . . Gurd, B. J. (2013). Changes in mechanisms proposed to mediate fat loss following an acute bout of high-intensity interval and endurance exercise. *Appl Physiol Nutr Metab*, 38(12), 1236-1244. doi:10.1139/apnm-2013-0101
- Wolfe, R. R., Peters, E. J., Klein, S., Holland, O. B., Rosenblatt, J., & Gary, H. (1987). Effect of short-term fasting on lipolytic responsiveness in normal and obese human subjects. *Am J Physiol*, 252(2 Pt 1), E189-196.
- Yoshimura, S., Fujita, Y., Hirate, H., Kusama, N., Azami, T., & Sobue, K. (2014). A short period of fasting before surgery conserves basal metabolism and suppresses catabolism according to indirect calorimetry performed under general anesthesia. *J Anesth*. doi:10.1007/s00540-014-1950-3
- Zauner, C., Schneeweiss, B., Kranz, A., Madl, C., Ratheiser, K., Kramer, L., . . . Lenz, K. (2000). Resting energy expenditure in short-term starvation is increased as a result of an increase in serum norepinephrine. *Am J Clin Nutr*, 71(6), 1511-1515.
- Zinczenko, D., & Moore, P. (2013). *The 8-Hour Diet: Watch the Pounds Disappear Without Watching What You Eat!* : Rodale Books.
- Zurlo, F., Schutz, Y., Frascarolo, P., Enzi, G., Deriaz, O., & Jequier, E. (1986). Variability of resting energy expenditure in healthy volunteers during fasting and continuous enteral feeding. *Critical care medicine*, 14(6), 535-538.

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