A SINGLE SESSION OF SPRINT INTERVAL TRAINING INCREASES TOTAL DAILY ENERGY EXPENDITURE

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ABSTRACT

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**Background:** Sprint interval training (SIT) is known to elicit favorable physiological adaptations, including improved insulin sensitivity and glucose tolerance. Its utility for weight maintenance is unclear. Presumably any effects would be mediated by increased energy expenditure during both the exercise itself, and during recovery. Therefore, the objective of this study is to determine the effects of a single bout of SIT on 24-hour energy expenditure (EE).

**Methods:** 24-hour EE was determined in 12 healthy men (age: 26 ± 2 years; body mass index: 23.6 ± 0.5 kg/m²; mean ± SE). After three days of controlled diet and maintenance of energy balance, subjects were studied in a whole-room indirect calorimeter for two consecutive days. One of these days (random order) began with a single bout of SIT (5 x 30 second “all-out” exertions on a cycle ergometer against a resistance equivalent to 7.5% body mass, separated by 4 minutes of loadless cycling). Subjects spent the other day in the calorimeter without exercising.

**Results:** An acute bout of SIT increased 24-hour EE in all subjects by an average of 226 ± 15 kcal during an otherwise sedentary day (Control: 2189 ± 58 vs. SIT: 2415 ± 62 kcal/day; \( P < 0.001 \)). There was also a non-significant (\( P = 0.054 \)) decrease in fat balance on the exercise day (control = -9.7 ± 7.9 g/day vs. SIT = -20.6 ± 8.2 g/day).

**Conclusions:** Our data provide support for SIT as a time-efficient exercise to increase total daily energy expenditure and may aid in the maintenance of health.
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CHAPTER 1 - REVIEW OF LITERATURE

Excess weight in adulthood

Currently, over two thirds of adult Americans are overweight (BMI of ≥ 25 kg·m⁻²) and half of the overweight individuals are classified as obese (BMI ≥ 30 kg·m⁻²).¹ In the United States the prevalence of obesity has more than doubled since the 1970s.² Many other nations are experiencing similar trends,³ resulting in the estimate of 1.46 billion overweight adults worldwide.⁴ Healthcare for obese individuals is exponentially more expensive than individuals of normal weight⁵ due to many common comorbidities.⁶,⁷ The global prevalence of overweight adults has not yet reached the prevalence seen in the United States, but if global trends prevail, the burden of excess weight will continue to mount.

Excess weight is due to the overconsumption of calories relative to energy expenditure. Modern careers and lifestyles tend to be sedentary; often requiring very little physical activity.⁸ It should be no surprise that the percentage of individuals with excess weight (however defined) has been increasing in recent decades. The problem with very low levels of physical activity is that energy intake tends to exceed energy expenditure.⁹,¹⁰ The implication is that caloric intake should not be isolated as a cause of weight gain. Initial support for this hypothesis came from a study that slightly increased energy expenditure in sedentary genetically obese mice, and documented a decrease in energy intake.¹¹ In a 16 day study of men and women, exercise caused energy intake to increase, but by only 30% of the increase in energy expenditure.¹² It appears that energy intake is best regulated when energy expenditure is at levels higher than are common in sedentary individuals. What this means is that we must turn our attention to increasing physical activity and exercise to promote health and prevent disease.

In an effort to promote health benefits of exercise the U.S. Department of Health and Human Services recommends 150 minutes of moderate intensity exercise or 75 minutes of vigorous intensity exercise per week.¹³ Despite a simplified public health message and many well-known health protective effects of exercise,¹⁴-¹⁷ less than 10% of Americans achieved the recommended level of physical
A perceived lack of time is the most prevalently cited of many factors accounting for a low level of physical activity. Current rates of weight gain can be used to make recommendations for the amount of increased energy expenditure needed to achieve energy balance. One longitudinal study reported that young adults aged 18-40 years gained 14-16 lb over 8 years. If linear weight gain was assumed, a positive energy balance of only 50 kcal per day may have been responsible for the changes over the extended time period. This implies that an increase in energy expenditure by as few as 50 kcal per day may blunt or prevent weight gain. This prediction assumed no compensatory increase in energy intake, or decrease in non-exercise physical activity.

Together these findings illustrate the importance of designing exercise programs that offer benefits to the people not currently meeting the recommended levels of physical activity. Convincing more people to exercise will curb excess weight gain and promote health. Additional exercise programs should minimize time commitment yet maximize energy expenditure.

**Sprint Interval Training**

Sprint interval training (SIT) is a time-efficient very-high intensity exercise that typically involves no more than 20-25 minutes of exercise and rest periods. The fact that this type of exercise is of short duration may help some individuals to significantly increase their energy expenditure without the time commitment that is often characteristic of endurance exercise sessions. Besides the possibility that SIT will aid in increasing energy expenditure, research has documented improvements similar to endurance exercise in physical fitness, metabolic parameters, and factors associated with cardiovascular health. There are a variety of SIT protocols. Unless otherwise noted, this review will focus on the most common form of SIT; consisting of 4-8, 30 second “all-out” exertions on a cycle ergometer against a resistance equivalent to 7.5% body mass, separated by an active recovery of 4-4.5 minutes of loadless cycling.
**Physiology of Energy Expenditure - During Exercise**

*Adenosine triphosphate (ATP) demand* - Sprint interval training is performed at a supramaximal intensity, meaning the external work rate is greater than the work rate attainable at maximum aerobic capacity (VO2max). During exercise the greatest contribution to whole-body ATP demand comes from contracting skeletal myocytes, which consume as much as 90% of the total whole-body VO2 at VO2max.38 Within contracting muscles Na+/K+-ATPase, Ca++-ATPase and actinomyosin are the predominate consumers of ATP.39 Cardiac myocytes will contribute to exercise-induced ATP demand to maintain increased energy expenditure.38 To a lesser degree hepatocytes contribute to ATP demand from upregulated gluconeogenesis.40

*ATP sources* – Since the vast majority of exercise-induced ATP demand is from skeletal myocytes38 the multiple ATP sources used will be reviewed. The 3 ATP sources are: 1) high energy phosphagen pools: phosphocreatine (PCr) and basal levels of ATP, 2) glycolytic substrate level phosphorylation of ATP, and 3) oxidative phosphorylation of ATP.41 The following descriptions of ATP sources during and immediately following repeated sprints compiles results from three studies39,42,43 to illustrate the integrated energy systems.

In the initial few seconds of a sprint interval, basal ATP is rapidly catabolized and the predominate source of high energy phosphates for ATP resynthesis comes from readily available PCr.39,41 PCr concentrations have been observed at ~17% and ~20% of pre-sprint interval concentrations during the first and second sprint intervals, respectively.43 During recovery intervals resynthesis of PCr will begin. In response to a 25 second sprint interval, PCr and ATP stores were mostly resynthesized within 1.5 minutes of rest in type I muscle fibers (slow twitch oxidative), but not type IIa (fast twitch oxidative glycolytic) or IIb (fast twitch glycolytic) fibers.42 In a separate study that made no distinction between fiber types, a muscle biopsy showed that PCr and ATP stores were still not at pre-exercise levels after a 4 minute recovery.43 As PCr is being rapidly depleted, energy demands are being met by the upregulation of glycolytic substrate level phosphorylation, and oxidative phosphorylation.39,41
Fatigue – The inability to maintain power output is very obvious during and between sprint intervals despite a great deal of encouragement to sprint ‘all-out’ for every second of the sprint. Fatigue is likely a result of decreased PCr and cellular inhibition of muscular contractions. In particular type IIb and to a lesser extent in type IIa muscle fibers are prone to fatigue.\textsuperscript{49} Rapid hydrolysis of ATP leads to increased plasma and cytosolic hydrogen ions (H\textsuperscript{+}) as well as increased cytosolic inorganic phosphate (Pi) during sprint exercise.\textsuperscript{43,50} Cytosolic H\textsuperscript{+} and Pi are associated with myocyte fatigue by decreasing the binding affinity of Ca\textsuperscript{++} to actinomyosin.\textsuperscript{51–53} To compensate for fatigue an increase in VO\textsubscript{2} during a second sprint interval suggests that there may be additional recruitment and reliance on type I muscle fibers for energy supply.\textsuperscript{43}

**Physiology of Energy Expenditure - After Exercise**

Energy expenditure after exercise is marked by an elevation of VO\textsubscript{2} during recovery that is greater than pre-exercise or resting VO\textsubscript{2}. This phenomenon is known as excess post-exercise oxygen consumption (EPOC). The magnitude of EPOC is positively correlated to exercise intensity, and EPOC duration is positively correlated to both exercise length and intensity.\textsuperscript{54,55} Some research has measured the magnitude and duration of EPOC during supramaximal exercise similar to SIT. EPOC lasted for 4 hours after in an exercise protocol of three 2 minute intervals at 108% VO\textsubscript{2max} on a cycle ergometer.\textsuperscript{56} In another study, supramaximal interval running, compared to submaximal continual running, resulted in an EPOC of just over twice the caloric expenditure and an 8-fold longer duration.\textsuperscript{57} Specific mechanisms for elevated energy expenditure after exercise consist of some well documented and some hypothetical phenomenon. Exactly what mechanisms occur after SIT has yet to be documented. Although the underlying mechanisms responsible for any prolonged elevation of energy expenditure following high intensity exercise were not examined in the current study, these mechanisms are relevant to the current review and are discussed below.

*ATP demand: Rapid phase* - The initial rapid phase of EPOC is characterized by relatively high energy demands of short duration, generally lasting between 10 and 60 minutes.\textsuperscript{54} Two processes most likely
contributing to high energy demand during the rapid phase are resynthesizing high-energy phosphagen pools and glycogen restoration. Rephosphorylation of ATP is stimulated via multiple metabolites during and after exercise to reestablish homeostatic levels. As basal ATP levels are restored, mass action of ATP and Cr induces the reversible reaction of creatine kinase to re-synthesize PCr.

Glycogen restoration is another important component of energy demand immediately after exercise. Gluconeogenic precursors alanine and lactate in the liver, and glutamine in the kidney, help to maintain plasma glucose and replenish depleted liver glycogen. Gluconeogenesis is thermodynamically unfavorable. Energy demands are increased due to reactions catalyzed by the enzymes: pyruvate carboxylase, phosphoenolpyruvate carboxykinase, fructose 1,6 bisphosphatase, and glucose 6-phosphatase. Gluconeogenesis is stimulated by exercise-induced elevations of plasma epinephrine and norepinephrine and by mass action of available precursors. Glycogenesis in skeletal muscle is rapidly upregulated after exercise to replenish the decreased glycogen stores. Rapid upregulation is mediated by the lower glycogen content, accumulating glucose-6-phosphate due to decreased glycolysis, and increased activity of glycogen synthase after exercise. The energy demand of glycogenesis will continue after the rapid phase of EPOC yet at a lower rate and stimulated indirectly by insulin if a meal is consumed.

**ATP demand: Slow phase** - The next period of EPOC is known as the slow phase. These remaining energy demands likely began immediately after cessation of exercise; however, the energy demands are low in magnitude, and may persist for hours to days. Uncoupled cellular respiration is defined as the dissipation of mitochondrial proton gradients by routes other than ATP synthase. If uncoupled cellular respiration occurs, additional energy supply will be required to meet the same energy demand. Basal levels of uncoupling in hepatocytes and skeletal myocytes appear to account for a significant portion of basal metabolic rate, possibly as high as 20%. Exercise is hypothesized to increase the rate at which uncoupling occurs; many of the proposed methods will be reviewed next. Energy demand of exercise-induced uncoupling may persist for hours after activation.
An exercise-induced elevation of body temperature is one proposed cause of uncoupling. The temperature of liver and skeletal muscle during and after exercise has been measured to increase from \(~38^\circ\text{C}\) to \(~40^\circ\text{C}\),\(^{72}\) and to have remained significantly elevated for at least an hour following cessation of exercise.\(^{73}\) There are two hypotheses to explain the relation between temperature and uncoupling. Both hypotheses propose that uncoupling is due to rising temperature causing an increase in bi-lipid membrane fluidity.\(^{74,75}\) The first hypothesis speculates that increased mitochondrial membrane fluidity allows water molecules to infiltrate the nonpolar region for a brief period, facilitating the conductance of protons back to the matrix.\(^{75}\) A second proposed hypothesis is that increased fluidity allows phospholipids to flip-flop within the inner mitochondria membrane, translocating a proton from the inner mitochondrial space to the matrix.\(^{76,77}\)

Another candidate for increased uncoupling after exercise is the creation of reactive oxygen species (ROS). Increased generation of ROS is implicated in regulated mitochondrial uncoupling via uncoupling protein-3 (UCP3), and adenine nucleotide translocase (ANT).\(^{78,79}\) After SIT, increased ROS generation has been noted, based on the detection of elevated downstream products of ROS.\(^{80}\) As exercise energy demands decrease, mitochondrial proton gradients will begin to increase. High proton gradients, as well as fat oxidation, increase the likelihood of ROS production at complex I and III.\(^{81-83}\) Lipid peroxidation, a downstream consequence of ROS generation, can activate proton conductance via UCP3 and ANT.\(^{79}\) Once activated, proton transport by UCP3 and ANT does not appear to be capable of inhibition.\(^{71,84}\) Instead regulation appears to be by proteolytic degradation (UCP3 half-life of 1 hour).\(^{85}\)

De-esterification of triacylglycerol (TG) followed by re-esterification of free fatty acids (FFA) is another process, known as futile cycling, that contributes to the slow phase of EPOC. Futile cycling of TG and FFA is most likely due to early exercise beta-adrenergic stimulated de-esterification (lipolysis) of TG in adipocytes.\(^{23,86,87}\) However, circulation of released of FFA is attenuated due to alpha-adrenergic stimulated vasoconstriction of the vasculature in adipose tissue as a result of exercise intensity.\(^{23}\) During recovery, adipose tissue perfusion is reestablished, and trapped FFA enter circulation.\(^{23}\) Additional
lipolysis in adipocytes is stimulated by exercise intensity-induced growth hormone release. After exercise, some of the plasma FFA are oxidized; however, most are used to resynthesize TG in adipocytes or hepatocytes. The net increase in futile cycling of TG and FFA after exercise may account for up to half of the slow phase of EPOC.

After exercise, reestablishment of ion gradients across plasma membranes and compartmentalization within organelles contributes to energy demand due to active transport mechanisms that, directly or indirectly, require ATP. The major ion gradients include Ca$$^{2+}$$, Na$$^+$$, and K$$^+$$. Above and beyond reestablishing homeostasis increased core temperature may increase futile cycling of these ions.

The interplay between lactate and EPOC has largely been disconnected from a cause and effect relationship that was once standard dogma. Exogenous lactate infusion supports the hypothesis that lactate is glucose/glycogen sparing at moderate exercise intensities due to its direct oxidation and because lactate is a gluconeogenic precursor. This energy supply relationship is believed to persist in the post-exercise period. Cytosolic pH balance as a result of lactate metabolism is one of the only mechanisms identified that still indicates lactate may have a small effect on energy demand. Lactate transportation involves an isoform of the monocarboxylate transporter family (MCT) which symports a proton and lactate. Na$$^+$$/H$$^+$$ antiport and Na$$^+$$/K$$^+$$-ATPase are stimulated and require energy to maintain pH homeostasis.

Energy Supply – The greater the exercise intensity, the greater the contribution of fat to energy supply during recovery. Although fat oxidation is increased by exercise, 24-hour fat balance is typically unaffected when energy balance is maintained, i.e. when energy intake increases to compensate for the additional energy expenditure from exercise.
Energy expenditure associated with SIT

SIT has received a great deal of attention in the past 10 years due to the need for time-sensitive approaches to increase energy expenditure, and because of its beneficial health effects. An acute bout of SIT will conceivably result in large energy demands during the exercise itself, even though cumulative time spent sprinting is extremely brief (2-4 minutes). Between sprint intervals, each recovery will likely result in a significant rapid EPOC phase. Then, after completion of the entire bout of SIT, there will likely be high magnitude and long duration rapid and slow phases of EPOC.

A few studies have attempted to quantify changes in energy expenditure due to SIT. In data from our lab, we explored the possibility that the effect of SIT on EPOC would be long lasting. We found that an acute bout of SIT, performed immediately after a morning RMR measurement, did not affect RMR ~23 hours later.97 In a study using a slightly modified SIT protocol (7x30 second @ 120% VO$_{2\text{max}}$ with 15 second recovery) exercise energy expenditure above RMR was calculated to be 109 ± 20 kcal and recovery energy expenditure above RMR was 32 ± 19 kcal.98 By using RMR rather than measuring a net change in total daily energy expenditure with a control condition this protocol failed to produce relevant data. These data warrant further examination of the effects of SIT on total daily energy expenditure in the more commonly utilized 30 second “all-out” exertion protocol.

With regard to a different issue, one study determined that, although 2 minutes of SIT used less oxygen during exercise than 30 minutes of moderate-intensity exercise, there was no difference in 24-hour VO$_2$ as inferred from eight 30 minute breath-by-breath indirect calorimeter sessions before, during, and after exercise.99 The authors suggest similar 24-hour energy expenditure between SIT and an approximately equal duration moderate-intensity exercise. However, without VCO$_2$ measurements, substrate oxidation rates cannot be estimated and therefore energy expenditure cannot be accurately measured. Furthermore, a difference in exercise intensity-dependent fat oxidation47 between these two protocols during and after exercise makes an energy comparison based on VO$_2$ inappropriate. Accurate VCO$_2$ measurements during supramaximal exercise must deal with the complication of significant
bicarbonate buffering. Accurate energy expenditure of supramaximal exercise can be dealt with through the use of direct calorimetry, doubly labeled water, or 24-hour measurements using indirect whole-room calorimetry.  

24-hour energy expenditure during SIT

Despite what is already known from previous research about the beneficial effects of SIT, the effect of a short bout of SIT on 24-hour energy expenditure has not been directly examined. Knowing the effect of SIT on 24-hour energy expenditure is a starting point for future experiments seeking to utilize SIT for weight maintenance or weight loss. Additionally, the effect of SIT on fat balance is also important for body weight regulation. The present study compares the total daily energy expenditure and substrate oxidation on a day of sedentary activity compared to a day with a bout of SIT performed on the morning of an otherwise sedentary day, using a whole-room indirect calorimeter.
CHAPTER II - MANUSCRIPT

INTRODUCTION AND SPECIFIC AIMS

The prevalence of obesity in the United States has more than doubled since the 1970s.\(^2\) Currently, over two thirds of adult Americans are overweight (BMI of ≥ 25 kg·m\(^{-2}\)), half of the overweight individuals are classified as obese (BMI ≥ 30 kg·m\(^{-2}\)). These dismal statistics have led to physical activity guidelines designed to promote lifelong achievement and maintenance of a healthy body weight, and to lower the risk for a variety of chronic diseases.\(^{15-17}\) Despite these guidelines, only ~10% of Americans achieved recommended levels of physical activity.\(^{13}\) In this environment of excess weight and failure to achieve physical activity guidelines, future research needs to focus on novel ways to increase physical activity.

An important contributor of poor adherence to physical activity guidelines is a perceived lack of time in everyday life to begin and continue an exercise program.\(^{18}\) An analysis of a long term epidemiologic study\(^{19}\) of young adults estimated that 50 kcal/day of positive energy balance accounts for the weight gain witnessed.\(^{20}\) The implications of these findings for physical activity are quite clear - develop exercise programs that minimize time commitment and maximize energy expenditure (EE).

One possible short duration exercise program is sprint interval training (SIT). SIT is composed of supramaximal intensity cycle intervals interspersed with sets of active recovery. One important advantage of SIT over conventional exercise programs is that SIT can be readily accomplished within 20-25 minutes. Recent research has focused on comparing SIT to traditionally recommended forms of exercise which are of longer duration and constant pace. SIT appears to elicit similar increases in physical fitness,\(^{21,22}\) as well as improves metabolic parameters,\(^{22-30}\) and factors associated with cardiovascular health.\(^{31-37}\) Considering the beneficial health effects and minimal time commitment, SIT may help to increase physical activity and to meet a 50 kcal/day EE target.
The effect of SIT on 24-hour EE is unknown. Given the supramaximal intensity of SIT and thus the associated recovery, it is possible that the effect on total daily EE is greater than might be estimated based on the amount of external work accomplished during the sprints. Most importantly, if SIT has a consequential impact on 24-hour EE, the adoption of this exercise may be an appropriate approach for weight maintenance and health promotion. Using a whole-room indirect calorimeter, we propose to analyze the effect of SIT on total daily EE. We hypothesize that an acute bout of SIT will significantly elevate 24-hour EE relative to the non-exercise control day.
METHODS

Subjects – Male volunteers were recruited who were between the ages of 18-40 years, had a BMI less than 30 kg/m², were weight stable (± 2 kg) for at least the previous 6 months, and had the ability to complete supramaximal exercise as assessed by an exercise stress test. Volunteers were excluded if they used tobacco, had an acute or chronic disease, or if they used over-the-counter/prescribed medications that have known appetite, food intake or intermediary metabolic effects. Eligible volunteers were informed of the risks and provided written consent. Study approval was given by the Colorado Multiple Institutional Review Board and Colorado State University Institutional Review Board.

Experimental Design – A randomized crossover controlled protocol was used to quantify the effect of an acute bout of SIT on 24-hour EE and substrate oxidation. Diet was controlled to achieve energy balance for 3 days prior to the experimental protocol. All subjects spent two consecutive days in a whole-room indirect calorimeter with a single bout of SIT randomized to one of the mornings (control day vs. SIT day; figure 1). Energy intake in the calorimeter was estimated to achieve energy balance during a day of sedentary behavior. The goal was to have subjects in energy balance on control day, and maintain the same energy intake, inducing a negative energy balance, on the exercise day.

Figure 1. Flow chart of a day spent in the whole-room indirect calorimeter
Preliminary Assessments

Body Composition Analysis – Dual-energy X-ray absorptiometry (DXA) was used to determine fat and fat-free mass (Hologic, Discovery W, QDR Series, Bedford, MA, USA). Girths were measured at the waist (midway between the lowest rib and the iliac crest) and hip (maximum circumference at the buttocks) over light-weight workout clothing to the nearest millimeter using flexible measuring tape. Height was measured to the nearest millimeter using a stadiometer (Detecto, Webb City, MO, USA). Mass was measured to the nearest 100 grams using an electronic scale (Medway, Mettler-Toledo, Columbus, OH, USA).

Resting Metabolic Rate (RMR) – Subjects arrived at the Human Performance/Clinical Research Laboratory (HPCRL) on the campus of Colorado State University by car; having fasted for no less than 12 hours, and having abstained from exercise for at least 24 hours. Subjects were placed in a supine position on a comfortable bed in a thermoneutral environment. A plexiglass ventilated canopy was positioned over the subject’s head and, using the dilution method, oxygen consumption (VO2) and carbon dioxide production (VCO2) were measured continuously and averaged every minute for 45 minutes. The first 15 minutes were considered habituation and were excluded from analysis; thus, the last 30 minutes were used to calculate RMR. Any data outside of 2 standard deviations were excluded from the generation of an average. Exhaled gas was analyzed using a custom-built indirect calorimetry system (Nighthawk Design, Boulder, CO) that utilizes a respiratory mass spectrometer (PerkinElmer MGA 1100; MA Tech Services, St Louis, MO) and an ultrasonic flow sensor (Medizintechnik, Zurich, Switzerland). Gas and flow calibration was performed prior to use with precision mixed gases (Airgas Intermountain, Denver, CO) and a 3L syringe (Hans Rudolph, inc., Kansas City, MO). In previous studies, RMR measurements using this system had a coefficient of variation of 3.3% and the test-retest had a r² of 0.93. One subject resided more than an hour from the HPCRL. To minimize an elevated metabolic rate induced by driving/traveling, the subject’s RMR was measured closer to home at the Clinical and Translational Research Center (CTRC - Colorado University Denver Anschutz Medical Campus).
same protocol was used except that exhaled gas was analyzed using a metabolic cart (Parvo Medics, Sandy, UT, USA).

**Aerobic Capacity and Exercise Stress Test** – Peak aerobic capacity (VO\textsubscript{2peak}) was measured using a metabolic cart (Parvo Medics, Sandy, UT, USA) during incremental exercise on a cycle ergometer (Velotron Dynafit Pro, RacerMate, Inc., Seattle, WA, USA or Lode BV, Groningen, Netherlands). Cycling began at 0 W and difficulty increased by 30–40 watts·min\textsuperscript{-1} depending on the subject’s self-reported fitness level. Exercise continued until volitional fatigue or failure to maintain more than 40 revolutions per minute. Participants were approved for participation in high intensity exercise based on a cardiologist’s interpretation of a 12-lead electrocardiogram that was collected before (supine, sitting, and standing) and every 3 minutes during the VO\textsubscript{2peak} test.

**Experimental Protocol**

**Outpatient Pre-study Protocol** – Subjects completed an electronic food preference survey (Vanderbilt University, Nashville, TN). The data were used to create an isoenergetic (RMR·1.5) diet provided for 3 days prior to the whole-room calorimeter to stabilize macronutrient intake and energy balance. Macronutrient distribution was designed to represent a typical western diet consisting of 15% protein, 55% carbohydrates, and 30% fat (12-15% monounsaturated fat, 6-9% polyunsaturated fat, and less than 6% saturated fat). Two additional modules (200 kcal/each), with the same macronutrient composition as the diet, could be consumed if the subject felt hungry or engaged in exercise. No other food or caloric beverages were permitted. Meals were prepared by the study investigators in the research kitchen of the Food Science and Human Nutrition Department at Colorado State University. Normal physical activity was maintained on day 1 and 2; however, on day 3 subjects were to refrain from exercise to minimize any lasting effects.\textsuperscript{54}

**Inpatient Whole-room Indirect Calorimeter Protocol** – Calorimeter collection days were scheduled back-to-back at the Clinical and Translational Research Center (CTRC - Colorado University Denver Anschutz Medical Campus). The whole-room indirect calorimeter is a 12-by-12 foot room with a hospital bed,
desk, sink, toilet, telephone, TV, computer, and a large window. The calorimeter is a flow-through design, with excurrent air pulled out of the room and subsamples of gas is continuously analyzed. Incurrent air from outside of the building is drawn into the room. Air locks are used to transfer food into the calorimeter with minimal contamination of the air supply. Condition (SIT day or control day) was randomized to day 1 or day 2 at the time of scheduling.

Subjects arrived at 0700 after an overnight fast with no caffeine consumption. The subject was asked to urinate so that no urine from the previous day/night would be collected during the study. After standard hospital admission procedures, the subject entered the calorimeter at 0745. Data collection began at 0800. From 0800 to 0845, subjects were to engage in sedentary activities (talking on the phone, watching tv, or reading). Subjects were instructed to remain awake during all sedentary times of the day. Preparation for exercise occurred at 0845. Exercise or continued quiet sitting began at 0900 and continued until about 0930. Sedentary activities were resumed between all meals. To minimize differences in energy expenditure after exercise, computer use was only permitted prior to 0900 and after 1900. Meals were prepared by the CTRC Bionutrition Core, and were served at 1000, 1400 and 1900. Subjects were monitored to make sure food was consumed within 15 minutes of its arrival. All calorimeter meals were of the same macronutrient composition as the pre-study diet. The caloric content was reduced (RMR * 1.4) to reflect the lower levels of physical activity found in previous calorimeter studies in our laboratory on a control day. Subjects prepared for bed at 2200 and were to lie in bed by 2215. A wake-up call occurred at 0600, and they were instructed to urinate for the final serial urine collection. Data collection ceased when the subject exited the chamber at 0700. The subject was given a chance to shower and then the protocol was repeated for the alternate condition.

Sprint Interval Training – At 0858 subjects began a 2 minute loadless warm up on a cycle ergometer (Monark Exercise AB, Stockholm, Sweden). At 0900 subjects then completed 5 x 30 second “all-out” exertions on a cycle ergometer against a resistance equivalent to 7.5% body mass, separated by 4 minutes of loadless cycling. An “all-out” exertion means that the subjects were instructed to pedal as fast as possible during every second of the sprint without pacing themselves. Resistance was manually set by a
researcher (KS) external to the calorimeter by way of a leak-free port in the calorimeter wall. Significant encouragement was provided over an intercom to motivate all subjects to continue the pedal as fast as possible. To verify supramaximal intensity, peak, mean, and minimum power were measured by SMI Power (Sports Medicine Industries, St. Paul, Minnesota), using fly wheel revolutions via an infrared camera, and a known resistance. Fatigue index was calculated as the percent decrease from peak to minimum power.

Measurements

Energy Expenditure and Substrate Oxidation – VO₂, VCO₂, and urinary nitrogen were measured in the whole-room indirect calorimeter to calculate 24-hour energy expenditure and substrate oxidation using the Weir equation. Gas concentrations were measured in duplicate using CO₂ and O₂ analyzers (Sable Systems, Las Vegas, NV, USA). The calorimeter was validated monthly using the gold standard propane combustion test; O₂ and CO₂ measurements were within 2% of expected values for every propane burn during the duration of this study. No within subject differences were found when the calorimeter was validated against a metabolic cart at various physical activity levels. Data were averaged every minute, collected for 23 hours and extrapolated to create 24-hour values. Total 24-hour urinary nitrogen was assessed by measuring total urine volume, and aliquots were measured using gas-phase chemiluminescence. The 24-hour protein oxidation rate was determined as grams of urinary nitrogen multiplied by 6.25.

In addition to the 24-hour values, rates of energy expenditure and respiratory exchange ratios were averaged during the following segments of the day: exercise: 0855-0930, post exercise: 0930-1000, breakfast to lunch: 1000-1400, lunch to bedtime: 1400-2200, and sleep: 0100-0400. Not all hours of the day were accounted for in this segment of the day analysis. Due to individual variation on actual times of falling asleep, waking up, and morning activity we excluded the hours between 2200-0100 and 0400-0700. Individual data points were scanned to confirm a lack of metabolic rate increases that would have indicated a subject was awake during a period that was assumed to reflect sleeping metabolic rate. To
monitor physical activity, other than during SIT, an accelerometer (ActiGraph, Pensacola, FL, USA) was worn on the hip.

*Urinary Catecholamines* – Urine remained refrigerated during collection, storage and transportation. Epinephrine, norepinephrine, and creatinine were all analyzed by quantitative high performance liquid chromatography-tandem mass spectrometry. Catecholamine values were all expressed as a ratio to creatinine.

**Statistical Analysis**

Data were analyzed using Excel (Microsoft Excel, version 14) unless otherwise noted. Paired Student’s t-tests were used to compare within subject dependent variables between conditions (control day vs. SIT day). Energy expenditure and balance, substrate oxidation rates and balance, urinary catecholamines, and cycle ergometer power output were all analyzed. Significant differences between dependent variables with a p-value of less than 0.05 were assumed to be an effect of an acute bout of SIT. Power and fatigue during sprint intervals were compared to the number of sprints using a one way ANOVA in SAS (Statistical Analysis System Institute Inc., version 9.4). When comparing different segments of the day, a paired Student’s t-test with a Bonferroni correction was used. If statistical analyses were run using fewer than all 12 subjects (due to missing data), the number of subjects included is stated.
RESULTS

Subjects - Twelve moderately active young adult male volunteers were recruited. All subjects were lean and healthy. Their physical characteristics are presented in Table 1.

Table 1. Physical characteristics of 12 male subjects

<table>
<thead>
<tr>
<th></th>
<th>Average ± SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yr)</td>
<td>26 ± 2</td>
</tr>
<tr>
<td>Body mass (kg)</td>
<td>73.5 ± 2.2</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>176 ± 2</td>
</tr>
<tr>
<td>BMI (kg·m⁻²)</td>
<td>23.6 ± 0.5</td>
</tr>
<tr>
<td>Waist/hip ratio</td>
<td>0.83 ± 0.01</td>
</tr>
<tr>
<td>Body fat (%)</td>
<td>17.2 ± 0.8</td>
</tr>
<tr>
<td>Fat mass (kg)</td>
<td>12.8 ± 0.9</td>
</tr>
<tr>
<td>Fat-free mass (kg)</td>
<td>59.7 ± 1.5</td>
</tr>
<tr>
<td>RMR (kcal·day⁻¹)</td>
<td>1720 ± 52</td>
</tr>
<tr>
<td>VO₂peak (mL·kg⁻¹·min⁻¹)</td>
<td>53.0 ± 2.0</td>
</tr>
<tr>
<td>HRpeak (beats·min⁻¹)</td>
<td>181 ± 3</td>
</tr>
<tr>
<td>RERpeak</td>
<td>1.13 ± 0.02</td>
</tr>
</tbody>
</table>

Sprint interval training (SIT) - All subjects completed five 30 second sprints. The entire exercise bout was designed to be completed in 24.5 minutes including a 2 minute warm-up, five 30 second sprints, and five 4 minute active recovery intervals. One subject experienced nausea; his recovery intervals were extended to facilitate completion. Accelerometer measurements showed there was no difference in physical activity (excluding 0900-0930) while in the calorimeter between Control and SIT day ($P = 0.69$, $n = 9$). SIT was completed at supramaximal intensity, peaking at an average work rate of 208%, and 30 second average work rate of 132% of the cycle ergometer power output capable at VO₂peak. Fatigue was evident in all subjects within and between sprint intervals based on decreasing power output and an increasing fatigue index (Table 2). Cycle ergometer power output data were only available for 9 subjects due to technical difficulties.
Table 2. Power output during 5 sprint intervals. Data are presented as an average ± SEM (n = 9)

Peak \( (P = 0.0498) \) and mean \( (P < 0.001) \) power decreased while fatigue increased \( (P < 0.01) \) as the number of sprints increased.

<table>
<thead>
<tr>
<th>Interval</th>
<th>(^1\text{Peak power} \text{(Watts)})</th>
<th>(^2\text{Mean power} \text{(Watts)})</th>
<th>(^3\text{Fatigue index} \text{(%)})</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sprint 1</td>
<td>805 ± 58</td>
<td>560 ± 25</td>
<td>49.4 ± 3.6</td>
</tr>
<tr>
<td>Sprint 2</td>
<td>763 ± 44</td>
<td>493 ± 22</td>
<td>53.2 ± 4.4</td>
</tr>
<tr>
<td>Sprint 3</td>
<td>708 ± 34</td>
<td>448 ± 26</td>
<td>54.8 ± 3.8</td>
</tr>
<tr>
<td>Sprint 4</td>
<td>694 ± 33</td>
<td>430 ± 23</td>
<td>56.9 ± 3.8</td>
</tr>
<tr>
<td>Sprint 5</td>
<td>723 ± 46</td>
<td>434 ± 26</td>
<td>60.1 ± 3.6</td>
</tr>
</tbody>
</table>

Energy expenditure and balance – The primary outcome was to determine the effect of SIT on 24-hour EE. A single bout of SIT increased EE in every subject (Figure 2); the average increase above a sedentary control was 226 ± 15 kcal (Table 3). Figure 3 shows that the effect of SIT was large in magnitude yet of a very short duration. Further analysis of this time period showed a transient 5-fold average increase of metabolic rate during exercise and a small 1.4-fold average elevated metabolic rate immediately after exercise as sedentary activities were resumed (Table 4). In an attempt to explain the effect of SIT on EE, cycle ergometer power output was analyzed. During sprinting, neither mean power (Watts; \( r = -0.07, P = 0.85 \)) nor relative mean power (Watts/kg; \( r = -0.04, P = 0.91 \)) predicted the change in EE.

In the calorimeter, subjects were given an energy intake equivalent to their RMR * 1.4, a value based on prior chamber studies.\(^{104} \) However, the actual average elevation above RMR during Control day in this study was only 1.28. As a result of lower than expected EE, average energy balance was positive on Control day; regardless, the addition of exercise achieved a negative energy balance on SIT day (Table 3).
Figure 2. Total daily EE of individuals (lines) and the sample averages ± SEM (n = 12)

Table 3. Total daily energy balance

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>SIT</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Energy balance (kcal·day⁻¹)</strong></td>
<td>158.2 ± 43.9</td>
<td>-77.8 ± 43.7</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td><strong>Energy expenditure (kcal·day⁻¹)</strong></td>
<td>2189 ± 58</td>
<td>2415 ± 62</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td><strong>Energy intake (kcal·day⁻¹)</strong></td>
<td>2347 ± 69</td>
<td>2337 ± 72</td>
<td>0.36</td>
</tr>
</tbody>
</table>
Figure 3. Average rate of EE during 23 hours of data collection (n = 12)

Table 4. Rates of EE during different times of the day. Data are presented as an average ± SEM (n = 12)


<table>
<thead>
<tr>
<th>Time of Day</th>
<th>Control</th>
<th>SIT</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Exercise</strong> (kcal·min⁻¹)</td>
<td>1.5 ± 0.1</td>
<td>7.5 ± 0.4</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td><strong>Post exercise</strong> (kcal·min⁻¹)</td>
<td>1.6 ± 0.1</td>
<td>2.2 ± 0.1</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td><strong>Breakfast to Lunch</strong> (kcal·min⁻¹)</td>
<td>1.7 ± 0.1</td>
<td>1.8 ± 0.0</td>
<td>1</td>
</tr>
<tr>
<td><strong>Lunch to lights out</strong> (kcal·min⁻¹)</td>
<td>1.7 ± 0.0</td>
<td>1.7 ± 0.0</td>
<td>1</td>
</tr>
<tr>
<td><strong>Sleeping</strong> (kcal·min⁻¹)</td>
<td>1.1 ± 0.0</td>
<td>1.2 ± 0.0</td>
<td>0.31</td>
</tr>
</tbody>
</table>
Substrate oxidation and balance – Figure 4 demonstrates that SIT resulted in a prolonged elevation of RER. When RER was analyzed during different times of the day, it was found to be significantly elevated for 5 hours compared to Control ($P < 0.001$; Table 5). Despite transient effects of SIT, average 24-hour RER was unaffected (Control: 0.85 ± 0.01 vs. SIT: 0.86 ± 0.01, $P = 0.19$). Another a priori outcome of importance was fat balance. Approaching significance, fat balance appears decreased in most individuals on the SIT day compared to the Control day (SIT: -20.6 ± 8.2 vs. Control: -9.7 ± 7.9 g fat·day$^{-1}$; $P =$ 0.054; Figure 5). Total daily substrate oxidation rates did not statistically differ on SIT day compared to Control day. Considerable individual variation of protein oxidation was observed between conditions ($P = 0.70$; Figure 6A). Carbohydrate and fat oxidation trended toward an increase on SIT day (carbohydrate: $P = 0.09$, fat: $P = 0.07$; Figure 6B & 6C).

Figure 4. Average respiratory exchange ratio during 23 hours of data collection, $n = 12$
Table 5. Respiratory exchange ratio during different times of the day. Data are presented as mean ± SEM (n=12). Exercise: 0900-0930, Post exercise: 0930-1000, Breakfast to lunch: 1000-1400, Lunch to lights out: 1400-2200, sleep 0100-0400.

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>SIT</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Exercise</td>
<td>0.80 ± 0.01</td>
<td>0.99 ± 0.02</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Post exercise</td>
<td>0.81 ± 0.01</td>
<td>0.99 ± 0.02</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Breakfast to Lunch</td>
<td>0.83 ± 0.01</td>
<td>0.89 ± 0.01</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Lunch to lights out</td>
<td>0.86 ± 0.01</td>
<td>0.85 ± 0.01</td>
<td>0.88</td>
</tr>
<tr>
<td>Sleeping</td>
<td>0.85 ± 0.01</td>
<td>0.84 ± 0.01</td>
<td>0.12</td>
</tr>
</tbody>
</table>

Figure 5. Fat balance of individuals (lines) and the sample averages ± SEM ($P = 0.054$; n = 12)
Figure 6. 24-hour substrate oxidation rates of individuals (lines) and the sample averages ± SEM measured using whole-room indirect calorimetry (n = 12): A) protein oxidation: $P = 0.70$; B) carbohydrate oxidation: $P = 0.09$; C) fat oxidation: $P = 0.07$. 
Twenty four hour urinary catecholamines - SIT did not alter the concentration of total daily urinary epinephrine or urinary norepinephrine relative to the Control day (epinephrine: Control: 4 ± 1 ug/g CRT vs. SIT: 5 ± 1 ug/g CRT, \( P = 0.16 \), and norepinephrine: Control: 19 ± 3 ug/g CRT vs. SIT: 20 ± 3 ug/g CRT, \( P = 0.33 \), n = 10).
DISCUSSION

The purpose of this study was to determine if sprint interval training could increase 24-hour EE by at least 50 kcal. The main finding was that five “all-out” 30 second sprints with 4-minute active recovery intervals between each sprint increased 24-hour EE by an average of 226 kcal. The total time required for our single bout of SIT was 24.5 minutes. The effects of SIT on EE were divided between the exercise bout itself (2 minute warm-up period and 4 minute active recovery intervals between sprints), and a brief period of time after exercise. During the exercise time period, EE was elevated 5-fold higher than the EE of the same subjects during the same time of day in the non-exercise control condition. In the 30 minutes following SIT metabolic rate remained modestly elevated at 1.4-fold above the non-exercise control.

The magnitude of the contribution of sprint interval exercise to total daily EE is of interest, with considerable disparity in findings among recent studies. Compared with previous studies, our subjects had a much greater increase in EE resulting from an acute bout of SIT. SIT protocols with different work rates and different recovery periods may have led to conflicting results among different studies. One study in question reported that exercise EE above RMR was calculated to be an average of ~141 kcal/day. The protocol used 7x30 second cycling intervals at 120% VO2max with a 15 second passive recovery.98 This average EE was a little over half of what our study found despite a greater number of sprints compared to our protocol. In contrast, our protocol used all-out sprints that did not limit the work rate during cycling. Averaged over 5 sprints, subjects in our study had a peak work rate of 208%, and 30 second work rate of 132% of the work rate attained at VO2peak. A possible second source of variability is the length of recovery. If EE is elevated after cycling intervals then longer recovery periods would likely result in a greater EE.57 Our study utilized 4 minute active recovery compared to a 15 second passive recovery.

Use of a linear mathematic model has also estimated a lower caloric expenditure than our reported findings. A bout of SIT (6x30 second sprints with 4 minute recoveries) was estimated to produce a net average EE of 141.9 ± 11.9 kcal (mean ± SD).107 The mathematic model used assumed a common
efficiency for all people at rest and a linear relationship between cycling work rate and EE during exercise.\textsuperscript{108} A linear model may have underestimated EE. Previous studies have demonstrated an exponential relationship between work rate and EE at higher submaximal exercise intensities.\textsuperscript{109} In our study, we performed only 5 sprints, yet found that EE was substantially higher than predicted with a linear model. Neither average power (work rate) nor average relative power outputs were correlated with the change in energy expenditure. These regression analyses fail to support a linear relationship between work rate and the effect of SIT on total daily EE.

There are a number of strengths of the present study. 24-hour whole-room indirect calorimetry, together with the assessment of urinary nitrogen, allow for the precise measurement of EE and substrate oxidation.\textsuperscript{110} Exhaled gases were collected and analyzed during relatively free-living exercise and sedentary behavior conditions. We collected data from all individuals on two consecutive days in which EI and EE were held constant for 23 hours of data collection except for the randomized performance of a single bout of SIT on the exercise day.

In applying our findings to the issue of weight maintenance a few caveats should be addressed. There may be changes in the behavior of free-living individuals as a result of SIT that affect energy balance. Our experimental design controlled non-exercise physical activity (NEPA) by carefully imposing sedentary activities, and confining individuals to a small room for two consecutive days. This control was justified by a study indicating exercise, irrespective of duration and/or intensity, does not appear affect NEPA.\textsuperscript{111} The effect of SIT on NEPA; however, has not yet been studied. We also controlled energy intake (EI) by feeding meals of identical macronutrient composition on both days. Controlling EI was appropriate because in a short term study of SIT (6x30 second intervals), ad lib energy intake was unaffected over the course of 3 meals compared to a non-exercise control.\textsuperscript{107} In a longer 16 day study of moderate and high intensity exercise, EI increased, but subjects compensated for only about 30% of the energy expended during exercise.\textsuperscript{12} The current literature cannot rule out confounding changes in NEPA
and EI. The implications for weight maintenance are speculative until running long term study for at least 6 months of SIT in free-living conditions.

The effect of SIT on substrate oxidation and fat balance is unclear in our study. Either: 1) SIT does not affect substrate oxidation; or 2) variability of energy balance between subjects led to variability of substrate oxidation and balance. 24-hour fat oxidation is unaffected by exercise when energy balance is maintained, and meals are given at intervals similar to free living conditions. Negative energy balance causes increased fat oxidation to meet energy demands, and results in negative fat balance. We attempted to attain energy balance on control day and to feed the same number of calories on SIT day. Therefore, fat balance would be affected by SIT exercise-induced negative energy balance. We chose to not increase EI because research discussed earlier found that SIT did not result in EI compensation in the short term. An EI equivalent to RMR * 1.4 was used in an attempt to match EE of a sedentary day in the calorimeter. Previous studies have validated the use of an individual’s RMR *1.4 to maintain energy balance in the indirect calorimeter used in this protocol. Unlike our protocol, a platform stepping exercise was used to induce physical activity levels of a normal free-living sedentary day. We chose not to include stepping in our protocol to ensure that the detection of elevated metabolism would not be interrupted by additional physical activity. Regardless of this difference in protocols exact energy balance is not feasible. An average error of 119-141 kcal/day is common for methods attempting to attaining 24-hour energy balance. Our method was only slightly beyond this range; we reported an average positive energy balance of 158 kcal on control day. Despite the fact that our study had variability of energy balance on SIT day, a strong trend toward a decreased fat balance was noted. Based on our results and the current literature, we would hypothesis that when SIT induces a negative energy balance, fat oxidation will increase to meet energy demands resulting in a negative fat balance.

Conclusions

An acute bout of SIT increased 24-hour EE by an average of 226 kcal. Our results demonstrate that SIT contributes to a meaningful increase in 24-hour EE, which could impact energy balance. Future
studies should examine the potential for long term SIT adherence to impact energy balance and body composition.
LITERATURE CITED


97. Sevits, K. J. et al. Total daily energy expenditure is increased following a single bout of sprint interval training. *Physiol. Reports* 1, n/a–n/a (2013).


APPENDIX A – INFORMED CONSENT

Consent and Authorization Form Approval

Date: SEP 14 2012

COMIRB

Study Title: Influence of a Single Session of Sprint-Interval Training on Total Daily Energy Expenditure

Principal Investigator: Edward Melanson, Ph.D.

COMIRB No: 11-1109

Version Date: December 14, 2011

Version #: 3

You are being asked to be in a research study. This form provides you with information about the study. A member of the research team will describe this study to you and answer all of your questions. Please read the information below and ask questions about anything you don’t understand before deciding whether or not to take part.

Why is this study being done?

This study is being done to determine how many more calories and fat you burn on a day when you do very high intensity, sprint-like exercise on a stationary bicycle. You are being asked to be in this research study because you are a healthy adult between the ages of 18 and 40 years.

Other people in the study

Up to 18 people from the Fort Collins and/or Denver area will participate in this study.

What happens if I join this study?

If you join the study, you will be asked to do the tests described below. It is expected that most individuals will be able to complete the study within 1 or 2 months.

If you agree to participate, you will be asked to schedule two preliminary study visits at the Department of Health and Exercise Science at Colorado State University. During these visits, the following tests will be performed:

1. Health History and Physical Examination: This visit will occur at CSU. If you consent to participate in this study, the supervising physician assistant will give you a physical examination to confirm that you are in a good state of health. You will be asked about the medical history of you and your family.

2. Graded Exercise Test: This visit will also occur at CSU. This test will tell us if your heart is healthy. You will be asked to walk on a motorized treadmill or ride an exercise cycle (cycle ergometer) for approximately 10-12 minutes. The exercise will become more difficult every 2 minutes. While you are walking/riding we will measure your heart rate with an electrocardiogram (ECG) and your blood pressure with a cuff placed around your upper arm. Dr. Denis Larson, a physician, will supervise the test. If we do not think your heart is healthy you will be referred to your primary care physician for further testing.

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There is a chance that you may not be allowed to take part in our study. You will be asked to do this test once; it lasts roughly 1 hour.

3. Resting metabolic rate: This visit will also occur at CSU. This is the amount of calories your body uses at rest. We will place a clear ventilated plexiglass hood over your head for 45 minutes and collect the air you breathe out. This will allow us to determine how many calories your body burns at rest.

4. Body Composition Assessment: This visit will also occur at CSU. After you have been cleared to participate in the study, we will determine the amount of body fat you have using a test called dual-energy x-ray absorptiometry (DXA). You will lie on a hospital-type bed and a small amount of x-ray will be passed through your body to measure the amount of fat, muscle, and bone you have in your body. You will have to lie very still for about 5 minutes. There is no pain with this procedure.

After completing the preliminary study visits, you will be asked to schedule a two-day study visit at the University of Colorado Anschutz Medical Center in Denver, CO. This stay can begin on any day of the week that is most convenient for you. This study visit will be conducted in the Clinical and Translational Research Center (CTRC), located on the 12th floor of the University of Colorado Hospital. You will be asked to stay in a special room (metabolic room) that accurately measures the number of calories you burn by measuring how much oxygen your body uses and how much carbon dioxide your body produces. A picture of the room is on the right. The room is sealed, but fresh air is constantly drawn in. The room is 12 feet by 12 feet and contains a regular hospital bed, a desk, a toilet, a telephone, a flat screen TV with a DVD player, and a computer with internet access. The room also has wireless internet access, so you can bring your own laptop to do work if you wish. An air lock allows food to be passed to you. There are curtains over the windows so that you may have privacy. The room is also equipped with a closed-circuit camera that can be viewed in the control room and at the nursing station. This camera permits the nurses to monitor you while you are in the room. However, the control for the camera is located inside the room, and you can turn the camera off anytime you want privacy, for example, when going to the bathroom.

One study will be an exercise day, and one day will be a day without exercise (control day). To decide the order of the study days, we will use a method of chance. This method is like flipping a coin or rolling dice. Some subjects will have the control first, and other subject will have the exercise day first.

On the first day, you will arrive at the CTRC at 7 AM, and you will enter the room at 8 AM. You will stay in the room for two consecutive days (including overnight), and will exit the room at 7:00 AM.

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AM on the morning of the third day. During each day, you will mimic your normal daily activities by doing 2 bouts (20 minutes each) of bench-stopping exercise at a comfortable pace. During your stay, you can watch TV or movies, read, or use the computer. At the beginning of the second day you will perform very high intensity, sprint-like exercise on a bicycle.

- For three days before the 2 day study visit in Denver, you will be asked to consume only food that we provide. You will be asked to report to the Department of Health and Exercise Science at CSU each morning to pick up your meals. The number of calories in the diets will be estimated to meet your daily energy needs and therefore maintain a stable body weight. These meals will be provided as 3 regular meals (breakfast, lunch, and dinner) plus some light snacks for between meals.

- Beginning on the first day of the diet and continuing through the study day, we will also ask you to wear a monitor that records your level of physical activity. The monitor is about the size of a book of matches. It is carried in a cell phone case, which you will clip to your belt or pants. There are no buttons to push and no displays.

- You will be asked to follow a standardized activity protocol during each day of the calorimeter stay (see table below).

<table>
<thead>
<tr>
<th>CONTROL DAY</th>
<th>EXERCISE DAY</th>
</tr>
</thead>
<tbody>
<tr>
<td>8:00 AM Start study</td>
<td>9:00 AM Start study</td>
</tr>
<tr>
<td>8:00-8:45 AM Quiet sitting</td>
<td>8:00-8:45 AM Quiet sitting</td>
</tr>
<tr>
<td>8:45-9:00 AM Prepare for exercise</td>
<td>8:45-9:00 AM Prepare for exercise</td>
</tr>
<tr>
<td>9:00-10:00 AM Quiet sitting</td>
<td>9:00-10:00 AM Exercise</td>
</tr>
<tr>
<td>9:25-9:40 AM Wash, clean up from exercise*</td>
<td>9:25-9:40 AM Wash, clean up from exercise</td>
</tr>
<tr>
<td>9:40-10:15 AM Quiet sitting*</td>
<td>9:40-10:15 AM Quiet sitting</td>
</tr>
<tr>
<td>10:00-10:15 AM Breakfast</td>
<td>10:00-10:15 AM Breakfast</td>
</tr>
<tr>
<td>10:15 AM-2:00 PM Quiet sitting*</td>
<td>10:15 AM-2:00 PM Quiet sitting*</td>
</tr>
<tr>
<td>2:00-2:15 PM Lunch</td>
<td>2:00-2:15 PM Lunch</td>
</tr>
<tr>
<td>2:15-7:00 PM Quiet sitting*</td>
<td>2:15-7:00 PM Quiet sitting*</td>
</tr>
<tr>
<td>7:00-7:15 PM Dinner</td>
<td>7:00-7:15 PM Dinner</td>
</tr>
<tr>
<td>7:15-10:00 PM Free to move around the room</td>
<td>7:15-10:00 PM Prepare for bed</td>
</tr>
<tr>
<td>10:00 PM Prepare for bed</td>
<td>10:00 PM Prepare for bed</td>
</tr>
<tr>
<td>10:15 PM Lie down in bed</td>
<td>10:15 PM Lie down in bed</td>
</tr>
</tbody>
</table>

- The physical activity protocol will be programmed into the computer in the calorimeter, and you will be prompted by a command and audio sound when they can move about the room. Upon starting the study each day, you will be instructed to sit quietly in the room. You may watch TV or read during this time, but will not be permitted to use the computer. At 8:45 AM, you will prepare for exercise (change clothes, turn on bike, etc). On the control day, we will ask you to mimic exercise preparation. Exercise will be performed at 9:00 AM; on the non-exercise control day, you will resume quiet sitting. After the exercise, you will be permitted time to wash and clean up from exercise, and then return to quiet sitting until breakfast is served (10:00 AM). After breakfast, you will remain quietly sitting until lunch (2:00 PM). After lunch, you will return to quiet sitting again until dinner is served (7:00 PM). At 45 minutes of each hour, you will be permitted

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to use the bathroom and computer (check email on internet, etc). After dinner, you will be free to move about the calerimetre, although you will be instructed to sustain sedentary activities (watching TV, reading, or using the computer). At 10:00 PM, you will be asked to prepare for bed, and at 10:15 PM, you will be instructed to lie down. You can turn off the lights whenever you wish, but will be instructed to do this at the same time each night.

- During each day in the metabolic room, you will be also asked to collect all of your urine in jugs that we provide. This is done so we can measure how much protein your body uses, which helps measure how many calories are being burned, and from what source (i.e. fats, sugars or proteins).

Sprint Exercise: At 9 o'clock on the second morning you will be asked to perform 5 sprints on an exercise bike. Each bout will last 30-seconds and will be separated by 4-minutes. The exercise intensity during these 30-seconds will be very, very high. You will be asked to exercise as hard as possible.

What are the possible discomforts or risks?

Discomforts and risks you may experience while in this study may include:

DXA: As part of this study we will perform 1 DEXA scan of your body. DEXA is a way of looking inside the body by using X-rays. X-rays are a type of radiation. Your natural environment has some radiation in it. This DEXA will give you about the same amount of radiation that you would get from your environment in 2 days.

RMR: During the resting metabolic rate (RMR) testing, a clear, ventilated plexiglass hood will be placed over your head for 45 minutes. Some volunteers find this procedure slightly claustrophobic.

Metabolic room: Some people may feel claustrophobic in the room. You will not be locked in the room; the door can be opened from the inside. There is also a call button and telephone that connects the metabolic room directly to the nursing station. However, if you leave the testing room early the testing session is over.

Graded Exercise Test: There is a very small chance of an irregular heartbeat during exercise (< 1% of all subjects). Other rare risks of a stress test are heart attack (< 5 in 10,000) and death (<2 in 10,000). Wearing a mouthpiece and nose-clip can sometimes cause dryness in the mouth and mild discomfort.

Sprint Interval Training: In addition to the risks described above, sprint interval training may result in sore legs, and excessive tiredness and sensations of loss of energy. Brief feelings of dizziness and nausea (feeling sick and unwell) are also possible.

What are the possible benefits of the study?

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This study is not designed to treat any illness or to improve your health. Also, there may be risks, as discussed in the section describing the discomforts or risks.

Who is paying for this study?
This research is being supported by the Investigators. Support for the use of the CTRC is provided in part by a grant from the National Institutes of Health.

Will I be paid for being in the study?
You will be paid $100 if you complete all parts of this study. If you leave the study early, or if we have to take you out of the study, you will not be paid. It is important to know that payment for participation in a study is taxable income.

Will I have to pay for anything?
It will not cost you anything to be in the study.

Is my participation voluntary?
Taking part in this study is voluntary. You have the right to choose not to take part in this study. If you choose to take part, you have the right to stop at any time. If you refuse or decide to withdraw later, you will not lose any benefits or rights to which you are entitled. If there are any new findings during the study that may affect whether you want to continue to take part, you will be told about them.

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Can I be removed from this study?

The study doctor may decide to stop your participation without your permission if the study
doctor thinks that being in the study may cause you harm, or for any other reason. Also, the
sponsor may stop the study at any time.

What happens if I am injured or hurt during the study?

If you have an injury while you are in this study, you should call Edward Melanson immediately.
His phone number is (303) 724-0935. You can also contact the supervising physician assistant
at (303) 266-1096. We will arrange to get you medical care if you have an injury that is caused
by this research. However, you or your insurance company will have to pay for that care.

Who do I call if I have questions?

You may have questions about your rights as someone in this study. You can call Edward
Melanson with questions. While your primary source of information pertaining to participation
in this study is the principal investigator, Dr. Melanson, a Research Subject Advocate is also
available on the CTRC at (720) 848-6662 to answer questions relating to participation in this
study. You can also call the Colorado Multiple Institutional Review Board (COMIRB). You can
call them at 303-724-1055.

Who will see my research information?

The University of Colorado Denver and the hospital(s) it works with have rules to protect
information about you. Federal and state laws including the Health Insurance Portability and
Accountability Act (HIPAA) also protect your privacy. This part of the consent form tells you
what information about you may be collected in this study and who might see or use it.
The institutions involved in this study include:

- University of Colorado Denver
- University of Colorado Hospital

We cannot do this study without your permission to see, use and give out your information. You
do not have to give us this permission. If you do not, then you may not join this study.

We will see, use and disclose your information only as described in this form and in our Notice
of Privacy Practices; however, people outside the University of Colorado Denver and its affiliate
hospitals may not be covered by this promise.

We will do everything we can to keep your records a secret. It cannot be guaranteed.

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The use and disclosure of your information has no time limit. You can cancel your permission to use and disclose your information at any time by writing to the study’s Primary Investigator, at the name and address listed below. If you do cancel your permission to use and disclose your information, your part in this study will end and no further information about you will be collected. Your cancellation would not affect information already collected in this study.

Edward L. Melanson, Ph.D.
Division of Endocrinology, Metabolism, and Diabetes
University of Colorado Denver
12801 East 17th Ave, RC1 South RM 7103, MS 8106
Aurora, CO 80045
Phone: (303) 724-0935
FAX: (303) 724-3920

Both the research records that identify you and the consent form signed by you may be looked at by others who have a legal right to see that information.

- Federal offices such as the Food and Drug Administration (FDA) that protect research subjects like you.
- People at the Colorado Multiple Institutional Review Board (COMIRB)
- The National Institutes of Health, which provides financial support for the University of Colorado CTRC.
- The study doctor and the team of researchers.
- Officials at the institution where the research is being conducted and officials at other institutions involved in this study who are in charge of making sure that we follow all of the rules for research.
- The investigator (or staff acting on behalf of the investigator) will also make all or some of the following health information available to Colorado State University.

We might talk about this research study at meetings. We might also print the results of this research study in relevant journals. But we will always keep the names of the research subjects, like you, private.

You have the right to request access to your personal health information from the Investigator.

The investigator (or staff acting on behalf of the investigator) will also make all or some of the following health information about you available to Colorado State University.

Information about you that will be seen, collected, used and disclosed in this study:

- Name and Demographic Information (age, sex, ethnicity, address, phone number, etc.
- Your social security number
- Portions of my previous and current Medical Records that are relevant to this study, including but not limited to Diagnosis(es), History and Physical, laboratory or tissue studies, radiology studies, procedure results

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- Research Visit and Research Test records
- Billing or financial information

Agreement to be in this study and use my data

I have read this paper about the study or it was read to me. I understand the possible risks and benefits of this study. I understand and authorize the access, use and disclosure of my information as stated in this form. I know that being in this study is voluntary. I choose to be in this study. I will get a signed and dated copy of this consent form.

Signature: ___________________________ Date: ________

Print Name: ___________________________

Consent form explained by: ___________________________

Print Name: ___________________________

Investigator: ___________________________ Date: ________

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APPENDIX B – HEALTH HISTORY/SCREENING FORM

Email/Telephone Screening Form
You may refuse to answer any of the following questions. However, please be aware that your refusal may prevent researchers from safely assessing your suitability for participation in this investigation.”

The information you provide will be kept private to the extent allowed by the law. Your information will be combined with information from other people taking part in the study. When we write about the study to share it with other researchers, we will write about the combined information we have gathered. You will not be identified in these written materials. We may publish the results of this study; however, we will keep your name and other identifying information private.

We will make every effort to prevent anyone who is not on the research team from knowing that you gave us information, or what that information is. For example, your name will be kept separate from your research records and these two things will be stored in different places under lock and key. You should know, however, that there are some circumstances in which we may have to show your information to other people. For example, the law may require us to show your information to a court.

If you are not eligible to participate in this study your information will remain private.

Name:
Phone:
Address:
E-mail:
Emergency contact:
Age:
Date of Birth:
Gender:
Handedness (left or right):
Ethnicity:
Occupation:
Approximate weight:
Approximate height:
BMI (Wt/Ht2):

GENERAL HEALTH QUESTIONS:

Do you use tobacco or have you ever used it?
If so, how long ago did you quit?
Are you regularly exposed to second hand smoke? (at home, work, restaurants, bars)
   If so, about how many total hours per week?

Do you drink alcohol?
If so, how many times per week?

Previous exercise stress test?
If yes, what were the results?

Please list any medications you are currently taking (prescription or otherwise):

Do you have any of the following conditions (Y/N)?

High blood pressure
Resting BP_______/_______
High cholesterol
Kidney disease?
Diabetes?
Pulmonary/Lung Disease?

Thyroid disease?
   If yes, are you taking any medication for it (e.g., synthroid, thyroxin)?
Allergies?
   If yes, do you take medication for them?
Orthopedic problems (e.g. arthritis, osteoporosis, joint disorders)

CARDIOVASCULAR DISEASE QUESTIONS
Diagnosed with cardiovascular disease, heart murmur or arrhythmia?
Do you have surviving relatives with heart disease, or a family history of sudden death?
Do you suffer excessive fatigue?
When you exercise or exert yourself do you ever experience any of the following?
  light headed or faint
  chest pain
  respiratory problems

Do you have any history of sickle cell disease (anemia)?

Have you ever experienced acute mountain sickness or any other unfavorable reaction to low-oxygen environments?

Have you ever experienced a seizure and/or have taken, or are currently taking, medication for seizure?

Have you a history of allergic reaction, hypersensitivity or idiosyncratic reaction to Methazolamide and/or Aminophylline, or an allergy to any sulfa or sulfonamide derivatives?

Do you regularly use Aspirin?

Have you made a blood or plasma donation, or have experienced a significant loss of blood, within the previous 30 days?

When was the last time you were ill or required medical attention?
DIETARY HABITS
Are you currently taking vitamin or dietary supplements?
Please list kind, dosage and for how long

Do you eat a special diet (vegetarian, vegan, Atkins, etc.)?
Has your weight been stable over the last 6-12months?

PHYSICAL ACTIVITY QUESTIONS
Please describe the type, frequency (#of sessions/week), duration (time/session) and intensity (low, moderate, high) of any physical activity you perform.

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SUBJECT TOLERANCE QUESTIONS
Are you willing to fast overnight?
Are you comfortable with needles and/or having blood drawn?
Are you willing to eat a controlled diet?
What days are you available for testing?
How did you hear about the study?

Are you currently participating in any other studies?