Effects of High-Intensity Interval Exercise (HIIE) vs Moderate-Intensity Continuous Exercise (MIE) on Postprandial Substrate Oxidation After Consumption of an Isocaloric High Sugar/ Fat Meal in Healthy Adults

by

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ABSTRACT

Obesity prevalence is high in the United States, in part due to increased fat storage following consumption of high fat/carbohydrate (sugar) foods. Following a meal, carbohydrate stimulates its own oxidation, while simultaneously suppressing fat oxidation, ultimately leading to fat storage. Aerobic exercise preceding a meal increases fat oxidation in the postprandial period, which may reduce fat storage. The ideal exercise prescription for optimal postprandial fat oxidation is unknown. The effect of low and moderate intensity continuous exercise (MIE) has been studied extensively, while the effects of high-intensity interval exercise (HIIE) on post-prandial substrate oxidation has not been examined. The purpose of this study was to compare the effects of MIE and HIIE on postprandial substrate oxidation after consumption of an isocaloric meal (2 glazed donuts; 520 kcal) in healthy adults. Ten subjects (8 males, 2 females; age=24yr, BMI=24 kg/m^2) completed three conditions in random order: 1) no exercise control; 2) MIE: cycling at 60-75%HR_{max}; 3) HIIE: cycling at 90-95%HR_{max}. The duration of each exercise bout was sufficient to expend approximately 520 kcal, the energy equivalent of the donuts, which were consumed 1 hour post-exercise. Immediately after consuming the donuts, pulmonary ventilation and gas exchange were measured breath-by-breath continuously and recorded (min-by-min) for 5 hours. Repeated measures analysis of covariance was used to compare the mean differences in outcome variables accounting for gender. Absolute postprandial fat oxidation (g/5 hours) was 17.3±5.4, 27.1±9.6 and 23±1.2 for control, MIE and HIIE trials respectively, with the postprandial fat oxidation significantly greater for the two exercise conditions compared to control. Relative to baseline values, both exercise conditions resulted in cumulative net postprandial fat

oxidation significantly greater than control (control = -1.79±3.99g; MIE = 11.51±8.41g, HIIE= 9.51±5.20g). Therefore, results indicate that exercise most certainly increases postprandial fat oxidation, and that exercise type, either MIE or HIIE, is not as important as total energy expended. The fact that exercise of ~1 hour was required to oxidize the amount of fat in two donuts, that required only a few minutes to consume, highlights the challenges of using exercise for weight control in an obesogenic environment.

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CHAPTER 1

INTRODUCTION

Can fat burning be optimized in humans in order to enhance postprandial fat oxidation and reduce chances of positive fat balance? Since 1973, computer-monitored substrate oxidation has been a scientifically employed method to evaluate the macronutrient metabolism of humans (Beaver, Wasserman, & Whipp, 1973). Throughout that time, metabolism has been studied to identify differences attributable to sex, ethnicity, age and body mass index (BMI). Substrate oxidation has been an important tool for studying different disease states such as diabetes, coronary artery disease or congestive heart failure, those with metabolic syndrome, or obesity (Moholdt et al., 2009; Munk, Staal, Butt, Isaksen, & Larsen, 2009; Warburton et al., 2005; Wisloff et al., 2007). Substrate oxidation has also been used in studying exercise performance (Jacobs et al., 2013; Lindsay et al., 1996; Stepto, Hawley, Dennis, & Hopkins, 1999), and skeletal muscle metabolism (Stepto, Martin, Fallon, & Hawley, 2001; Westgarth-Taylor et al., 1997).

Obesity prevalence is high in the United States, and this may be due in part to increased fat storage following consumption of foods high in fat and carbohydrate (sugar) like that characteristic of the western diet. It is known that carbohydrate stimulates its own oxidation while at the same time suppressing fat oxidation (Bobbioni-Harsch et al., 1997; E Jequier, 1993). Exercise increases fat oxidation both during and after exercise (Bahr & Sejersted, 1991; W. J. Tucker, S. S. Angadi, & G. A. Gaesser, 2016), which may reduce fat storage.

Postprandial substrate oxidation has been experimentally tested for decades, and substrate oxidation has been examined in conjunction with different durations and intensities of exercise (W. J. Tucker et al., 2016). Available research has also compared various durations and intensities of exercise with a variety of meals of different compositions either following or preceding exercise to look at energy expenditure and substrate oxidation. To date, there has yet to be a randomized clinical trial that examines how exercise of different intensities, specifically high-intensity interval exercise, affects the utilization of nutrients with subsequent ingestion of a meal comprised of mostly fat and sugar. A previous study conducted by Hansen suggests that exercise may increase the ratio of fat to carbohydrate that is oxidized postprandial (Hansen, Zhang, Gomez, Adams, & Schoeller, 2007). It is possible that exercise preceding a meal consisting primarily of sugar and fat will increase the nutrient partitioning and oxidation of fat, which potentially could affect fat storage and, ultimately, body composition over time.

Purpose and Hypothesis

This study compared the effect of high-intensity interval exercise (HIIE) and moderate-intensity continuous exercise (MIE) on postprandial substrate oxidation/nutrient partitioning after consumption of an isocaloric high sugar/high fat meal ~520 calories (2 glazed donuts) in adults 18-45 (20-28) years of age.

Hypothesis 1: Because evidence supports that the higher the intensity of the exercise bout the more elevated the subject's excess post-exercise oxygen consumption (EPOC) (Bahr & Sejersted, 1991), a larger postprandial oxidation of lipids will be demonstrated after the HIIE isocaloric exercise bout compared to the MIE isocaloric exercise.

Definition of Terms

Excess post-exercise oxygen consumption: The volume of oxygen consumed after exercise above resting metabolic rate.

VO_{2max}: Maximal Volume of oxygen consumed per minute. Maximal VO_2 is an indicator of cardiovascular fitness.

Moderate intensity continuous exercise (MIE) (Crespo, Mullane, Zeigler, Buman, & Gaesser, 2016; Knurick, Johnston, & Gaesser, 2016; Tucker, Sawyer, Jarrett, Bhammar, & Gaesser, 2015)**:** Subjects exercise at a continuous pace at approximately 50% of their VO_{2max}.

High intensity interval exercise (HIIE): Subjects alternate 1-minute work 90-95% VO_{2max} and 1-minute rest period (Esfandiari, Sasson, & Goodman, 2014; Gibala, Little, Macdonald, & Hawley, 2012; Gillen et al., 2012; Jacobs et al., 2013; Little et al., 2011; Skelly et al., 2014; Tucker et al., 2015).

Nutrient Partitioning: Metabolism of macronutrients, including oxidation, storage and conversion to other substrates.

Substrate Oxidation: The metabolism or breakdown of the macronutrient substrates carbohydrate and fat, this is often measured by indirect calorimetry.

Substrate Utilization: Similar to substrate oxidation, specifically how the particular substrate is used.

Isocaloric: Isocaloric translates to “same calories”. It is used to compare the amount of calories expended from exercise matches the amount of calories ingested from the meal.

Post-absorptive: The metabolic environment in which the dietary ingested nutrients have been absorbed.

Postprandial: The metabolic environment following the ingestion of dietary nutrients.

Respiratory Quotient (RQ): Ratio of carbon dioxide produced to oxygen consumed at the metabolic level or cellular level of an individual at a given time. This ratio illustrates the ratio of carbohydrate and fat being utilized for energy at a given time (0.7 being exclusively fat, 1.0 being exclusively carbohydrate, 0.85 being a 50:50 mix of both carbohydrate and fat.

Respiratory Exchange Ratio (RER): Carbon dioxide expired to oxygen consumed of an individual at the lung; the RER is measured from inspired/expired breath and is used to estimate RQ. RER can be >1.0, notably during a maximal exercise test.

Delimitations

Participants were male or female between the age of 18 (20) and 45 (28) years of age who are fit to perform exercise, as established by the completion of a physical activity readiness questionnaire (PARQ, see appendix D). Since this was a pilot study, having a wide representation of young to middle aged individuals fit for exercise is warranted.

Limitations:

Limitations of this study include self-reported dietary, sleep and exercise journals and compliance to the pre-study protocol. Levels of ingested caffeine, stress levels, and physical activity prior to the lab visit. The 5-hour postprandial data may not comprehensively collect the data until the substrate utilization returns to baseline. Malfunction of the Oxycon mobile measurement device that resulted in the loss of data (a few minutes to even >1 hour on one occurrence). A metabolic chamber may be the gold

standard for measuring energy expenditure and metabolism, however the cost and availability makes it unfeasible at this time.

CHAPTER 2

REVIEW OF LITERATURE

According to the 2011-2014 statistics provided from the Centers for Disease Control and Prevention (CDC) it is crudely estimated that 36.5% of adults in the United States is obese. (Ogden, Carroll, Fryar, & Flegal, 2015) The medical costs associated with obesity was estimated to be ~147 billion U.S. dollars per year in 2008. (Finkelstein, Trogdon, Cohen, & Dietz, 2009) An individual who is clinically obese is also more likely to exhibit metabolic syndrome than a healthy weight individual. This is based on the characteristics of diagnosis from the American Heart Association, that elevated waist circumference (>40 in for men, >35 in for women) accounts for one out of three characteristics (five total) that are required, and waist circumference is directly correlated with central adiposity or central obesity. (Grundy et al., 2005) These comorbidities of obesity cause increased healthcare visits and costs for Americans. A way to circumvent these conditions and improve the overall health of these populations in society is to improve the body composition of the individuals. Two lifestyle factors that affect body composition are dietary and exercise habits.

Substrate Oxidation

The three major macronutrients that make up the food that we ingest are carbohydrate, fat/lipids, and protein. Carbohydrate and fat can be reincorporated into glycogen and adipose tissue respectively for storage, or oxidized for fuel depending on metabolic demands and where the nutrients are needed in the body (Bessesen, Bull, & Cornier, 2008). Carbohydrates may be converted to fat under conditions of excessive carbohydrate intake over several days, but this process (*de novo* lipogenesis) is not

common in humans (Hellerstein, 1999). Digested proteins result in amino acids, which are then used as the building blocks to synthesize new proteins.

Indirect calorimetry measures the inspiratory and expiratory gases, oxygen (VO_2) and carbon dioxide (VCO_2) respectively, and the ratio thereof to produce substrate oxidation rates. (H. A. Haugen, L. N. Chan, & F. Li, 2007) The Respiratory Quotient (RQ) is the ratio of VCO_2/VO_2 and is reflective of a particular substrate oxidation ratio of carbohydrates and lipids. Only under conditions of low carbohydrate availability (prolonged exercise, fasting) are amino acids oxidized to any appreciable extent, (Jungas, Halperin, & Brosnan, 1992) which is why non-protein RQ is used for most conditions and is often used interchangeably. An RQ of 1.0 represents exclusive metabolism of carbohydrate as fuel source and 0.7 represents exclusive metabolism of lipid as fuel source (H. A. Haugen et al., 2007). The RQ is a theoretical value that holds the assumption that only the exchange of oxygen and carbon dioxide derives from the metabolic processes in the body. A more practically measurable value that reflects the VCO_2/VO_2 at the mouth is the Respiratory Exchange Ratio (RER).

The RER can be measured in a whole room calorimeter or via a wearable mask that collects and analyzes ventilation and VCO_2/VO_2 rates such as the Oxycon Mobile system. The physiological limitations of RER involve the body's bicarbonate pool, which utilizes CO2 when necessary to maintain homeostasis. During hyperventilation CO2 is eliminated in excess of that produced by oxidative metabolism, and there is a decrease in the bicarbonate buffer pool. The excess VCO_2, coupled with VO_2 representing oxidative metabolism results in a higher than accurate representation of RQ. Hyperventilation is followed by a subsequent hypoventilation phase that aids in the

replenishment of bicarbonate by shunting the CO2 product of metabolism from exhalation toward the reformation of the bicarbonate pool which typically occurs during high-intensity exercise and is represented by a lower than accurate RQ (Eric Jequier, Acheson, & Schutz, 1987). Use of RER is not acceptable for determining substrate oxidation rates during this hyper-hypo-ventilation cycle. The RER value can be over 1.0 and is usually around 1.15 when maximum VO_2 is achieved (Howley, Bassett, & Welch, 1995). RER values greater than 1.0 are due to greater use of oxygen in the working muscles and greater expulsion of carbon dioxide from hyperventilation secondary to increased CO_2 production from bicarbonate buffering of acid accumulation (Eric Jequier et al., 1987). The maximum volume of oxygen consumed by an individual during a cardiovascular fitness test performed to volitional exhaustion is represented as the "VO_{2max}". The VO_{2max} value is the gold standard used (H. A. Haugen, L.-N. Chan, & F. Li, 2007; Shephard et al., 1968) to represent the cardiovascular fitness of an individual. The VO_{2max}, or maximum heart rate (HR_{max}) can be used in conjunction with the maximum intensity that an individual is able to achieve upon exhaustion to determine a workload intensity that should elicit the desired metabolic response for either MIE or HIIE. (Rowell, 1974)

Substrate Oxidation in an Energy Neutral State

Bobbioni-Harsch assessed energy expenditure and substrate oxidation for eight hours postprandial following meals that differed markedly in macronutrient composition (Bobbioni-Harsch et al., 1997). The ten non-obese subjects (5 male, 5 female) were instructed by a dietitian how to consume a diet composed of 50% carbohydrate, 30% lipid, and 20% protein (representing a daily intake of 28 kcal/kg bodyweight) for the

three days leading up to the experiment. Standardizing the composition of the diet prior to the trials creates a similar baseline of substrate for the body between subjects. The experiment began after a 10 hour fast, at which time the rate of available glucose for the body will have leveled off (Ruderman, Aoki, & Cahill, 1976). The subjects were then randomized to either a glucose load providing 637 kilocalories, lipid load providing 626 kilocalories, or a mixed load (glucose and lipid) providing 612 kilocalories.

The results of this study illustrate that the ratio of carbohydrate and lipid oxidation can be modified dependent upon the macronutrient distribution of the meal ingested. These meals were unique because the "high carb" or high fat" were composed 100% of glucose or lipid cream. Also, this study looked exclusively at the full effects of oxidation and utilization of nutrients along with different blood parameters related to macronutrient uptake and utilization for a single meal over the course of eight hours. This time frame allowed for data to be gathered after the body would return to a post-absorptive or fasted state. The important results from this study illustrate that carbohydrate stimulates its own oxidation whether taken exclusively, or in combination with the ingestion of fat. This study supports data from a previous study (Griffiths, Humphreys, Clark, Fielding, & Frayn, 1994) that found free fatty acids (FFA) remained significantly elevated postprandial with the fat and (to a lesser extent) mixed meals compared to the carbohydrate meal; the increase in FFAs did not result in any appreciable increase in fat oxidation (Bobbioni-Harsch et al., 1997). The mixed meal, containing ~50% glucose, suppressed FFA concentration in comparison to the fat meal. The increased available glucose explains, in part, the reduced fat oxidation after the mixed meal.

A study conducted by Hill and colleagues (Hill et al., 1991) examined the effects of various diet compositions of meals meeting the energy requirements without over feeding in eight obese volunteers (three men, five women). All subjects were studied for fourteen days, while 6 subjects were studied for a total of twenty-one days. The subjects were randomized before receiving either diet A or B (described below) for seven days, followed by a seven-day washout period and then administration of the other diet. Diet C (described below) was always administered last. Subjects consumed all food during the testing period at the Vanderbilt Clinical Research Center and spent days three and seven of each experimental feeding protocol in the whole-room calorimeter. Resting Metabolic Rate, body composition and 24-h energy expenditure were measured one to two days before each experimental feeding protocol to obtain a baseline.

Diets A, B, and C consisted of a constant percentage of dietary protein (20%), while differing in the ratio of fat and carbohydrate. Diet A (high carbohydrate) consisted of 60% kilocalories from carbohydrate and 20% kilocalories from fat. Diet B (high fat) consisted of 60% kilocalories from fat and 20% kilocalories from carbohydrate. Diet C (less-skewed mixed) consisted of 35% kilocalories from carbohydrate and 45% kilocalories from fat.

The results of this study determined that daily energy expenditure was not affected by the composition of the diet when caloric energy needs are being met without being exceeded. The key finding of this study was that 24-hour Respiratory Quotients trended with the composition of the diet in the absence of an overfed state. During the baseline period the mean RQ was 0.837. During the high carbohydrate diet, the RQ at day three and seven was slightly elevated at 0.856 suggesting a relative shift toward

carbohydrate oxidation during this period. During the high fat diet, the RQ at day three and seven was reduced to 0.749 and 0.747 respectively suggesting a relative shift toward fat oxidation during this period. During the mixed diet period, the RQ at day three and seven was reduced slightly to 0.780 and 0.782 respectively. It appears possible to shift the body's preferred substrate for metabolism, by adjusting the composition of the diet in an energy neutral state.

A possible explanation for the adjustment in substrate oxidation comes from studying the metabolic activity in active tissues such as skeletal muscle. The following study (Bergouignan et al., 2012) controlled for confounding variables well and included 10 lean and 9 obese healthy, non-diabetic volunteers that were between the age of 20 and 45 years, weight-stable, and leading a sedentary lifestyle. The subjects completed three separate four-day trials that were separated by one to three weeks, and although a complete adaptation to an isocaloric high-fat diet may take more than one week to achieve, several studies have demonstrated that a rapid and dramatic shift in substrate oxidation occurs within the first 24-48 hours (Hill et al., 1991; Roy et al., 1998; Patrick Schrauwen, Wagenmakers, van Marken Lichtenbelt, Saris, & Westerterp, 2000) leading to the confidence placed behind obtaining results after 96 hours of testing. Subjects first performed a low-fat diet as a baseline, followed by either an exercise (E. L. Melanson et al., 2009), or high fat diet (Bergouignan et al., 2012) which was randomly selected. The high fat diet will be discussed here, whereas the exercise intervention will be discussed later.

The study diets were prepared using primarily whole foods, and the fat content was modified by substituting low fat dairy products with higher fat alternatives. Both

diets also consisted of equivalent energy and protein but varied in the carbohydrate and fat contents. The low-fat diet consisted of 20% fat and 65% carbohydrate, whereas the high fat diet consisted of 50% fat and 35% carbohydrate. All food was packaged and provided to the subjects, and they were required to return the empty food containers to the study conductors to show compliance.

The subjects entered the calorimeter at 8:00 and exited at 7:00 the following morning. While inside, three exercise sessions of twenty minutes of bench stepping were performed and designed to mimic activities of daily living. Their meals were delivered upon a scheduled time, and subjects were instructed not to nap, and go to bed at the same time every night, which was documented by the subject. Blood samples and muscle biopsies were taken from the subjects, and RNA extraction and immunoblotting techniques were utilized to measure gene expression and total amounts of AMPK, CD36, COX4, LPL, PDK4, PGC1a, and SIRT, all of which play a role in fat oxidation.

The subsequent switch to the high fat diet elicited a reduced RQ after two days in both the obese and lean groups, with the relation of change between the two groups not significant. In other words, the muscle biopsies found that an increase in dietary fat significantly increased the expression of CD36 and PDK4 in both obese, and lean populations. The CD36 had no change between the lean and obese populations. Alternatively, PDK4 expression was higher in obese individuals than of lean subjects. Important conclusions to be drawn from the collective findings of this study is that the increase in fat oxidation that arises from an increase in dietary fat consumption (without an excess in energy intake) is in part due to the physiological adaptations in the muscle that reflect the shift toward oxidative metabolism. Also, this response does not vary

between lean and obese populations, suggesting that the ability for obese individuals to adapt to short-term increase in dietary fat consumption is not hindered.

In an energy neutral state there appears to be a shift of substrate utilization toward the predominant macronutrient of the diet, with carbohydrate stimulating its own oxidation whether taken alone or with a meal of mixed macronutrient distribution. These are known to be the case under an energy neutral state, however a large factor that contributes to a weight gain is excess energy intake.

Substrate Oxidation in an Overfed State

In the overfed state, it has been demonstrated that carbohydrate oxidation is adjusted to dietary intake over a 24-hour period (Flatt, 1988), however additional fat above maintenance does not increase postprandial oxidation of fat after 9 hours (Flatt, Ravussin, Acheson, & Jequier, 1985). Similarly, consuming 3 meals with fixed 50%, 35%, and 15% kilocalories from carbohydrate, fat, and protein respectively, and a 106 g fat supplement (954 kcal) above maintenance, did not increase 24-hour fat oxidation (increased by ~1g) (Y Schutz, Flatt, & Jéquier, 1989). A trial in 5 young men that began with a 13-day dietary maintenance period followed by a 9-day overfeeding period (1.6x maintenance energy intake) of a mixed diet concluded that the rate of carbohydrate oxidation increases until carbohydrate balance is achieved. Carbohydrate intake at maintenance was 300g/day and increased to ~460g/day during overfeeding. Carbohydrate utilization rapidly adjusted to the increased intake, such that after 9 days, utilization matched the higher intake. By contrast, lipid intake increased from ~120g/day during maintenance to ~180g/day during overfeeding, during which time oxidation decreased

from 100g/day during the maintenance phase until virtually all lipid consumed was stored, and only ~25g/day were utilized (E Jequier, 1993; Ravussin et al., 1985).

In relation to the metabolic response, an increase in carbohydrate ingestion results in an increase in insulin that facilitates glucose uptake into muscle, liver and other tissues of the body. Insulin also acts to inhibit the process of lipolysis, and reduce fat oxidation (Acheson, Flatt, & Jequier, 1982). This makes sense from an energy efficiency standpoint, because it would be inefficient to break down lipids and create free fatty acids for energetic use, when the ingestion of a meal including carbohydrate provides the preferred form of energy in an available state.

Though carbohydrate intake stimulates its own oxidation, dietary fat intake responds in quite the contrary manner, and was shown to lead to direct storage of lipid with no influence on lipid oxidation.

Horton and colleagues (Horton et al., 1995) furthered the research of energy storage and utilization in the overfed state. This study recorded much more detail and shared very detailed methods, and results. This study included nine lean or ideal body weight subjects, and seven obese subjects. The subjects completed two trial periods of fourteen days of overfeeding that were preceded by a week of baseline maintenance diet. The maintenance or baseline diet was determined from an accurate fourteen-day, weighted diet diary. The subjects met with a dietitian who showed them how to accurately weigh and record the food. One week after completing the weighted diary the first baseline phase began. The food was made by the clinical research center's kitchen and one meal per day was required to be eaten at the center, while the rest could be eaten at home or in the workplace. Daily bodyweight was measured to ensure weight stability

and a meeting with the dietitian would correct any imbalances in the diet that led to a weight fluctuation. The subjects' usual fitness was measured one week prior using accelerometers that were provided.

The fourteen-day overfeeding phase provided subjects with 150% of their estimated energy requirements based on the activity and energy expenditure data that was previously gathered. The additional 50% of energy was provided in the form of exclusively fat macronutrient, or exclusively carbohydrate macronutrient, and whole room energy expenditure and substrate oxidation was measured at days one, seven, and fourteen. Also, an accelerometer measured activity the last seven days of the overfeeding phase.

The second fourteen-day overfeeding period was separated by a four-week washout, with three weeks choice eating and the baseline week repeated. The second overfeeding period was administered exactly like the first except the 50% of calories from overfeeding came from whichever macronutrient (carbohydrate or fat) that the subject didn't previously receive.

The nutrient storage was measured from the amount of energy provided by the diet minus the energy expended, as measured day one, seven and fourteen. Carbohydrate overfeeding led to a two-fold increase in the amount of carbohydrate oxidation over the fourteen-day overfeeding period. The carbohydrate oxidation doubled on day one of over feeding, and by day 7 had peaked. The fat oxidation had the reverse effect, being reduced by 50% on day one, and by 50% again after one week. On the contrary, during the fat overfeeding trial the results were more static, with less change exhibited throughout. The carbohydrate overfeeding period showed carbohydrate oxidation

increased from ~5 MJ/day to ~10 MJ/day at one week, while fat oxidation decreased from ~6 MJ/day to about 2 MJ/day at one week. The fat overfeeding period showed fat oxidation trended upwards slightly day one and then leveled off at the week point, changing from 5 MJ/day to ~6 MJ/day, while carbohydrate oxidation had decreased from ~5 MJ/day to ~4.5 MJ/day. The slight increase in fat oxidation was not nearly enough to offset the increase in fat intake during the overfeeding period. Comparing the fat intake to fat oxidation throughout this study showed that the degree of positive fat balance during overfeeding was between 90-95% of the total excess energy consumed (Horton et al., 1995).

The results of the aforementioned study (Horton et al., 1995) indicate that an increase in body weight, primarily fat mass, occurred in both overfeeding groups, but fat gain was much greater during the fat-overfeeding phase. Additional studies that investigated a single meal (Chuck Bennett et al., 1992; Flatt et al., 1985), and another which examined an entire day (Y Schutz et al., 1989) show that an increase in dietary fat intake results in only a slight increase in fat oxidation, considerably less than the amount of fat consumed. The interpretation of these findings is that as carbohydrate metabolism ramps up to oxidize additional carbohydrate in the diet, the remaining dietary fat is stored. In other words, fat oxidation occurs secondarily to increases in body fat mass during a period of dietary overfeeding. (Yves Schutz, Tremblay, Weinsier, & Nelson, 1992)

Patterns of Dietary Intake in America

Data from 56 countries during 1971-2010, illustrate an increase in food energy supply to be associated with an average increase in body weight, specifically, the

association between change in food energy supply and change in body weight was statistically significant overall and for high-income countries ($P<0.001$) (Vandevijvere, Chow, Hall, Umali, & Swinburn, 2015). A separate USDA analysis of nutrient content of foods in the US from 1909-2000, lends further support by showing that per capita food energy in the US food supply has been increasing from about 3,100 kcal/day in 1965 to a peak of 3,900 kcal/day in 2000 (Gerrior, Bente, & Hiza, 2004). As per capita food energy becomes more readily available, daily energy intake also is trending positively in part due to increased portion sizes (Livingstone & Pourshahidi, 2014). Both the National Health and Nutrition Examination Surveys (NHANES) (Yang et al., 2003) and CDC data (Control & Prevention, 2004) indicate an increase in daily energy intake between 1971 and 2000, with the CDC eluding 168 kcal in men and 335 kcal in women (Glenn A Gaesser, 2007). A randomized controlled clinical trial overfed 10 male and 13 female subjects by 500 kcal for 11 days, with reported sensations of decreased hunger and increased satiety, though these sensations were not sufficient enough to adjust intake. The failure to follow hunger fullness cues, along with increased portion sizes is positively correlated with concurrent trends in obesity (Rolls, Roe, & Meengs, 2007).

The increase in reported energy intake since 1970 occurred in conjunction with an increase in the consumption of carbohydrates, which rose by 60 to 70 g/day, or as a percentage of macronutrient distribution of the diet rose from 42% - 50% (Control & Prevention, 2004; Ford & Dietz, 2013). By contrast, reported absolute intake of fat, in grams per day, remained relatively stable between 1971 and 2000 (Control & Prevention, 2004). Consequently, fat intake, as a percentage of total energy, actually decreased from

approximately 34% - 31% during this period." (Ford & Dietz, 2013; Glenn A Gaesser, 2007)

Exercise and Substrate Oxidation

The argument has been made that a moderate-intensity exercise is most effective to elicit fat oxidation, with one article testing 5 subjects for 30 min at 25%, 65% and 85% VO_{2max} which demonstrated the exercise at 65% VO_{2max} resulted in 60% more fat oxidation than 25% VO_{2max} exercise, and 85% VO_{2max} resulting in 10% more fat oxidation then the 25% VO_{2max} exercise (Romijn et al., 1993). Another article further established the "fat-burning zone" that is widely propagated by fitness enthusiasts and personal trainers to be between 55% and 72% of VO_{2max}, and 68% and 79% of HR_{max} (Achten, Gleeson, & Jeukendrup, 2002). Though technically these moderate range intensities elicit greater fat oxidation during exercise than high-intensity exercise, elevated metabolism and a decreased RER (or increased fat oxidation rate) following high-intensity exercise results in a greater 24-hour energy expenditure and overall fat oxidation over a 24-hour period.

Excess Post-Exercise Oxygen Consumption

Excess post-exercise oxygen consumption (EPOC) is the term that explains the elevated inspired VO_2 that persists for a period of time after a bout of exercise. The practical significance of the EPOC is that the more oxygen a person is consuming, the more metabolic processes are at work, which means an elevated metabolic rate. One study comparing exercise intensities and EPOC (Bahr & Sejersted, 1991) engaged six healthy males in three separate 80 minute exercise bouts on a cycle ergometer maintaining ~75 rpm at 29%, 50% and 75% VO_{2max} followed by bed rest for twelve hours

and forty minutes without sleeping. Subjects received three meals of bread, jam and skim milk throughout the experiment with an average caloric content per meal of 1075 kcal, and a macronutrient distribution of 5% fat, 81% carbohydrate and 14% protein. Consequently, EPOC was elevated for an average of 1.3 ± 0.5 L, 5.7 ± 1.7 L and 30.1 ± 6.4 L between the 29%, 50%, and 75% exercise intensities respectively. These data illustrate that the higher intensity of exercise matched for duration, the greater volume of EPOC ultimately results in more calories burned (with a hypothetical RER of 0.85—29%: 6 kcal, 50%: 28 kcal, 75%: 146 kcal) and more fat oxidized (with a hypothetical RER of 0.85—29%: 0.66 g, 50%: 3 g, 75%: 16 g) during the post-exercise period alone. Prolonged exercise intensity above 40% to 50% of VO_{2max} is necessary to elicit the metabolic processes responsible for having a prolonged EPOC for longer than two hours post-exercise (Bahr & Sejersted, 1991). Alternatively, low-intensity exercise does not allow for a heightened metabolism post-exercise and an additional caloric deficit to manifest throughout the day. The EPOC has shown an exponential growth relationship with increased exercise intensity, and linear growth with increased exercise duration (Knuttgen, 1970; Laforgia, Withers, & Gore, 2006).

Post-Exercise Substrate Oxidation

Low-intensity cycling with concurrent consumption of a high-fat diet was examined over five days and six nights (Hansen et al., 2007). There were ten sedentary female volunteers with a BMI between 20-30, without metabolic disease and exhibiting regular menstruation. Subjects' spent time during the experiment at the University of Wisconsin Sports Medicine Clinic in a metabolic chamber, except data was not measured day 3 as subjects were allowed a day off from measurements for their psychological well-

being. Subjects were instructed to not partake in any physical activity beyond activities of daily living on the day of admission and fast for four or more hours prior to admission.

The crossover design had subjects participate in the two groups that performed 2 bouts of MIE (45% VO_{2max} --one in the morning, one in the evening) of exercise expending either 150 kcal or 300 kcal for group one and two respectively. The weight maintenance meals consisted of 30% of energy from fat and provided on average 2025 kilocalories. The breakdown of the three treatment diets for Sedentary, Exercise1, and Exercise2 were 1976, 1991, and 2008 kilocalories per day respectively, as subjects were instructed to eat only until satiety. All low-fat meals prior to testing were designed to provide 30%, 55%, and 15% of energy from fat, carbohydrate, and protein respectively, whereas all high-fat meals were designed to provide 50%, 35%, and 15% of energy from fat, carbohydrate, and protein respectively.

The study found that the non-protein RER decreased with the introduction of the high-fat diet by -0.03 and -0.045 in the sedentary group and both exercise groups respectively. High fat diet day two showed another decrease of -0.01 in RER for all groups. Day three was unmeasured, and day four showed a -0.001, -0.005 and -0.15 changes in RER from day 2 in the sedentary, exercise group 1 and exercise group 2 respectively. The results support that there is a positive linear correlation between exercise time and proportion/amount of lipid oxidation. More importantly, fat oxidation when transitioning from the low-fat diet to high-fat day 1 resulted in greater fat oxidation across groups (Sedentary: 18 ±11 g/d, Ex1: 35 ± 28 g/d, Ex2 36 ± 1 g/d). Specifically, moderate-intensity exercise increases the rate and total amount of fat oxidation when

women switch to a high-fat diet (Hansen et al., 2007). The same result is shown by another study in the male population (Smith et al., 2000).

Fasted or Fed Effects on Substrate Oxidation

Various studies have looked at postprandial substrate oxidation following a single meal. 5g/Kg bodyweight of a pasta meal was provided for a control, or prior to 90-min MIE at 50% VO_{2max} in six male and six female subjects (N Folch, F Peronnet, D Massicotte, S Charpentier, & C Lavoie, 2003). Fat oxidation was significantly higher and carbohydrate oxidation significantly lower following the MIE compared to the control trial. Another study with 8 lean and 10 obese subjects had them complete both a control and a 200 kcal exercise bout at 65% $VO_{2reserve}$ on the cycle ergometer following a single protein or carbohydrate dominant meal of 400 kcal each (Petra Stiegler, S Andrew Sparks, & Adam Cunliffe, 2008). The subjects were randomly assigned to either meal group that provided 400 kcal regardless of the trial completed and had 4-hour substrate oxidation, glucose and insulin levels measured. Energy expenditure was not affected by the exercise trials, however postprandial fat oxidation was increased, and carbohydrate oxidation decreased with exercise. Regarding meal composition, postprandial substrate oxidation following the protein dominant meal led to a greater increase in fat oxidation than following the carbohydrate dominant meal. Lastly, exercise increased postprandial fat oxidation regardless of the meal composition.

A different study examined the postprandial substrate oxidation effects of a mixed meal isocalorically matched at 300 kcal following two types of exercise eliciting 300 kcal energy expenditure: low-intensity (35% VO_{2max}), high-intensity (70% VO_{2max}) and a control trial (Pillard et al., 2010). Fat oxidation for the 70% VO_{2max} exercise was

significantly higher than the 35% VO_{2max} and the control. This implies that exercise intensity plays a larger role than duration when the exercise is matched for energy expenditure.

The previous three studies examined postprandial substrate oxidation while exercising in the post-absorptive (fasted) state, whereas the following study (Gregory et al., 2011) had subjects exercise in the postprandial state following the meal. Eight women completed two trials, both a low-fat (80% carb, 5% fat) and a high-fat (37% carb, 48% fat) diet followed by 30 min of 60-65% VO_{2max} MIE that was determined to be best (Achten et al., 2002) at eliciting fat oxidation during exercise. The RER was lower (rate of fat oxidation higher) in the high-fat trial compared to the low-fat trial, suggesting that MIE exercise in the post-prandial state results in RER that trends with dietary composition.

24-Hour Isocaloric Energy State and Substrate Oxidation

A study that examined the effects of post-absorptive and postprandial exercise consisting of 60 min at 50% VO_{2max} found that the energy expenditure during exercise and for 24 hours was equivalent, however the key finding was that more fat was oxidized during exercise and for 24 hours when performed in the post-absorptive (fasted) state (Shimada et al., 2013). The same held true when the 50% VO_{2max} cycling was extended to 3 hours (Bielinski, Schutz, & Jequier, 1985). Another study (K. Iwayama et al., 2015) looked at the effects of 24-hour fat oxidation and how pre-meal exercise affected this parameter, specifically they compared breakfast, lunch and dinner. The results demonstrated that fat oxidation over a 24-hour period was only increased when the exercise was performed before breakfast, and not before lunch or dinner. Expanding

upon post-absorptive effects of exercise before breakfast, 60-min at 50% VO_{2max} in female subjects elicited 30% greater 24 hour fat oxidation compared to control group (Kaito Iwayama et al., 2017). Two-a-day one-hour (morning and evening) 45% VO_{2max} cycling bouts produced greater overall 24 hour fat oxidation in conjunction with high-fat meals (K. Hawkins, K. Hansen, D. Schoeller, & J. Cooper, 2012). Another 24 hour energy maintained trial examining a single 60 min MIE at 55% VO_{2max} in 10 lean-trained, 10 lean-sedentary and 7 obese-sedentary found no significant difference between fat oxidation among any conditions (Edward L Melanson et al., 2009). In an energy neutral state no significant difference in 24 hour fat oxidation was found between a 60-minute weightlifting circuit, 50-min cycling at 70% VO_{2max}, or control day (Melanson et al., 2002).

It seems a consensus in the literature that exercise in the postprandial state does not influence 24-hour fat oxidation. The evidence however is conflicting on whether post-absorptive exercise has a positive or null effect on postprandial fat oxidation when energy balance is maintained throughout the day.

24-Hour Energy Deficit State and Substrate Oxidation

The literature is more congruent that 24-hour energy expenditure and fat oxidation increases when exercise creates a negative energy balance for the day. This held true examining various populations of 10 lean, 10 obese-sedentary, 9 reduced-obese and 7 physically active reduced-obese where 60% VO_{2max} exercise prescribed to initiate a 15% energy deficit for 24-hours (Bergouignan, Kealey, Schmidt, Jackman, & Bessesen, 2014). Specifically, it was noted that the exercise bouts did not affect RQ during the day, however during sleep all subjects experienced reduced RQs. Additional analysis between

these populations found that the physically active reduced-obese individuals oxidized more fat overnight than reduced-obese individuals during control and exercise conditions even after adjustment for fat-free mass. Physical activity has positive metabolic effects independent of weight loss, and these positive effects appear to manifest overnight, suggesting an important role of sleep in the regulation of lipid metabolism (Bergouignan et al., 2014). Another study of the obese population compared HIIE glycogen exhaustive exercise (50% W_{max} warm-up for 5min, followed by alternating 2 min of 80% W_{max} and 60% W_{max} until volitional exhaustion, lastly 70% W_{max} until unable to maintain cadence >60RPM) with a reduced fat (30%) and high fat (60%) for which they consumed for 3 days (P. Schrauwen, Lichtenbelt, Saris, & Westerterp, 1998). RER decreased on both trials with high-fat diet compared to reduced fat, and furthermore overall fat oxidation in the control trial (no exercise) was less than fat intake, however during exhaustive exercise fat oxidation matched fat intake. This suggests that obese subjects are capable of adjusting fat oxidation to fat intake on a high-fat diet when glycogen stores are lowered.

Saturated vs. Unsaturated Fat Oxidation

Palmitate and Oleate are common fatty acids that are found in a variety of food sources and represent saturated and unsaturated fatty acids respectively. Palmitate oxidation has not been shown to be affected by prior exercise regardless of intensity (Votruba, Atkinson, Hirvonen, & Schoeller, 2002; Votruba, Atkinson, & Schoeller, 2003, 2005), whereas oleate oxidation throughout the day increases in response to any intensity of exercise, and its oxidation is positively correlated with intensity (Votruba et al., 2002; Votruba et al., 2003). Further oleic acid oxidation research has shown a possible diurnal effect of fat oxidation. Following post-absorptive exercise before breakfast, oleate

oxidation remained elevated throughout the day, with a peak following the lunch (Votruba et al., 2005). This research may help to explain differences amongst fat oxidation between some studies, as Votruba shows that all FFA are not alike when it comes to the effect of exercise on fat oxidation.

High-Intensity Exercise and Substrate Oxidation

The classifications of aerobic exercise intensity (as a % of VO_{2max}) provided by the American College of Sports Medicine are: very light (<37%), light (37-45%), moderate (46-63%), vigorous [high] (64-90%), near-maximal to maximal (≥91%) (Garber et al., 2011). High-intensity exercise also includes high-intensity interval exercise (HIIE), which consists of alternating exercise bouts of ~30 seconds to 4 minutes at a very high intensity, following by active recovery intervals of ~1-4 minutes at a low-intensity (G. A. Gaesser, Angadi, & Sawyer, 2011; Wesley J Tucker, Siddhartha S Angadi, & Glenn A Gaesser, 2016).

There is a gap in the scientific literature surrounding postprandial substrate oxidation and high-intensity exercise, the aforementioned studies except for one including what they deemed exhaustive exercise (P. Schrauwen et al., 1998) pertained to low or moderate intensity exercise. Also, no studies have examined the influence of high-intensity interval exercise on postprandial substrate oxidation. This type of exercise is becoming increasingly more popular. Thus, comparing the effects of moderate-intensity continuous exercise and high-intensity interval exercise, matched for total energy expenditure, on postprandial substrate oxidation following a meal that is conducive to suppressing fat oxidation, is warranted.

CHAPTER 3

METHODOLOGY

Ten sedentary or recreationally active male or female, between the age of 18 (20) and 45 (28) years volunteered to participate in this study. Exclusion criteria for subjects were elite athletes training to compete and those unwilling to cease exercise for two days prior to each lab visit, a medical condition preventing consumption of a glazed donut, and those unfit to perform exercise as determined by a "yes" to any of the questions on the Physical Activity Readiness Questionnaire (PAR-Q) (Thomas, Reading, & Shephard, 1992). At the consenting lab visit each subject was provided a description of the study including purpose of research, length of trial, experiment protocols, and potential harms and benefits to the subject. Subjects were instructed not to perform any exercise 48 hours prior to the next visit. Subjects were also provided with and instructed on how to complete dietary intake food journals for each day preceding a lab visit.

This study was approved by the Arizona State University Institutional Review Board (IRB #:STUDY00005552).

Recruitment

Ten subjects were recruited via flyers, social media, emails and listservs that were disbursed throughout the greater Phoenix and Tempe areas surrounding the Arizona State University campus. Following a potential subject's initial inquiry, a pre-screening Qualtrics survey was provided to the individual via email or text message to prevent unnecessary lab visits for those who do not meet the inclusion criteria for this study. Recruitment began January 2017.

Study Protocol

This pilot study was a randomized controlled clinical trial consisting of four separate test days with at least 72 hours, and no more than 14 days between lab visits. Lab visit one was the baseline test period and the remaining 3 visits were randomized via a random number generator (SPSS). All experimental conditions were performed in a climate controlled Healthy Lifestyles Research Center (22* C) at the Arizona State University Downtown Campus in ABC-1, Room 166. The following outcome measures were assessed at baseline and throughout each trial: total calories expended per hour (Kcal/hour), calories of fat expended per hour (Kcal Fat/hour), calories of carbohydrate expended per hour (Kcal CHO/hour), grams of fat oxidized per hour (G Fat/hour), grams of carbohydrate oxidized per hour (G CHO/hour), net substrate oxidation relative to baseline per hour (ΔG Fat/hour & ΔG CHO/hour).

Visit One

All participants who passed the eligibility requirements after completing the pre-screening survey reported to the Healthy Lifestyles Research Center where they signed consent forms and had the study protocol explicitly explained. Subjects filled out and signed the PAR-Q with only their non-identifiable ID to ensure they are eligible for enrollment and to maintain confidentiality throughout. When deemed eligible, height and weight were measured using a stadiometer and a standard beam scale respectively. Lastly, a VO_{2max} test (explained in detail below) was performed. The inclusion of this test on the day of consent reduced the number of lab visits required by each participant.

At the conclusion of visit one, the subsequent 3 lab visits were scheduled. The subjects were given food records and instructed to complete them the day prior to each

lab visit. It was advised for them to choose food and drink that they could easily replicate the day before each lab visit for the remainder of their participation. The subjects arrived to the remaining 3 trials following a 12 hour fast of all food and drink except water, and a 14 hour fast of caffeine.

VO_{2max} test

Subjects were equipped with a ventilation mask attached to a hose, and a Polar heart rate monitor (Polar, Lake Success, NY, USA) for the metabolic measurement device (Parvomedics TrueOne 2400, Sandy, Utah) to measure ventilation and respiratory gas exchange and heart rate data continuously. This system had been previously validated against the Douglas bag method and was shown to underestimate VO_2 at high workloads above 200 W and overestimates RER at all workloads during interval and steady state endurance exercise. (Perret & Mueller, 2006). The Parvomedics device was calibrated using the standard calibration gas mixture of 5% CO_2, 16% O_2 and 79% N_2 and appropriate values of calibration were less than 1% difference.

After 2 minutes of seated rest, subjects pedaled on an electronically braked cycle ergometer (Viasprint 150P; Ergoline, Bitz, Germany) at a cadence of their choice (50-90 rpm) for 5 minutes at 50 W. After this warm-up, power was increased in ramp fashion by 30 W/min until each subject reached volitional fatigue despite strong verbal encouragement. After a 5-10-minute cool-down period of cycling at 50 W, participants performed a verification phase at a constant power of 100% of the peak power reached during the incremental ramp test until they reached volitional exhaustion. Participants were asked to keep their cadence above 50 rpm and pedal for as long as possible during the verification phase.

VO_{2max} was defined as the average of the 2 highest consecutive 15- second averages achieved for VO_2 during either the ramp or verification phase of the maximal exercise test. HR_{max} was determined using the highest HR achieved during either the ramp or verification phase.

Control Visit

The control visit began 2 hours later than the exercise visits to standardize time for donut ingestion and 5-hour postprandial period to account for any diurnal effects. Subject was fitted with the polar HR monitor and Oxycon Mobile device to measure HR, Ventilation, and gas exchange for 30 minutes to collect baseline data, and 5 hours postprandial (approximately 6 hours [30 min baseline, 5 min donut consumption, 5-hour postprandial measurement]). Gas exchange and HR were monitored continuously, with an optional break (approximately 5 minutes) every 60 minutes to drink water or use the restroom.

HIIE

Subjects were fitted with the same mask and HR monitor that was used for the all of the experimental conditions. After 30-minutes of seated rest, the subject walked to the mechanically braked, calibrated cycle ergometer (Ergomedic 828E; Monark, Vansbro, Sweden) and began a 5-minute warm up at a workload which elicited 50% of the subjects VO_{2max} (50-90 rpm preferential cadence). Then, the appropriate number of intervals (1-minute work at 90-95% of HR_{max} followed by 1-minute of active recovery cycling at 1 or 0.5 Kp resistance) required to expend 520 calories was performed, followed by a 5-minute cool-down that was identical to the warm-up procedure. This completed the HIIE exercise protocol. 60 minutes post-exercise the mask was removed, and 2 glazed donuts

were consumed within 5 minutes, and the mask replaced. Continuous measurement of HR, ventilation, and gas exchange were measured for 5 consecutive hours with an optional break (approximately 5 minutes) every 60 minutes to drink water or use the restroom.

MIE

Subjects were fitted with the same mask and HR monitor that was used for the all of the experimental conditions. After 30-minutes of seated rest, the subject walked to the mechanically braked, calibrated cycle ergometer (Ergomedic 828E; Monark, Vansbro, Sweden) and began a 5-minute warm up at a workload which elicited 50% of the subjects VO_{2max} (50-80 rpm preferential cadence). Then, the appropriate minutes of cycling work at a workload that produced 60-75% of HR_{max} was performed, followed by a 5-minute cool-down that was identical to the warm-up procedure. This concluded the MIE exercise protocol. 60 minutes post-exercise the mask was removed, 2 glazed donuts were consumed within 5 minutes and the mask replaced. Continuous measurement of HR, ventilation, and gas exchange was measured for 5 consecutive hours with an optional break (approximately 5 minutes) every 60 minutes to drink water and remove the mask. .

Independent Variable

The study treatments (HIIE: [13-35] x 1 min at 90-95% HR_{max}, MIE: [36-94] min at 60-75% HR_{max}, Control: no exercise) were performed on different laboratory days at least 72 hours apart, no more than 14 days apart. The HIIE protocol was individually calculated from the subjects VO_{2max} to determine the number of intervals required to meet the isocaloric needs of the post-exercise meal. A protocol of 8-10, 1-minute (90-95% VO_{2max} intensity) exercise followed by 1-minute active recovery cycling is a standard

HIIE procedure, that has been successfully performed at 16 intervals (Tucker et al., 2015). Both the HIIE and the MIE have been predetermined and isocalorically matched with the 520 calories that are consumed from the glazed donut meal.

Laboratory Analyses

Pulmonary ventilation and gas exchange were measured breath-by-breath via the Oxycon Mobile for detection of VO_2 and carbon dioxide production (VCO_2), which were used to compute (Lusk, 1924) EE (kcal/min), and along with the RER determined substrate oxidation rates (g/hour). Furthermore, Δ substrate oxidation was determined by subtracting the value at each hour from baseline. VO_2 was expressed as 60-s averages of breath-by-breath data using JLab software. Since blood bicarbonate levels have been reported to return to resting levels within 30 min after cessation of high-intensity exercise (Phelain, Reinke, Harris, & Melby, 1997) and arterial CO_2 partial pressure has been shown to not be different from resting control conditions from 60-120 min after HIIE (Malatesta, Werlen, Bulfaro, Chenevière, & Borrani, 2009), a 60-min post-exercise period was allotted prior to donut consumption to allow the body to restore acid-base balance prior to the postprandial period.

Statistical Analyses

All data were analyzed using SPSS Software (SPSS 24.0, IBM Corporation, Armonk, NY). A repeated measures analysis of covariance was performed to account for any differences in Gender. Sample size (n = 10) was determined based on previous studies that have assessed fat oxidation differences between exercise protocols (Burns, Oo, & Tran, 2012; Chan & Burns, 2013; Hazell, Olver, Hamilton, & Lemon, 2012; Skelly et al., 2014; W. J. Tucker et al., 2016) and postprandial fat oxidation differences

between meals of different macronutrient composition (Bobbioni-Harsch et al., 1997; Hill et al., 1991; E Jequier, 1993).

CHAPTER 4

RESULTS

The purpose of this randomized controlled trial was to determine the effect of high-intensity interval exercise (HIIE) and moderate-intensity continuous exercise (MIE) on postprandial substrate oxidation after consumption of an isocaloric high sugar/high fat meal ~520 calories (2 glazed donuts) in adults 18-45. This chapter presents the data collected from the experimental protocol including subject demographics/fitness characteristics, exercise expenditure averages, total substrate oxidation patterns between conditions (in grams of fat and grams of carbohydrate). Furthermore, the repeated measures design of this study resulted in each subject acting as their own control to increase the validity of the dependent variable while decreasing the noise from a highly variable intersubject resting RER.

Subject Demographics

There were 23 responses to the Qualtrics survey that was emailed in the listserv advertisement. Out of the 23 respondents, 21 were eligible to participate in the study. A total of 15 volunteers signed a consent form and were enrolled in the trial. The 15 subjects had their trial order randomized via random number generator. Five subjects dropped out after enrolling due to scheduling conflicts. A total of 10 participants completed the study.

Age, gender, height, weight, BMI, as well as maximum values achieved during the VO_{2max} test: absolute and relative VO_2, heart rate, workload achieved in watts from the VO_{2max} test, and total exercise time and calories expended from both the MIE and

HIIE trials are displayed in table 1 for all subjects individually along with the total mean and standard deviation for all participants.

Table 1. Subject Baseline Demographic and Fitness Characteristic data

Gender	Age	Height (cm)	Weight (Kg)	BMI	VO_{2max} Absolute (L/min)	VO_{2max} Relative (ml/kg/min)	HR_{max}	$Watt_{max}$	HIIE Time (min)	MIE Time (min)	HIIE kcal	MIE kcal
M	23	177.30	72.85	23.17	3.45	47.36	196	270.69	48	58	503.77	519.81
M	26	172.60	65.75	22.07	3.95	60.08	206	315.75	44	47	488.89	459.80
M	28	192.20	103.85	28.11	4.86	46.80	191	382.28	38	42	505.77	547.61
M	21	176.10	66.55	21.46	3.17	47.63	191	248.38	56	61	510.90	515.59
M	26	181.40	86.15	26.18	3.43	39.81	181	270.27	47	57	491.56	532.94
M	23	175.80	76.85	24.87	3.15	40.99	183	288.83	54	62	522.46	508.62
M	24	161.80	65.20	24.91	2.62	40.18	192	233.32	70	77	492.30	526.51
M	23	178.00	72.70	22.94	4.13	56.81	185	330.53	40	48	515.94	504.48
F	20	155.80	51.05	21.03	2.02	39.57	194	159.67	80	99	492.76	510.03
F	21	161.70	59.15	22.62	2.55	43.11	190	196.09	66	73	498.85	490.50
	[a]23.50 ±2.55	[a]173.27 ±10.79	[a]72.01 ±14.75	[a]23.74 ±2.24	[a]3.33 ±0.84	[a]46.23 ±7.16	[a]190.90 ±7.16	[a]269.58 ±65.12	[a]54.30 ±13.81	[a]62.40 ±16.92	[a]502.32 ±11.44	[a]511.59 ±24.15

[a] Data are mean ± SD

Changes in Outcome Measures

An analysis of covariance test was conducted between gender and the outcome variables to determine whether gender needed to be controlled for. Gender showed no significant impact on any of the outcome variables. Any potential relationships between age, height, BMI, and physical fitness and the outcome variables were not considered in this exploratory pilot study with a relatively small sample size and minimal exclusionary criteria.

Substrate oxidation data in grams per hour are available for each hour postprandial, as well as baseline, total trial oxidation and averages for Control, MIE and HIIE trials in Table 2. Additionally, Δ Substrate oxidation and caloric expenditure data for the same variables and trials is available in Tables 3 and 4 respectively. Fat oxidation per hour is shown in Figure1. Fat oxidation decreased in the control trial during the first two hours, whereas fat oxidation increased in the two exercise conditions. Total fat oxidation is shown in Figure 2. Total fat oxidation in the postprandial period for the two exercise conditions (MIE: 27.1g ± 9.6, HIIE: 23.0g ± 6.0) was greater ($P < 0.001$ and $P = 0.003$ respectively) than during the control condition (17.3g ± 5.4).

Carbohydrate oxidation per hour is shown in Figure 3. Carbohydrate oxidation increased during the first two hours and decreased for the final three hours, with the exception of MIE remaining consistent with baseline for hour one. Total carbohydrate oxidation is shown in Figure 4. Total carbohydrate oxidation in the postprandial period for the two exercise conditions (MIE: 61.42g ± 25.68, HIIE: 64.46g ± 12.56) was greater than during the control condition (79.54g ± 22.42).

Δ Fat oxidation in relation to baseline oxidation is shown in Figure 5. Δ Fat oxidation decreased in the control trial during the first three hours, followed by an increased rate for hours 4 and 5. Conversely, Δ fat oxidation remained elevated for the 5 hours postprandial in the two exercise conditions. Total Δ fat oxidation in the postprandial period for the two exercise conditions (MIE: 11.51g ± 8.41, HIIE: 9.51g ± 5.20) was greater ($P < 0.001$) than during the control condition (-1.79g ± 3.99).

Δ Carbohydrate oxidation in relation to baseline oxidation is shown in Figure 6. Δ Carbohydrate oxidation increased in the control trial during the first four hours, followed by a decreased rate for hour 5. Conversely, Δ carbohydrate oxidation during MIE remained constant for hour 1, increased for hour 2, whereas HIIE increased for hours 1 and 2. Δ Carbohydrate oxidation during both exercise trials decreased continuously for hours 3-5. Total Δ carbohydrate oxidation in the postprandial period for the two exercise conditions (MIE: -11.90g ± 17.80, HIIE: -11.34g ± 15.17) was a smaller negative change than during the control condition (-17.34g ± 14.93).

Caloric expenditure from fat in relation to baseline caloric expenditure of fat is shown in Figure 7. Caloric expenditure from fat for both MIE and HIIE increased during hour postprandial hour 1, then decreased hour 2 (while remaining elevated greater than baseline), and continued to increase for hours 3, 4 and 5 postprandial. The Caloric expenditure from fat for the control trial decreased steadily hours 1 and 2 postprandial, followed by a steady increase hours 3, 4 and 5. Total caloric expenditure from fat in the postprandial period for the two exercise conditions (MIE: 244.3 ± 86.2 kcal, HIIE: 207.2 ± 54.4 kcal) was greater than during the control condition (155.5 ± 48.1 kcal).

Caloric expenditure from carbohydrate in relation to baseline caloric expenditure of carbohydrate is shown in Figure 8. Caloric expenditure from carbohydrate for all control, MIE and HIIE trials increased for the first 2 hours postprandial followed by a decrease for hours 3, 4 and 5. Total caloric expenditure from carbohydrate in the postprandial period for the control condition (318.2 ± 89.7 kcal) was greater than during the two exercise conditions (MIE: 245.7 ± 102.7 kcal, HIIE: 257.8 ± 50.2 kcal).

Table 2. Postprandial substrate oxidation including baseline, 5 hours postprandial and total postprandial oxidation for each experimental condition.

Substrate Oxidation	Control	MIE	HIIE
Carbohydrate (g)			
0 (BL)	12.44 ± 4.12	14.66 ± 5.67	15.16 ± 4.22
1	17.51 ± 4.93	14.61 ± 6.11	16.44 ± 3.94
2	19.93. ± 3.91	16.55 ± 4.85	16.94 ± 2.49
3	16.60 ± 5.31	12.27 ± 5.50	12.20 ± 2.34
4	13.61 ± 4.71	9.81 ± 5.19	10.10 ± 1.96
5	11.89 ± 3.56	8.19 ± 4.03	8.78 ± 1.83
Total PP	79.54 ±22.42	61.42 ± 25.68	64.46 ± 12.56
Avg PP	15.91 ± 4.48	12.28 ± 5.14	12.89 ± 2.51
Fat (g)			
0 (BL)	3.81 ± 1.13	3.13 ± 0.91	2.70 ± 1.10
1	2.91 ± 1.12	5.20 ± 1.93	4.06 ± 1.20
2	2.02 ± 0.74	4.30 ± 1.59	3.24 ± 0.86
3	2.99 ± 1.20	5.20 ± 2.20	4.72 ± 1.60
4	4.01 ± 1.01	6.02 ± 1.83	5.53 ± 1.33
5	5.36 ± 1.28	6.42 ± 2.07	5.48 ± 1.05
Total PP	17.28 ± 5.35	27.14 ± 9.62	23.02 ± 6.04
Avg PP	3.46 ± 1.07	5.43 ± 1.92	4.60 ± 1.21

[a] Data are mean ± SD; BL: Baseline, PP: Postprandial. There were significant differences between groups at baseline. Mean fat oxidation difference between control and MIE is 1.53 ($p<0.001$), control and HIIE is 0.77 ($p=0.003$) and MIE and HIIE is 0.76 ($p=0.004$).

Table 3. Δ Postprandial substrate oxidation including Δ baseline, Δ 5 hours postprandial, total Δ postprandial and average Δ oxidation for each experimental condition.

Δ Substrate Oxidation	Control	MIE	HIIE
Δ Carbohydrate (g)			
0 (BL)	0.00	0.00	0.00
1	5.07 ± 2.99	-0.06 ± 4.19	1.28 ± 3.46
2	7.49 ± 2.40	1.89 ± 3.89	1.78 ± 2.95
3	4.16 ± 3.61	-2.39 ± 3.57	-2.96 ± 3.26
4	1.17 ± 3.45	-4.86 ± 2.98	-5.07 ± 2.75
5	-0.55 ± 2.49	-6.48 ± 3.18	-6.38 ± 2.74
Total PP	17.34 ± 14.93	-11.90 ± 17.80	-11.34 ± 15.17
Avg PP	3.47 ± 2.99	-2.38 ± 3.56	-2.27 ± 3.03
Δ Fat (g)			
0 (BL)	0.00	0.00	0.00
1	-0.91 ± 0.98	2.07 ± 2.04	1.36 ± 0.98
2	-1.80 ± 0.78	1.17 ± 1.40	0.54 ± 1.09
3	-0.83 ± 0.81	2.08 ± 1.83	2.02 ± 1.17
4	0.20 ± 0.78	2.89 ± 1.44	2.82 ± 1.02
5	1.55 ± 0.64	3.30 ± 1.70	2.78 ± 0.94
Total PP	-1.79 ± 3.99	11.51 ± 8.41	9.51 ± 5.20
Avg PP	-0.36 ± 0.80	2.30 ± 1.68	1.90 ± 1.04

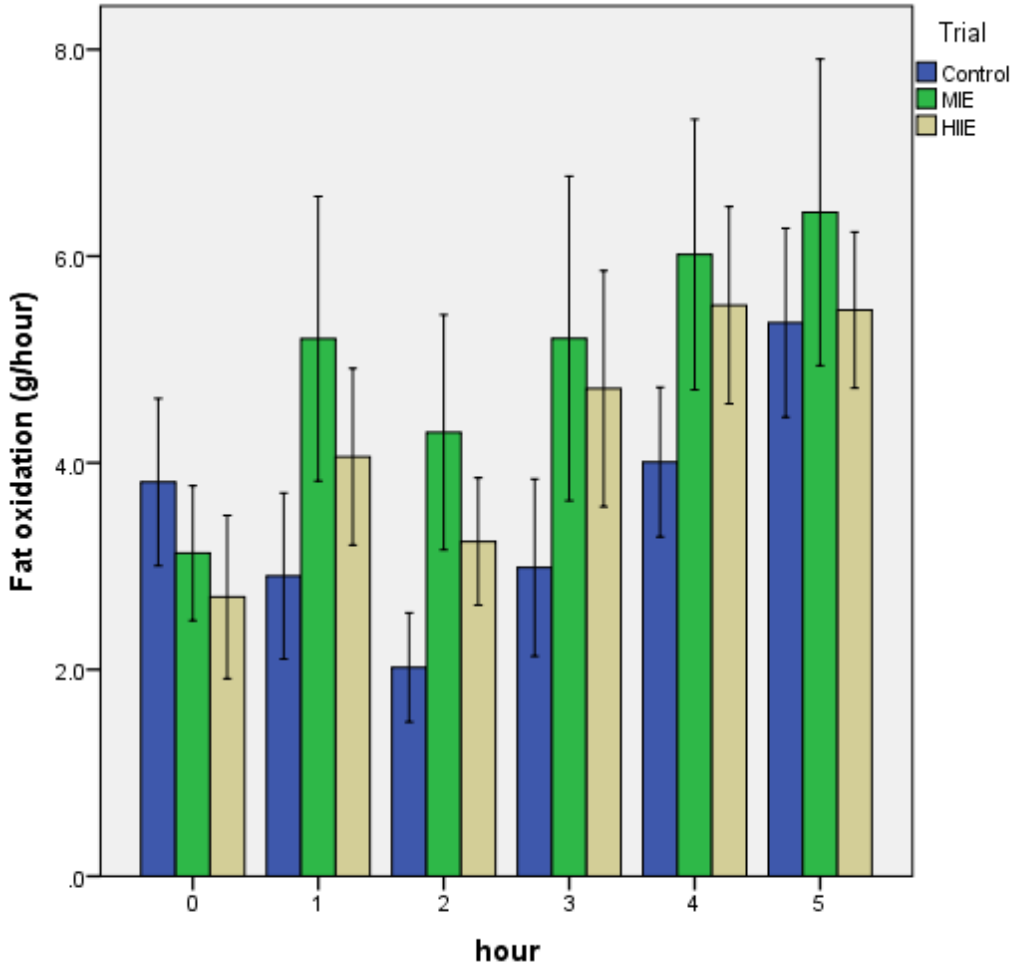
[a] Data are mean ± SD; BL: Baseline, PP: Postprandial. Δ substrate oxidation is determined by subtracting x hour from BL, accounting for BL variance. There were significant differences between groups at baseline. Mean Δ fat oxidation difference between control and MIE is 2.22 ($p<0.001$), control and HIIE is 1.88 ($p<0.001$) and MIE and HIIE is 0.33 ($p=0.69$).

Table 4. Postprandial caloric expenditure including baseline, 5 hours postprandial and total postprandial oxidation for each experimental condition.

Caloric Expenditure	Control	MIE	HIIE
Carbohydrate (kcal)			
0 (BL)	49.76 ± 16.47	58.65 ± 22.69	60.64 ± 16.86
1	70.05 ± 19.71	58.43 ± 24.46	65.77 ± 15.77
2	79.72 ± 15.65	66.19 ± 19.40	67.76 ± 9.96
3	66.39 ± 21.23	49.09 ± 21.98	48.81 ± 9.37
4	54.44 ± 18.85	39.23 ± 20.75	40.38 ± 7.82
5	47.57 ± 14.23	32.75 ± 16.12	35.12 ± 7.32
Total PP	318.18 ± 89.66	245.68 ± 102.70	257.84 ± 50.24
Avg PP	63.64 ± 17.93	49.14 ± 20.54	51.57 ± 10.05
Fat (kcal)			
0 (BL)	34.32 ± 10.18	28.14 ± 8.21	24.31 ± 9.94
1	26.15 ± 10.10	46.81 ± 17.33	36.53 ± 10.77
2	18.18 ± 6.65	38.66 ± 14.30	29.15 ± 7.76
3	26.87 ± 10.77	46.83 ± 19.76	42.46 ± 14.36
4	36.07 ± 9.09	54.15 ± 16.46	49.72 ± 12.00
5	48.20 ± 11.51	57.82 ± 18.66	49.31 ± 9.50
Total PP	155.47 ± 48.12	244.27 ± 86.15	207.18 ± 54.38
Avg PP	31.09 ± 9.62	48.85 ± 17.30	41.44 ± 10.88

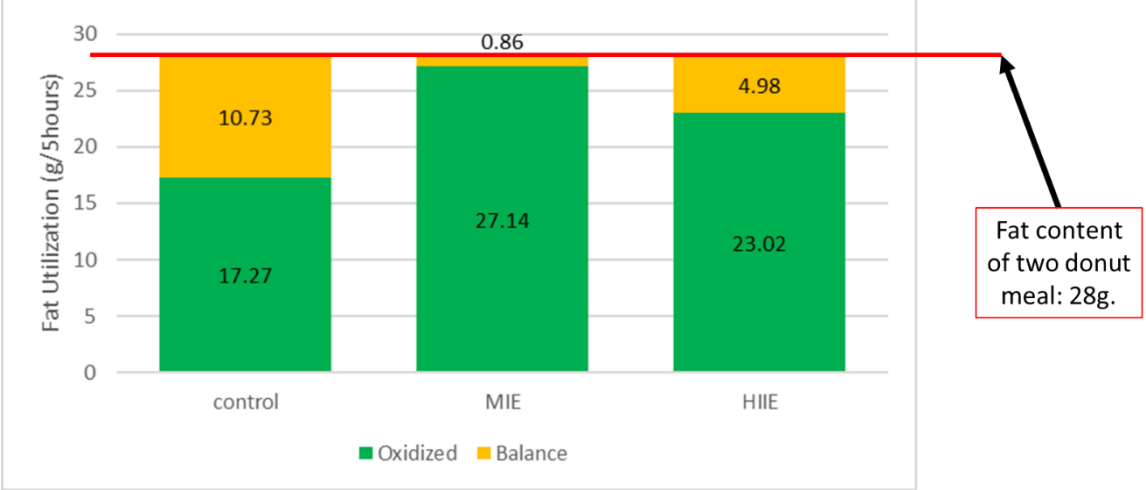
[a] Data are mean ± SD; BL: Baseline, PP: Postprandial. There were significant differences between groups at baseline.

Figure1: Postprandial fat oxidation (g/hour) for control and exercise conditions



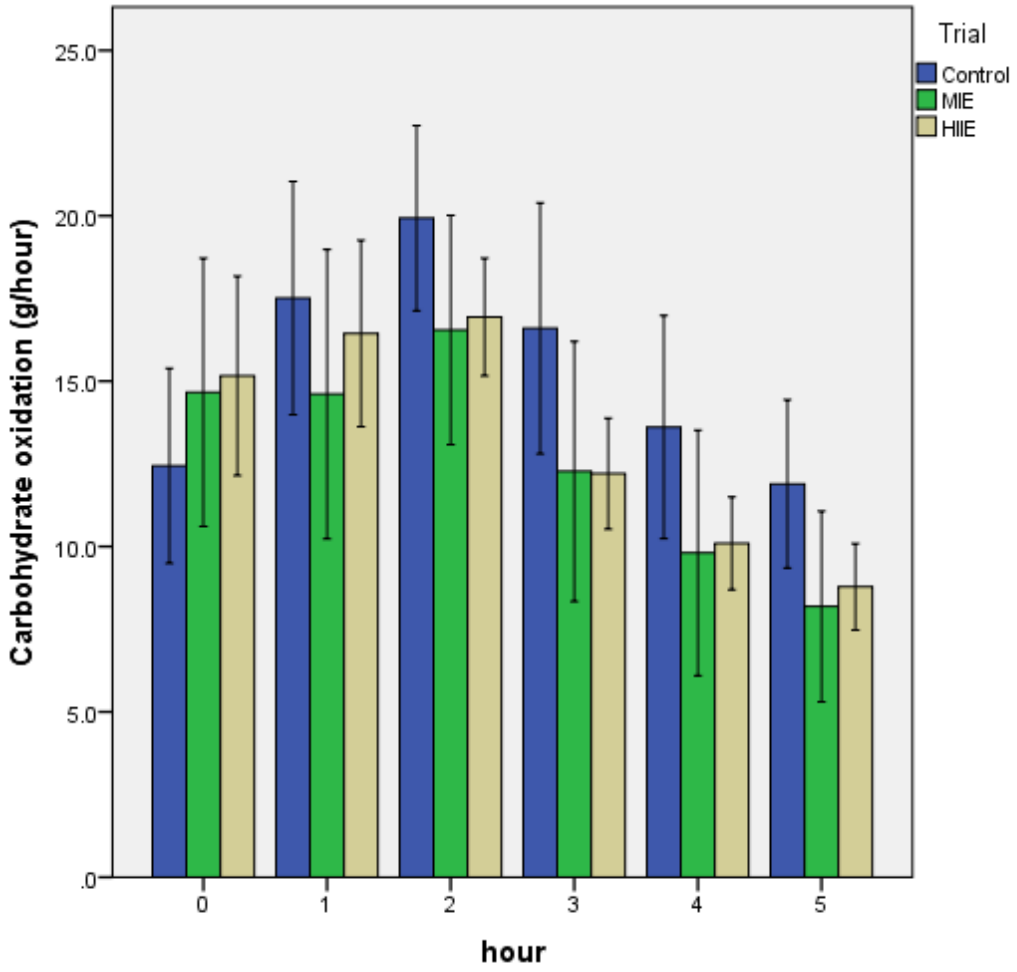
Substrate oxidation of fat (g) at baseline (0 hour), 1, 2, 3, 4 and 5 hours postprandial for control condition and two exercise conditions. For both exercise conditions the postprandial substrate oxidation was measured ~65 min post-exercise and the control condition (no exercise) was time matched. Error bars represent 95% CI. Mean fat oxidation difference between control and MIE is 1.53 ($p<0.001$), control and HIIE is 0.77 ($p=0.003$) and MIE and HIIE is 0.76 ($p=0.004$).

Figure 2: Total postprandial fat utilization (g/5 hours) for control and exercise conditions



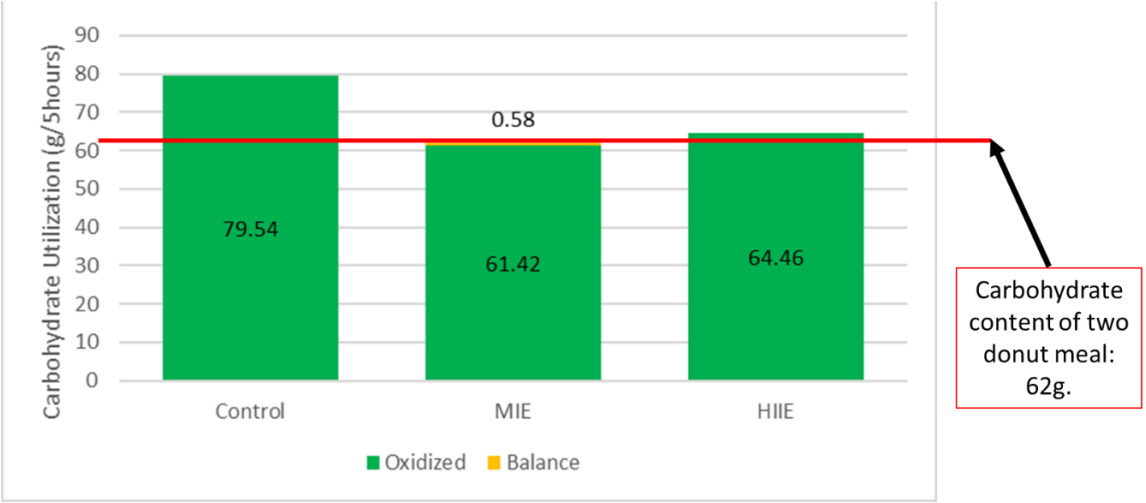
Substrate utilization of fat (g/5 hours) during the postprandial period. For both exercise conditions the postprandial substrate oxidation was measured ~65 min post-exercise and the control condition (no exercise) was time matched. Fat content of the two-donut meal (28g) is denoted by the red line. Fat oxidation is denoted by the green bar and the fat balance (remaining fat from the meal) is denoted by the orange bar.

Figure 3: Postprandial carbohydrate oxidation (g/hour) for control and exercise conditions



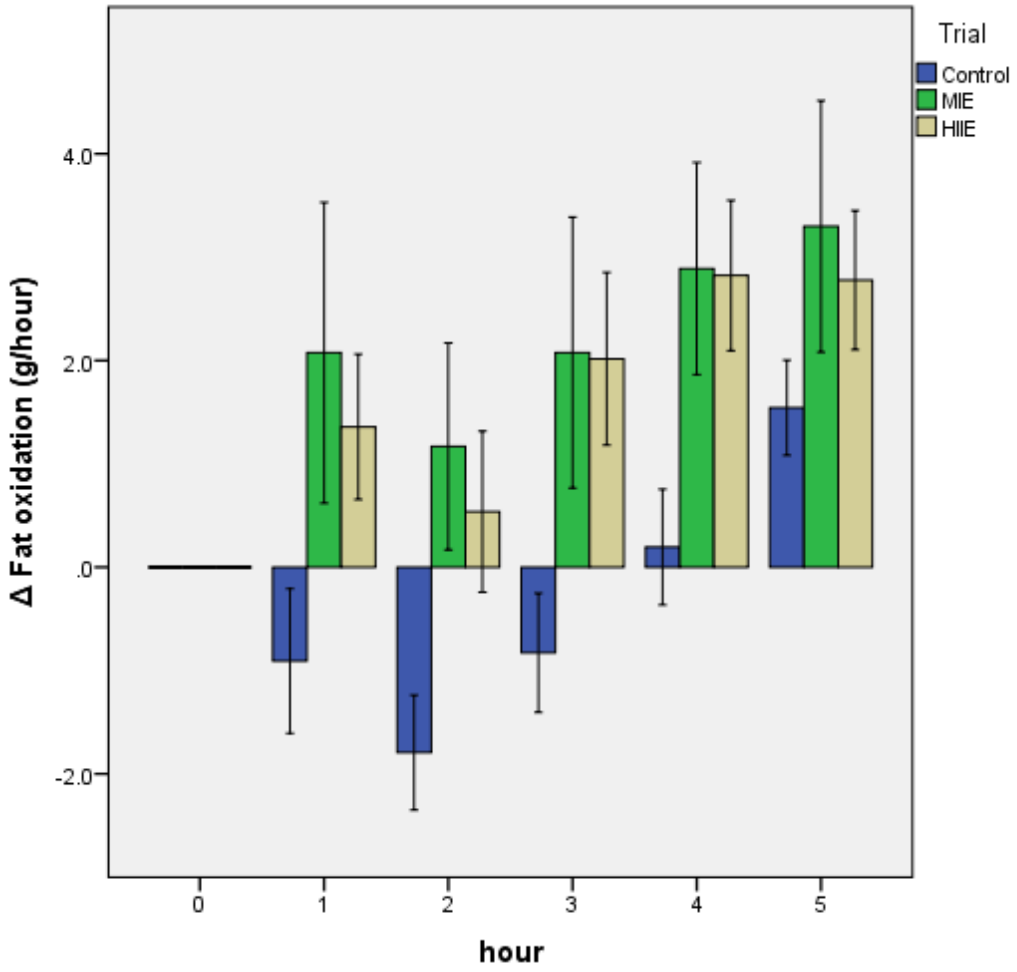
Substrate oxidation of carbohydrate (g) at baseline (0 hour), 1, 2, 3, 4 and 5 hours postprandial for control condition and two exercise conditions. For both exercise conditions the postprandial substrate oxidation was measured ~65 min post-exercise and the control condition (no exercise) was time matched. Error bars represent 95% CI.

Figure 4: Total postprandial carbohydrate utilization (g/5 hours) for control and exercise conditions



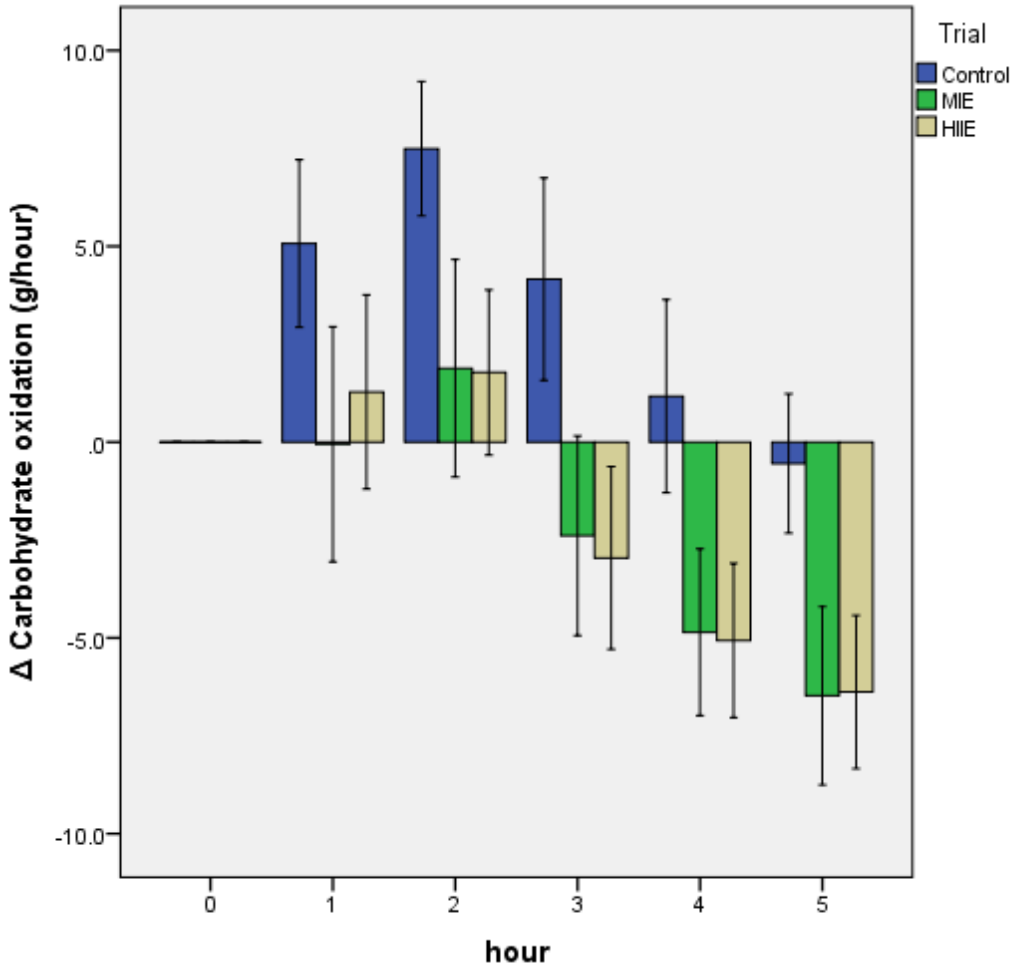
Substrate utilization of carbohydrate (g/5 hours) during the postprandial period. For both exercise conditions the postprandial substrate oxidation was measured ~65 min post-exercise and the control condition (no exercise) was time matched. Carbohydrate content of the two-donut meal (62g) is denoted by the red line. Carbohydrate oxidation is denoted by the green bar and the carbohydrate balance (remaining carbohydrate from the meal) is denoted by the orange bar.

Figure 5: Postprandial change in fat oxidation (g/hour), relative to baseline, for control and exercise conditions



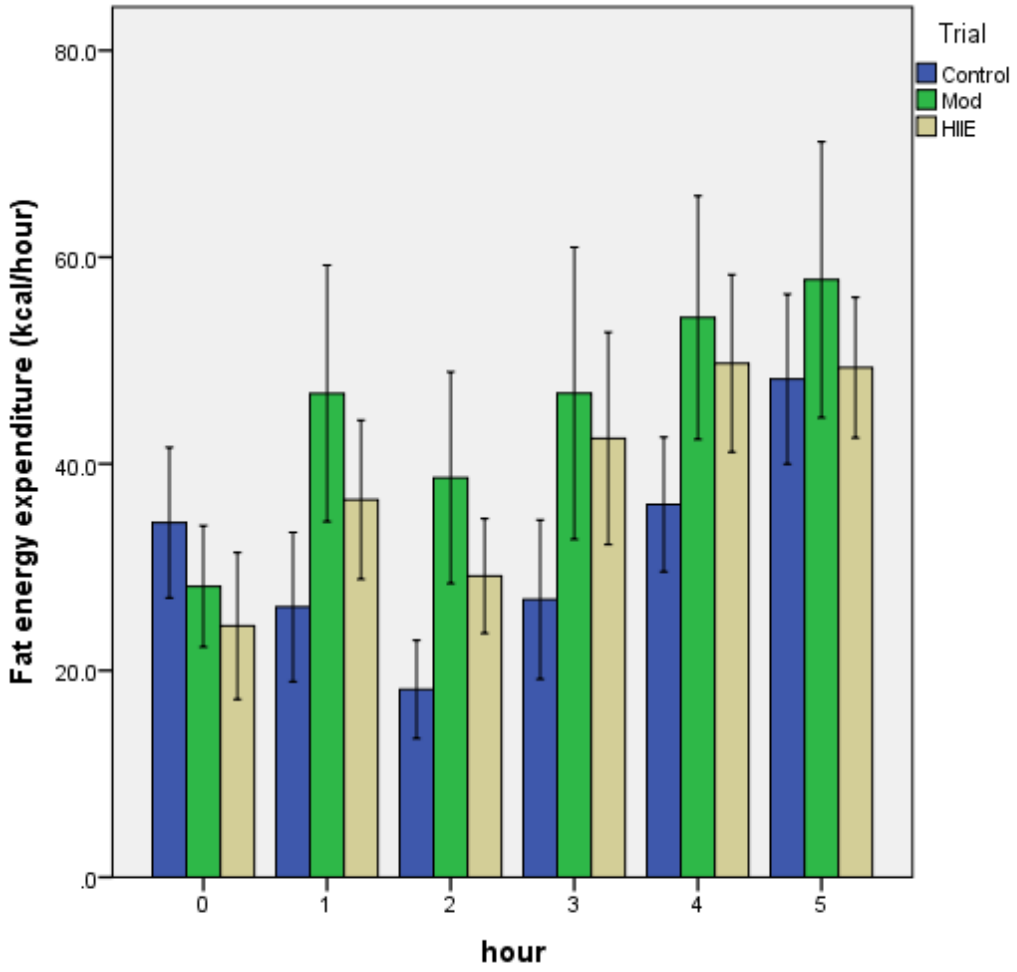
Δ substrate oxidation of fat (g) at baseline (0 hour), 1, 2, 3, 4 and 5 hours postprandial for control condition and two exercise conditions. For both exercise conditions the postprandial substrate oxidation was measured ~65 min post-exercise and the control condition (no exercise) was time matched. Error bars represent 95% CI. Mean Δ fat oxidation difference between control and MIE is 2.22 ($p<0.001$), control and HIIE is 1.88 ($p<0.001$) and MIE and HIIE is 0.33 ($p=0.69$).

Figure 6: Postprandial change in carbohydrate oxidation (g/hour), relative to baseline, for control and exercise conditions



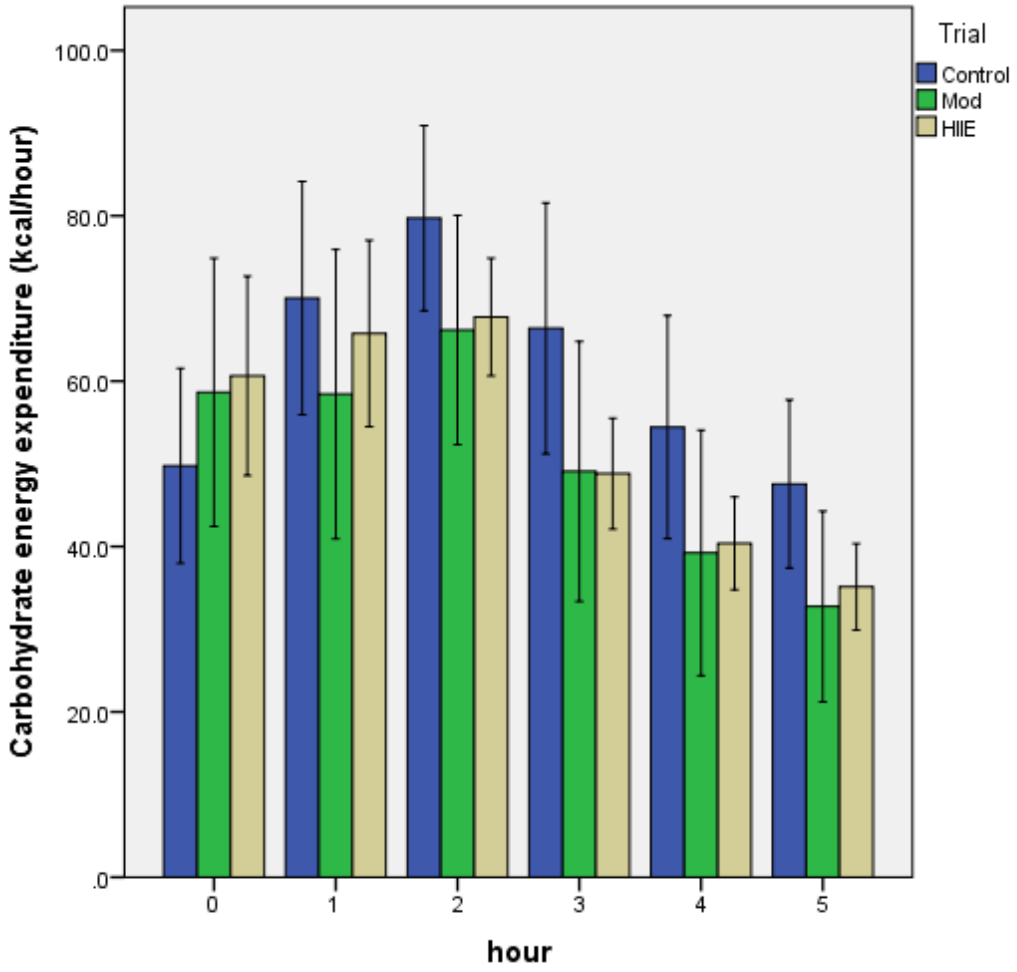
Δ substrate oxidation of carbohydrate (g) at baseline (0 hour), 1, 2, 3, 4 and 5 hours postprandial for control condition and two exercise conditions. For both exercise conditions the postprandial substrate oxidation was measured ~65 min post-exercise and the control condition (no exercise) was time matched. Error bars represent 95% CI.

Figure 7: Postprandial energy expenditure from fat (kcal/hour) for control and exercise conditions



Caloric expenditure of fat (kcal) at baseline (0 hour), 1, 2, 3, 4 and 5 hours postprandial for control condition and two exercise conditions. For both exercise conditions the postprandial substrate oxidation was measured ~65 min post-exercise and the control condition (no exercise) was time matched. Error bars represent 95% CI.

Figure 8: Postprandial energy expenditure from carbohydrate (kcal/hour) for control and exercise conditions



Caloric expenditure of carbohydrate (kcal) at baseline (0 hour), 1, 2, 3, 4 and 5 hours postprandial for control condition and two exercise conditions. For both exercise conditions the postprandial substrate oxidation was measured ~65 min post-exercise and the control condition (no exercise) was time matched. Error bars represent 95% CI.

CHAPTER 5

DISCUSSION

The purpose of this randomized controlled repeated measures crossover designed trial was to determine the effects of high-intensity interval exercise (HIIE) and moderate-intensity continuous exercise (MIE) on postprandial substrate oxidation/nutrient partitioning after consumption of an isocaloric high sugar/high fat meal ~ 520 calories (2 glazed donuts) in adults in their twenties. This Chapter presents the discussion including an analysis of the results, strengths and limitations of the study, future considerations and practical applications.

Analysis of Results

The hypothesis of this study, that a larger postprandial oxidation of lipids would be demonstrated after the HIIE isocaloric exercise bout compared to the MIE isocaloric exercise bout, was rejected. The overall fat oxidation in the 5-hour postprandial periods for the MIE and HIIE trials were not significantly different from each other when baseline caloric expenditure and substrate oxidation were accounted for. Importantly, both exercise conditions created significantly greater absolute postprandial fat oxidation compared to the control trial. This is most clearly demonstrated by analyzing the Δ Fat oxidation values (Table 3) or graph (Figure 3). In the control condition, fat oxidation was suppressed below baseline in the for the first 3 hours postprandial, and cumulative fat oxidation during the entire 5-hour postprandial period was less than the fat oxidation compared to the baseline fat oxidation rate. By contrast, fat oxidation during the two exercise conditions was higher during each hour postprandial compared to baseline fat oxidation rate (Table 3 and Figure 3).

The lack of difference in total postprandial fat oxidation, relative to baseline, between MIE and HIE suggests that exercise intensity is not an important determinant of postprandial substrate oxidation. The results indicate that total energy expenditure is the primary determinant of postprandial substrate oxidation, at least under isocaloric conditions and when exercise is performed prior to meal ingestion. These results are opposite to some previous research suggesting that exercise intensity plays a larger role than duration when the exercise is matched for energy expenditure (Pillard et al., 2010). The MIE required 42 to 99 minutes and HIIE required 38 to 70 minutes to complete. Although MIE required 8 minutes longer to expend ~500 kcal (i.e., 62.4 minutes vs. 54.3 minutes), this difference is relatively trivial in the context of the duration of exercise required to expend the equivalent number of kcal. The total postprandial carbohydrate oxidized in all trials was greater than or equal to (MIE= +0.58g carbohydrate balance) the carbohydrate ingested in the two donuts (Figure 2). This illustrates that the carbohydrate ingested from donuts has been completely oxidized within 5 hours postprandial. Although the total fat oxidized in both exercise conditions was not quite equal to the fat ingested in the two donuts (Figure 4), the present data indicated that fat oxidation was increasing each of the last two hours postprandial during the exercise conditions (Figure 3). This suggests that the fat ingested from the donuts would have been completely oxidized within 6 hours postprandial. By contrast, the present data indicate that it might require 7-8 hours to completely oxidize all the fat ingested during the control trial. How subsequent meals might alter the pattern of substrate oxidation during control and exercise conditions requires additional study.

Also, follow-up studies should compare more traditional HIIE protocols, with fewer intervals, that typically result in total energy expenditures of 250-300 kcal (Wesley J Tucker et al., 2016). Exercise protocols lasting ~60 minutes, like the present study, are much longer than typically utilized, and are well in excess of current public health recommendations.

Strengths & Limitations

The strengths of this current design lie within the randomized, repeated measures, crossover design which allow each participant to act as their own control. This became especially useful when accounting for the variance among baseline data. Accounting for diurnal variation (K. Hawkins et al., 2012; Votruba et al., 2005) within subjects by adjusting subject start time to reflect a consistent postprandial 5-hour period. Furthermore, the outcome measures were recorded continuously and saved as an average per minute, which were later converted to an average per hour for easier display, comprehension, and to maintain consistency amongst other academic research that reported on similar outcome measures.

Ingestion of a meal consisting mainly of fat and sugar has high ecological validity due to the high percentage of fat and sugar in the American diet, and the fact that carbohydrate stimulates its own oxidation and suppresses fat oxidation. Such a meal is ideal for testing the efficacy of exercise for altering postprandial fat and carbohydrate oxidation, which has implications for fat balance.

Pre-trial dietary intake was a limitation of this study. Some studies (Bergouignan et al., 2012; Hill et al., 1991; Horton et al., 1995) controlled the subjects' meals days in advance with preparation in lab and providing a consistent macronutrient composition

between subjects. This study attempted to maintain a consistent diet for a single day prior to each trial, so long as the subjects kept their dietary intake at the same time, frequency and volume the day prior to each trial with a 12-hour fast of all oral intake except water and a 14-hour fast from caffeine. The caloric and macronutrient composition of the pre-trial diet was undoubtedly different between-subjects. Subject compliance is a very common issue faced with reporting dietary intake given that for 39 years the National Health and Nutrition Examination Survey (NHANES) collected self-reported energy intake data that was determined to be physiologically implausible for over 58% of respondents (Archer, Hand, & Blair, 2013), even though the subjects' choice was thought to increase the likelihood of adherence. It is reasonable to believe that variation by omission or addition of dietary intake occurred between the pre-trial diet for some subjects. Lastly, women were not excluded from this pilot study (though only two of ten participants were women), due to the conflicting evidence regarding the variation of substrate oxidation utilization throughout the different stages of the menstrual is conflicting. It is worth mentioning that some studies have found an increased proportion of fat oxidation is present during the Luteal phase compared to the Follicular phase of menstruation (Isacco, Duché, & Boisseau, 2012).

Future Considerations

For future direction, it would be interesting to assess the comparison of HIIE and MIE with additional metabolic parameters in addition to substrate oxidation and energy expenditure. For example, blood samples for insulin and FFA analysis, as well as Flow-Mediated Dilation (FMD) which is a common non-invasive method to assess endothelial (dys)function. A commonly cited meta-analysis on FMD illustrates that a 1% decrease in

FMD equates to a 13% increase in cardiovascular disease risk (Inaba, Chen, & Bergmann, 2010). Along with addressing different outcome variables, the extension of postprandial measurement from 5-hours to 24-hours with the addition of a few more meals to replicate typical human meal patters. Previous research has demonstrated that positive metabolic effects from physical activity can manifest overnight suggesting sleep as an important factor in the regulation of lipid metabolism (Bergouignan et al., 2014). Additionally, regarding the 24-hour period, an energy balance state or energy deficit state could be explored.

A larger sample size would also be beneficial in a future study. The sample size of the current trial (n = 10) is consistent with other studies that examined the same outcome variables in humans (C. Bennett et al., 1992; N. Folch, F. Peronnet, D. Massicotte, S. Charpentier, & C. Lavoie, 2003; Gregory et al., 2011; Horowitz, Kaufman, Fox, & Harber, 2005; Kaito Iwayama et al., 2017; Melanson et al., 2002; Pillard et al., 2010; P. Schrauwen et al., 1998; Shimada et al., 2013; W. J. Tucker et al., 2016; Votruba et al., 2005). Nonetheless, similar studies have also had around double the sample size (Bergouignan et al., 2014; K. R. Hawkins, K. C. Hansen, D. A. Schoeller, & J. A. Cooper, 2012; Martins et al., 2016; E. L. Melanson et al., 2009; P. Stiegler, S. A. Sparks, & A. Cunliffe, 2008; Votruba et al., 2002; Votruba et al., 2003) which will only improve the power of the results to demonstrate significant findings.

Conclusion & Practical Applications

The present pilot study demonstrated that exercise, either MIE or HIE, results in significantly greater fat oxidation postprandial following an isocaloric high sugar, high carb meal of ~520 calories compared to a non-exercise control. The fact that MIE

resulted in slightly greater total postprandial fat oxidation was opposite to the result hypothesized, as HIIE has been reported to induce greater post-exercise fat oxidation compared to MIE (Schjerve et al., 2008; Tjønna et al., 2008; Wesley J Tucker et al., 2016). The present results indicate that either exercise protocol is sufficient to enhance postprandial fat oxidation. Whether more traditional exercise protocols lasting much less than the time required for the protocols used in the present study (mean ~1 hour), can produce similar results require additional investigation. The fact that exercise of ~1 hour was required to oxidize the amount of fat in two donuts, that required only a few minutes to consume, highlights the challenges of using exercise for weight control in an obesogenic environment. Lastly, to answer the initial question proposed at the beginning of this paper, "Can fat burning be optimized in humans in order to enhance postprandial fat oxidation and reduce chances of positive fat balance?", the present data indicate that exercise most certainly increases postprandial fat oxidation, and that exercise type, either MIE or HIIE, is not as important as total energy expended.

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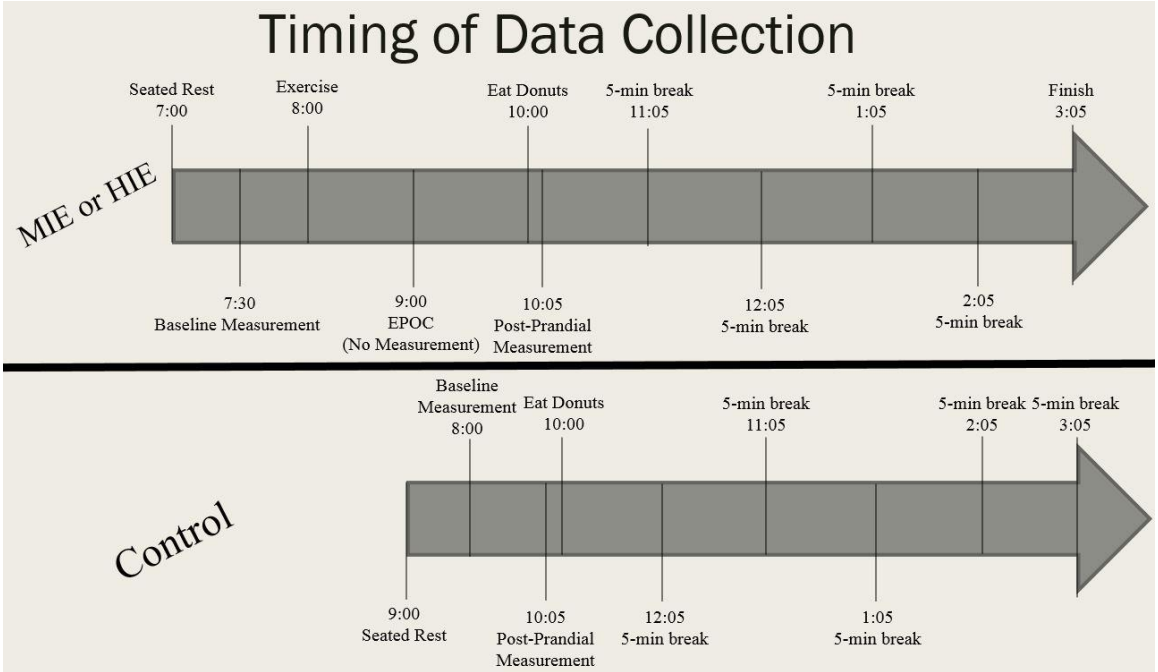
APPENDIX A

STUDY VISUAL TIMELINE

The individual timing of the trials varied between subjects, however within subjects the timing of trials remained consistent. The following timeline illustrates an example of a particular sequence of trials.

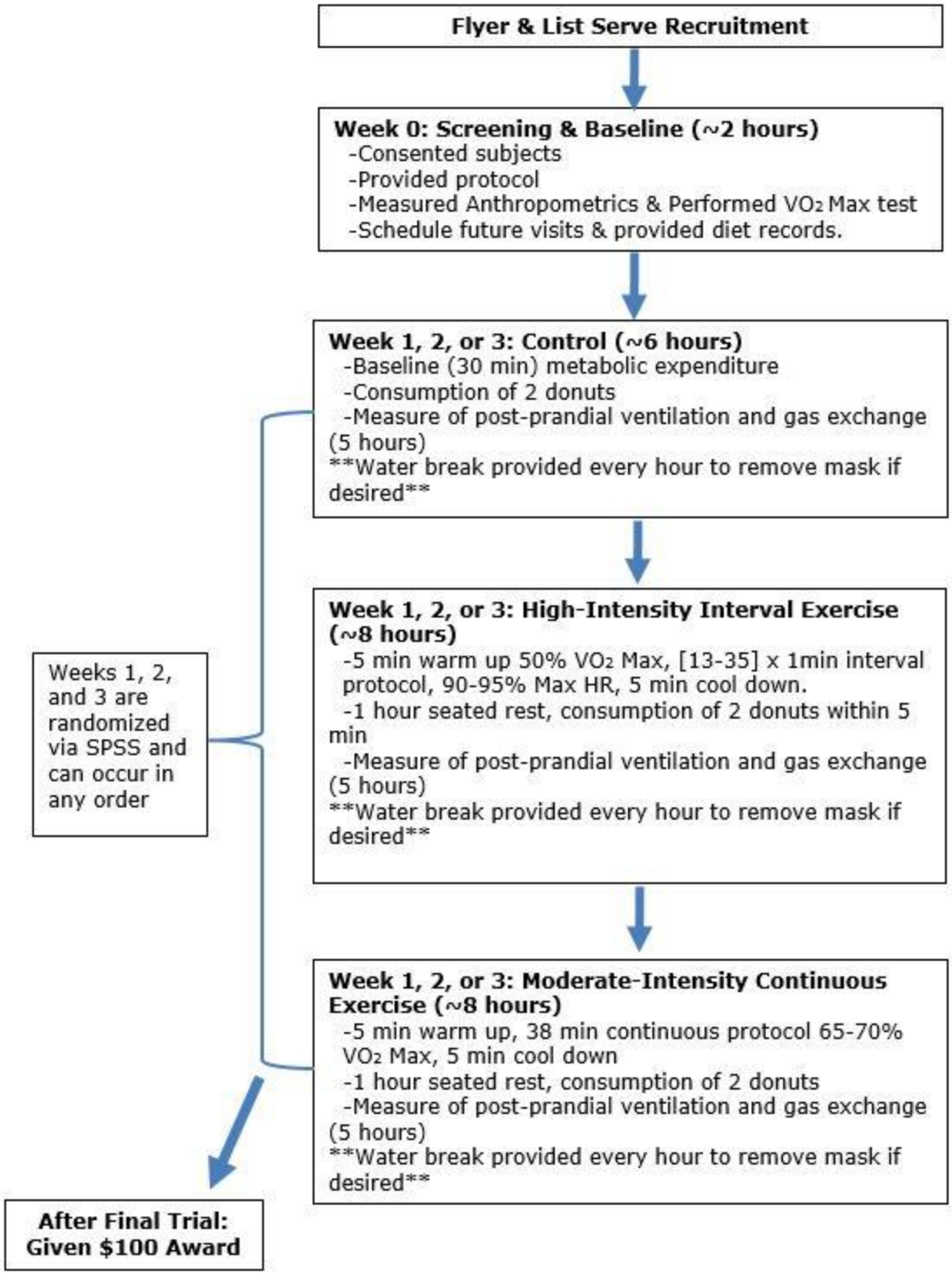


APPENDIX B

STUDY FLOW DIAGRAM

Study Protocol

**Week does not have to be a full 7-day week, as long as each visit is separated by at least 72 hours. **



APPENDIX C

INFORMED CONSENT

Consent Form: Bioscience

CONSENT FORM
Effects of high-intensity interval exercise and moderate-intensity continuous exercise on postprandial fat and carbohydrate oxidation in healthy adults

INTRODUCTION
The purposes of this form are to provide you (as a prospective research study participant) information that may affect your decision as to whether or not to participate in this research and to record the consent of those who agree to be involved in the study.

Researchers
Glenn Gaesser, PhD, professor, Siddhartha Angadi, PhD, assistant professor, Catherine Jarrett, doctoral student, and Jacob Fleming, masters student, all in the School of Nutrition and Health Promotion, have requested your participation in a research study.

Why am I being invited to take part in a research study?
We invite you to take part in a research study because you are between the ages of 18 & 45, healthy, non-smoker, do not have gluten intolerance, are not taking medications, and are fit enough to perform vigorous exercise.

Why is this research being done?
The objective of this study is to determine how two different types of exercise sessions affect the burning of fat and carbohydrate after eating two donuts (about 520 calories). These exercise bouts are as follows:

(1) High-intensity interval exercise: alternating 1-min intervals ranging between 50% of maximum heart rate and 90-95% maximum heart rate, for approximately 35 minutes (enough to burn 520 calories);
(2) Moderate-intensity continuous exercise at about 70% of maximum heart rate (enough to burn 520 calories).

Information from measurements during these exercise sessions will be compared to those obtained during a session that consists of resting quietly in a chair for an equal amount of time.

How long will the research last?
For each participant the study will take about 3-4 weeks to complete. It will require 4 visits, with anywhere from 3-10 days between visits. The total amount of time commitment is approximately 26 hours.

How many people will be studied?
We expect about 15-20 people here will participate in this research study.

What happens if I say yes, I want to be in this research?
It is up to you to decide whether or not to participate. Should you choose to participate, you will come to the ABC1 laboratory on the downtown Phoenix campus on 4 different days, with a minimum of 3 days between visits.

On visit 1:

You will have your height and weight measured, and an exercise test for determination of your maximum aerobic capacity (fitness level). For this test, you will be wearing a facemask attached to a

hose that collects the air you breathe out. You will also wear a heart rate monitor that consists of an elastic strap that wraps around your chest to measure your heart rate. You will sit quietly on the stationary cycle for 2 minutes then you will be asked to pedal at light resistance for 5 minutes for the warm-up phase. After the warm-up phase the resistance will increase continuously every minute until you cannot continue. We will encourage you to push yourself as hard as you feel comfortable. You will provide us a verbal or hand signal when you want to stop.

The whole test will take about 30 minutes.

This visit will take no longer than 2 hours.

Visits 2, 3 and 4:

These visits will be in random order, and consist of the following:

8 AM:	Arrival at laboratory in a fasted state (nothing but water after 8 PM the night before; no caffeinated beverages after 6 PM); sit quietly for 30 minutes
8:30 AM:	Resting metabolic rate measurement using the same equipment used for the maximum exercise test at visit 1
9:00 AM:	Begin either of two exercise sessions, or the resting control trial (described below)**
~9:45 10:00 AM	Begin 1 hour of recovery (after exercise sessions) while seated in a chair or continued rest (in the resting control trial)
10:45 - 11:00 AM	Exactly 1 hour after completion of the exercise sessions, or rest (in the non-exercise control session), you will consume 2 glaze donuts (520 calories total) and rest in a seated chair for 5 hours.
~4:00 PM	This ends the experimental procedures for that day

**The three experimental conditions consist of the following:

- For the resting control trial: seated rest throughout the time in the laboratory (8 AM to 4 PM)
- For the exercise conditions:
    - Exercise will be performed on a stationary cycle ergometer
    - 5 minutes of warm-up at a moderate intensity
    - ~35-40 minutes of exercise (enough to burn 520 calories), either continuously at ~70% maximum heart rate, or by alternating 1-min intervals at a high-intensity (90-95% maximum heart rate) and low-intensity (50% maximum heart rate);
    - 5 minutes of cool-down; the exercise sessions are expected to take 45-60 minutes, including warm-up and cool-down, depending on your fitness level
    - 6 hours of seated rest, until ~4 PM (to match the measurement period of the resting control trial)

During each condition you will wear the same facemask as in visit #1, that attaches to a hose that collects the air you breathe out.

You will be allowed to remove the facemask every hour for a few minutes to drink water, if desired, and you will be permitted restroom breaks as necessary.

During the time you are resting quietly in a chair you will be allowed to read, work on your laptop computer, use you phone, etc.

On the day before visit 2 you will be asked to record all the food and beverages you consume (breakfast, lunch, dinner, snacks). You will be asked to consume the same food and beverages on the day before visits 3 and 4.

Each of visits 2, 3 and 4 will take about 8 hours. Total time for these 3 visits combined will be approximately 24 hours.

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

If you decide to leave the research, contact the investigator so that the investigator can formally withdraw you from the study.

If you stop being in the research, already collected data may not be removed from the study database. You will be asked whether the investigator can use the previous collected data in the results. If you agree, this data will be handled the same as research data.

The reason for your withdrawal will be requested to be used in research reporting purposes only.

Risks
Research studies often involve some risks. The risks of exercise include local muscle soreness, abnormal changes in blood pressure, nausea, faintness, dizziness, irregular heartbeats (rare), and, in very rare instances, heart attack.

You will be monitored by trained investigators and if there are any adverse effects, the exercise testing or the exercise session will be halted.

Will being in this study help me any way?
There may be no direct benefits to you. However, results of your tests will be made available to you at your request, and any questions about that information will be answered by one of the study personnel.

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

After your participation in the study is complete, selected results will be available to you upon request requiring you to sign the Research Results Acknowledgement Statement.

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

COSTS AND PAYMENTS
All study procedures will be provided to you at no cost to you.
You will be paid $75 at completion of the study by either compensation-check or gift certificate. Partial payment will be prorated based on the number of visits you complete if you withdraw from the study ($5 after visit 1; an additional $15 after visit 2: an additional $20 after visit 3; an

additional $35 after visit 4, for a maximum total of $75) If you agree to participate in the study, then consent does not waive any of your legal rights. However, no funds have been set aside to compensate you in the event of injury.

Who can I talk to?

If you have questions, concerns, or complaints, or think the research has hurt you, talk to the research team at Dr. Glenn Gaesser, 550 N 3rd ST, Phoenix, AZ 85004; 602-827-2283; glenn.gaesser@asu.edu.

This research has been reviewed and approved by the Bioscience IRB ("IRB"). You may talk to them at (480) 965-6788 or research.integrity@asu.edu if:

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

Signature Block for Capable Adult

Your signature documents your permission to take part in this research.

Signature of participant	Date
Printed name of participant	
Signature of person obtaining consent	Date
Printed name of person obtaining consent	

APPENDIX D

PHYSICAL ACTIVITY READINESS QUESTIONNAIRE

Physical Activity Readiness Questionnaire (PAR-Q) and You

Regular physical activity is fun and healthy, and increasingly more people are starting to become more active every day. Being more active is very safe for most people. However, some people should check with their doctor before they start becoming much more physically active.

If you are planning to become much more physically active than you are now, start by answering the seven questions in the box below. If you are between the ages of 15 and 69, the PAR-Q will tell you if you should check with your doctor before you start. If you are over 69 years of age, and you are not used to being very active, check with your doctor.

Common sense is your best guide when you answer these questions. Please read the questions carefully and answer each one honestly:

YES	NO		
☐	☐	1.	Has your doctor ever said that you have a heart condition and that you should only do physical activity recommended by a doctor?
☐	☐	2.	Do you feel pain in your chest when you do physical activity?
☐	☐	3.	In the past month, have you had chest pain when you were not doing physical activity?
☐	☐	4.	Do you lose your balance because of dizziness or do you ever lose consciousness?
☐	☐	5.	Do you have a bone or joint problem that could be made worse by a change in your physical activity?
☐	☐	6.	Is your doctor currently prescribing drugs (for example, water pills) for your blood pressure or heart condition?
☐	☐	7.	Do you know of any other reason why you should not do physical activity?

If you answered:

YES to one or more questions

Talk to your doctor by phone or in person BEFORE you start becoming much more physically active or BEFORE you have a fitness appraisal. Tell your doctor about the PAR-Q and which questions you answered YES.

- You may be able to do any activity you want – as long as you start slowly and build up gradually. Or, you may need to restrict your activities to those which are safe for you. Talk with your doctor about the kinds of activities you wish to participate in and follow his/her advice.
- Find out which community programs are safe and helpful for you.

NO to all questions

If you answered NO honestly to all PAR-Q questions, you can be reasonably sure that you can:

- Start becoming much more physically active – begin slowly and build up gradually. This is the safest and easiest way to go.
- Take part in a fitness appraisal – this is an excellent way to determine your basic fitness so that you can plan the best way for you to live actively.

Delay becoming much more active:

- If you are not feeling well because of a temporary illness such as a cold or a fever – wait until you feel better; or
- If you are or may be pregnant – talk to your doctor before you start becoming more active.

Please note: If your health changes so that you then answer YES to any of the above questions, tell your fitness or health professional. Ask whether you should change your physical activity plan.

Informed use of the PAR-Q: Reprinted from ACSM's Health/Fitness Facility Standards and Guidelines, 1997 by American College of Sports Medicine

APPENDIX E

RECRUITMENT FLYER

ARIZONA STATE UNIVERSITY

Men & Women 18 – 45 years old and physically fit to perform about an hour of exercise and eat 2 donuts are needed for a study investigating the effects of different exercise protocols nutrient oxidation patterns

Compensation: $100

This study is designed to determine the effects of different intensity exercise protocols on post-prandial substrate oxidation followed by the consumption of two glazed donuts. This study includes 4 visits (at least 72 hours apart) to the Healthy Lifestyles Research Center on the Arizona State University Downtown Campus in Phoenix. Time commitment: 24 hours over the course of 2-4 weeks. **Your participation throughout the study is completely voluntary.**

Eligible subjects must be nonsmokers, in good health, have no restrictions for participating in vigorous intensity physical activity, willing to refrain from exercise 48 hours prior to each lab visit, and must not be taking any medications for blood pressure, cholesterol, diabetes or a heart condition.

Please contact:
Jacob Fleming (520-302-1395; Jmflemi2@asu.edu)

Jacob Fleming 520-302-1395 Jmflemi2@asu.edu	Jacob Fleming 520-302-1395 Jmflemi2@asu.edu	Jacob Fleming 520-302-1395 Jmflemi2@asu.edu	Jacob Fleming 520-302-1395 Jmflemi2@asu.edu	Jacob Fleming 520-302-1395 Jmflemi2@asu.edu	Jacob Fleming 520-302-1395 Jmflemi2@asu.edu	Jacob Fleming 520-302-1395 Jmflemi2@asu.edu	Jacob Fleming 520-302-1395 Jmflemi2@asu.edu	Jacob Fleming 520-302-1395 Jmflemi2@asu.edu	Jacob Fleming 520-302-1395 Jmflemi2@asu.edu	Jacob Fleming 520-302-1395 Jmflemi2@asu.edu	Jacob Fleming 520-302-1395 Jmflemi2@asu.edu

APPENDIX F

QUALTRICS SCREENING QUESTIONS

Substrate Oxidation Pre-Screening Questions Sheet Experiment

Short consent for participation in the pre-screening process

1) The following pre-screening questions will allow us to establish your eligibility for participation in our study (a brief description of the study is displayed in the flyer). Participation in the pre-screening process is completely voluntary and you may end the process at any time. The survey should only take a few minutes to complete.

Do you consent to participate in the pre-screening survey?

Screening Questions

1. How old are you?
2. Do you smoke?
3. Has your doctor ever said that you have a heart condition and that you should only do physical activity recommended by a doctor?
4. Do you feel pain in your chest when you do physical activity?
5. In the past month, have you had chest pain when you were not doing physical activity?
6. Do you lose your balance because of dizziness or do you ever lose consciousness?
7. Do you have a bone or joint problem (for example, back, knee or hip) that could be made worse by a change in your physical activity?
8. Is your doctor currently prescribing drugs (for example, water pills) for your blood pressure or heart condition?
9. Do you know of any other reason why you should not do physical activity?
10. Are you currently training for a specific athletic event, such as an endurance race or marathon?
11. Please briefly describe the specific athletic event for which you are training.
12. Are you currently dieting or trying to lose weight?
13. What is your name? (First Last)
14. What is your email address?
15. What is your phone number?
16. What is your preferred method for us to contact you?

APPENDIX G

SUBJECT CHARACTERISTICS, VO_{2max} & CALCULATIONS SHEET

Subject: _____ DATE: _____

DOB: _____ SEX: _____ ETHNICITY: _____

WEIGHT (KG): _____ HEIGHT (CM): _____ SEAT HEIGHT: _____

RANDOMIZED TRIAL ORDER: _____ _____ _____

VO2 MAX: _____ HR MAX: _____

WARM UP & COOL DOWN

MODERATE INTENSITY CONTINUOUS

60-75% HR (beats): _____ (watts): _____

55-60% VO2: _____

TIME EXERCISE (WARM-UP INCLUDED): _____

HIGH INTENSITY INTERVAL

90-95% HR (beats): _____ (watts): _____

67.4% VO2: _____

TIME EXERCISE (WARM-UP INCLUDED): _____

APPENDIX H

TIME RECORD SHEET

TIME RECORD SHEET

SEATED REST_____

BASELINE_____

BEGIN EXERCISE_____

FINISH EXERCISE_____

DONUTS_____

POST-PRANDIAL_____

FINISH_____

BATTERY/BATHROOM TIMES

_____

_____

_____

_____

_____

_____

_____

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_____

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_____

_____

_____

_____

APPENDIX I

MIE RECORD SHEET

	VO2	KP	Cadence	HR
Warm up				
Warm up				
Warm up				
Warm up				
Warm up				
1:00				
2:00				
3:00				
4:00				
5:00				
10:00				
15:00				
20:00				
25:00				
30:00				
35:00				
40:00				
45:00				
50:00				
55:00				
60:00				
65:00				
70:00				
Cool Down				
Cool Down				
Cool Down				
Cool Down				
Cool Down				

APPENDIX J

HIIE RECORD SHEET

Date Week/Session				
Time	Description	HR	Resistance	RPM
1	Warm up			
2	Warm up			
3	Warm up			
4	Warm up			
5	Warm up			
6	Interval 1			
7	Rest 1			
8	Interval 2			
9	Rest 2			
10	Interval 3			
11	Rest 3			
12	Interval 4			
13	Rest 4			
14	Interval 5			
15	Rest 5			
16	Interval 6			
17	Rest 6			
18	Interval 7			
19	Rest 7			
20	Interval 8			
21	Rest 8			
22	Interval 9			
23	Rest 9			
24	Interval 10			
25	Rest 10			
26	Interval 11			
27	Rest 11			
28	Interval 12			
29	Rest 12			
30	Interval 13			
31	Rest 13			
32	Interval 14			
33	Rest 14			
34	Interval 15			
35	Rest 15			
36	Interval 16			
37	Rest 16			
38	Interval 17			
39	Rest 17			
40	Interval 18			
41	Rest 18			
42	Interval 19			
43	Rest 19			
44	Interval 20			
45	Rest 20			
46	Cool Down			
47	Cool Down			
48	Cool Down			
49	Cool Down			
50	Cool Down			

APPENDIX K

3-DAY DIETARY RECALL

FOOD JOURNAL

INSTRUCTIONS

• Please write down all foods and beverages consumed for three 24-hour time periods preceeding the following lab visits. Each day starting at 12:00 am and ending at 11:59 pm (No food after 8pm, just water).

• You will be asked to record all vitamin, mineral, and herbal supplements you took at the end of each record.

• List the approximate **Time** the meal was consumed, **Place** where it was consumed (home, work, name of restaurant, church, etc.), and the type of eating occasion or **Meal** (breakfast, lunch, dinner, snack, or other).

• List each **Food/Beverage Item** you consumed, including foods eaten between meals and all drinks, even if it is a non-caloric item like water, coffee, tea, or sugar free gum.

• Specify **Details/Ingredients/Preparation** of each food or beverage consumed (1 large egg *fried w/ 1 tbsp butter,* 1 6 oz salmon fillet *baked with 0.5 oz olive oil and topped with lemon wedge*).

• Record the **Amount** of each food or beverage consumed. Portion sizes can be recorded in a variety of ways, please use the method that works best for you.

Portion sizes can be recorded using the following standard measurements: o Weight in grams or ounces (Not fluid ounces)

- o Solid foods – use volume in cups, tablespoons or teaspoons
- o Liquids – use volume in fluid ounces
- o Fraction of the whole (e.g. 1/8 of 9" pie)
- o Dimensions for the following shapes:

Example	Shape	Measurement Needed
Meatball	Sphere	Diameter
Meat Patty	Cylinder or disk	Diameter x thickness
Lasagna	Rectangle or cube	Length x height x width
Pie	Wedge	Length x height x width of arc

Example

Time	Place	Meal	Food/Beverage Item	Details/Ingredients/Preparation	Amount
8:00am	Home	Breakfast	Brown Sugar Instant Oatmeal	Made with water, nothing else added	2 packets
			Milk	Skim	8 fluid oz
			Coffee	Brewed, caffeinated	16 fluid oz
			Coffee Creamer	Fat Free, liquid hazelnut, Coffee Mate	1 Tbsp
12:00pm	Home	Lunch	Pizza	Frozen, thin crust, supreme pizza (Tony's Brand)	2 slices
			Water	Tap with ice	16 fluid oz

FOOD JOURNAL

Time	MEAL	FOOD/BEVERAGE ITEM	DETAILS/INGREDIENTS/PREPARATION	AMOUNT

FOOD JOURNAL

Time	MEAL	FOOD/BEVERAGE ITEM	DETAILS/INGREDIENTS/PREPARATION	AMOUNT

FOOD JOURNAL

Time	MEAL	FOOD/BEVERAGE ITEM	DETAILS/INGREDIENTS/PREPARATION	AMOUNT

APPENDIX L

MEAL NUTRITION INFORMATION

Item	Kcal	Kcal (Fat)	Kcal (CHO)	Fat (g)	Sat Fat (g)	Carb (g)	Sugar (g)	Fiber (g)	Pro (g)
2 Glazed Donut	520	260	248	28	12	62	24	2	6

CONTAINS ALLERGENS: Eggs, Milk, Soy, Wheat

INGREDIENTS: Donut: Enriched Unbleached Wheat Flour (Wheat Flour, Malted Barley Flour, Niacin, Iron as Ferrous Sulfate, Thiamin Mononitrate, Enzyme, Riboflavin, Folic Acid), Palm Oil, Water, Dextrose, Soybean Oil, Whey (a milk derivative), Skim Milk, Yeast, Contains less than 2% of the following: Salt, Leavening (Sodium Acid Pyrophosphate, Baking Soda), Defatted Soy Flour, Wheat Starch, Mono and Diglycerides, Sodium Stearoyl Lactylate, Cellulose Gum, Soy Lecithin, Guar Gum, Xanthan Gum, Artificial Flavor, Sodium Caseinate (a milk derivative), Enzyme, Colored with (Turmeric and Annatto Extracts, Beta Carotene), Eggs; Glaze: Sugar, Water, Maltodextrin, Contains 2% or less of: Mono and Diglycerides, Agar, Cellulose Gum, Citric Acid, Potassium Sorbate (Preservative), Artificial Flavor.