THESIS

SHORT-TERM SPRINT-INTERVAL TRAINING IMPROVES INSULIN SENSITIVITY IN YOUNG ADULT HUMANS

Submitted by

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WE HEREBY RECOMMEND THAT THE THESIS PREPARED UNDER OUR SUPERVISION BY TYLER JOHNSON ENTITLED SHORT-TERM SPRINT-INTERVAL TRAINING IMPROVES INSULIN SENSITIVITY IN YOUNG ADULT HUMANS BE ACCEPTED AS FULFILLING IN PART REQUIREMENTS FOR THE DEGREE OF MASTER OF SCIENCE.

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ABSTRACT OF THESIS

SHORT-TERM SPRINT-INTERVAL TRAINING IMPROVES INSULIN SENSITIVITY IN YOUNG ADULT HUMANS

Habitual endurance exercise has been shown repeatedly to increase insulin sensitivity, one of the principal determinants of blood glucose control. Many adults however, choose not to participate in this type of exercise, citing insufficient time as a perceived obstacle. A recent study has described improved blood glucose control post-consumption of a glucose beverage following 2-weeks of sprint-interval training (SIT), implying that SIT may increase insulin sensitivity.

PURPOSE: Using the gold standard measure, the hyperinsulinemic euglycemic clamp technique, we investigated the hypothesis that SIT will increase insulin sensitivity. METHODS: 12 healthy, sedentary or recreationally active adults (age: 27 ± 3 yr; body mass index: 26.2 ± 1.4 kg/m^2; VO_{2peak}: 36.0 ± 3.3 ml/kg/min (mean ± SE)) completed 6 sessions of repeated (4 to 7) 30-second bouts of extremely high-intensity cycle ergometer exercise (i.e. a Wingate protocol) over 14 days. Prior to and 72 hours following completion of SIT the glucose infusion rate (GIR) required to maintain a blood glucose concentration of 90 mg/dL during a standardized infusion of insulin was determined. In

order to quantify the effect of the most recent SIT bout on insulin sensitivity, GIR was determined in 7 adults (25 ± 1 yr; 25.7 ± 1.4 kg/m^2; 37.1 ± 4.3 ml/kg/min) prior to and 72 hours following a single bout of SIT. Finally, in order to establish the day-to-day variability in GIR, 9 adults (23 ± 2 yr; 26.8 ± 1.6 kg/m^2, 33.3 ± 2.2 ml/kg/min) served as a sedentary control.

RESULTS: Compared with baseline, insulin sensitivity was increased following short-term SIT (GIR: 6.2 ± 0.7 *vs.* 8.0 ± 0.8 mg/kg/min; $P = 0.02$) but was unchanged following a single bout of SIT (9.7 ± 1.3 *vs.* 10.7 ± 1.4; $P = 0.43$) or a period of inactivity (7.9 ± 0.9 *vs.* 8.3 ± 1.0; $P = 0.38$). Regardless of intervention, blood glucose concentration at the end of the hyperinsulinemic euglycemic clamp, body mass and fasting blood glucose concentration remained unchanged (all $P > 0.2$), with the exception of a small increase in fasting glucose following a single bout of SIT (73.6 ± 1.8 *vs.* 75.8 ± 1.3 mg/dL; $P = 0.01$).

CONCLUSION: These data, collected using the gold standard hyperinsulinemic euglycemic clamp technique, suggest that short-term SIT is a viable alternative to endurance training as a strategy to improve insulin sensitivity.

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CHAPTER I

REVIEW OF THE LITERATURE

Metabolic Syndrome

The combination of medical disorders that increase the risk of developing cardiovascular disease and diabetes is known as metabolic syndrome (MetS) or *syndrome X* (55, 108). An estimated 24% of all U.S. adults fall under the criteria for diagnosis of MetS set forth by the adult treatment panel III (ATP III) (45). Moreover, this percentage is likely underestimated because of the improbability of measuring for all of the MetS risk factors in every person with lifestyle rooted diseases.

Clinical diagnosis of MetS includes the presence of three or more of the following clinical features: fasting plasma glucose ($\geq$ 110 mg/dL), abdomen obesity (>102 cm in men or >88 cm in women), HDL-cholesterol (< 40 mg/dL in men or < 50 mg/dL in women), triglycerides (TG) ($\geq$ 150 mg/dL) and arterial blood pressure ($\geq$ 130/85 mmHg) (1, 55, 56). As many as 71% and 44% of U.S. adults have at least one or two of the established risk factors, respectively (45). In addition to the main causes, physical inactivity and a high-fat diet, elements such as smoking, alcohol, and genome play a role in the development of MetS.

It is extremely difficult to identify the national health costs associated with MetS because its diagnosis warrants suffering any three of the mentioned physiologic

complications. An individual treated for complications associated with cardiovascular disease (CVD) most likely exhibits 3+ of MetS risk factors, but unless each factor is diagnosed, MetS will not be the diagnosis. Similarly, an individual suffering from type 2 diabetes (T2D) will most certainly be afflicted with most of the MetS risk factors, as they are commonly seen in T2D patients (80, 118, 125). However, unless these disparities are identified, MetS will not be listed as the cause for medical treatment. Nevertheless, an estimated $80 billion or greater is spent annually on the combined MetS risk factors (124). Of this, $27 billion is spent solely on prescription drugs for the treatment of the related disparities (124). The average annual medical expense for individuals with MetS is $5477, of which $1832 is attributable to prescription costs (124). While this is a tremendous cost for the U.S., the majority of expenses are spent on disease management and not on possible solutions to eliminate the disease.. It is crucial that more time and funding be spent on the most efficient ways to prevent and eliminate these disease risk factors.

MetS is also termed, "*insulin resistance syndrome*" because insulin resistance occurs in a majority of individuals with MetS (41, 55, 108). MetS is associated with a 2- to 3-fold and 3- to 4-fold increased risk in the development of CVD and T2D, respectively (14). As identified in The Framingham Study, chronic illness and mortality associated with diabetes is principally attributed to CVD (79, 80). Diabetes is listed as the seventh leading cause of death in the United States. However, because of its association with CVD, the number one cause of death, diabetes is most likely underreported as the actual cause of death (107).

Insulin Resistance and Type 2 Diabetes

Insulin resistance leading to clinically diagnosed T2D is a continually growing health epidemic. An estimated 24 million people in the United States had diabetes as of 2007, with 90-95% of diagnosed cases consisting of T2D (107). The prevalence of insulin resistance syndrome is currently ~150 million and is predicted to reach 366 million by 2030 (133). An individual with a fasting plasma glucose concentration of 100-125 mg/dL is considered to have impaired fasting glucose (IFG) (1). When insulin resistance causes fasting plasma glucose to increase to $\geq$ 126 mg/dL, T2D is diagnosed (1). An estimated 57 million individuals $\geq$ 20 years old have IFG and are on track to join the over 24 million people with T2D (107). Serious physiologic complications associated with insulin resistance include: heart disease and stroke, high blood pressure, kidney disease, blindness, nervous system disease, dental problems, amputations and pregnancy complications (107). Research for the treatment of insulin resistance and diabetes has become a primary healthcare concern because diabetes' costs reached an estimated annual expenditure of $174 billion in 2007 (107).

Individuals with insulin resistance have impaired insulin action on whole-body glucose uptake, which may result in a hyperglycemic state leading to numerous morbidities and mortality (118, 138). A long-term cohort following newly diagnosed T2D's observed a 50% increased mortality rate in individuals with a chronic higher fasting blood glucose (FBG) (>140 mg/dL) compared to those with a lower FBG (<140 mg/dL) (2). Any means to attenuate fasting plasma glucose will lower mortality rates of individuals with low-level insulin resistance or T2D.

In recent years, the vastly growing health and economic impact T2D has on a global scale has forced the disease to the frontlines of medical research and treatment. The aim to discover and administer effective treatments for T2D has directed millions of researchers and individuals in the medical profession to explore the direct cause or causes driving the development of insulin resistance and T2D. The etiology of T2D must be identified in order to fashion the most efficient modalities for treatment.

Physiological Disparities and Insulin Resistance

The exact pathway through which insulin resistance leads to T2D is not exactly known. When cells become insulin resistant they are poorly stimulated by insulin to trigger the insulin signaling cascade and thus, uptake glucose into the cells. Continuous hyperglycemia leads to a lack of peripheral insulin-responsiveness and may progress to inadequate insulin secretion. A number of studies have determined defects in: mitochondrial activity, mitochondrial biogenesis and metabolic signaling as the primary causes of tissue level insulin resistance (59, 86). Furthermore, impaired mitochondrial activity has been identified in T2D patients and their insulin resistant offspring (8). Decreased insulin-stimulated mitochondrial ATP production in diabetic patients compared to nondiabetics strengthens the hypothesis that insulin resistance has a direct link to impaired mitochondrial function (123). Mitochondrial dysfunction as an underlying cause of insulin resistance is further supported by evidence suggesting that muscle oxidative capacity is the best predictor of insulin sensitivity (15, 17). However, it cannot be dismissed that other factors lead to insulin resistance and it is insulin resistance that leads to mitochondrial complications (87).

Mitochondrial dysfunction is thought by many as the main cause of insulin resistance, however, intramuscular TG content can independently affect metabolic function as it is strongly associated with insulin resistance (52). This accumulation of intracellular lipids suggests a state of impaired fat oxidation associated with impaired metabolic flexibility, the cell's ability to select the most widely available substrate for fuel metabolism (16, 52). Lipid accumulation may impair the action of numerous proteins in the insulin signaling cascade, leading to less GLUT-4 translocation and reduced glucose uptake (48). Metabolic flexibility can be enhanced through an intervention of physical activity and diet (16, 17, 51). Metabolic flexibility is a persons' ability to adjust the level of lipid and carbohydrate oxidation necessary to meet metabolic demand based on fuel availability. Impaired metabolic flexibility is to be expected in the presence of dysfunctional mitochondria and complications in the insulin signaling cascade.

Increases of the enzyme activity in pathways leading to enhanced metabolic capacity have been observed concomitantly with increases in exercise and decreases in insulin resistance (60, 67, 86). Current research is looking for the most effective method of training for the greatest improvements in insulin resistance (119). Exercise interventions although effective, have been difficult for T2D patients to adhere to and physically complete (13, 120). These long-term interventions have shown as little as ~50% completion rates (120). Currently, pharmacologic interventions are the most commonly used intervention for management of T2D.

Pharmacologic Treatment

Several of the most commonly taken oral anti-hyperglycemic agents act via stimulation of varying components of the insulin signaling pathway, leading to an increase in whole-body glucose uptake and metabolism. Metformin is currently the most widely prescribed anti-diabetic drug in the world. This oral anti-diabetic treatment is annually prescribed to more than 120 million people worldwide (129). Metformin is a peripheral insulin-sensitizing biguanide that acts via the stimulation of 5' AMP-activated protein kinase (AMPK), an important enzyme in the insulin signaling cascade, carbohydrate and lipid metabolism and in energy homeostasis (90, 129). Metformin also suppresses hepatic glucose production, aiding in the attenuation of hyperglycemia associated with insulin resistance (84). Treatment with Metformin can lower the fasting blood glucose of T2D's up to 50-70 mg/dL (84). A serious complication associated with the use of metformin is its association with increased blood lactate levels and metabolic acidosis while at rest (93). Metabolic acidosis is a normal physiologic process during exercise, but is a life threatening condition if it occurs at rest, having a 30-50% mortality rate (93). Metformin treatment is also not possible in individuals with liver or kidney complications, which commonly accompany insulin resistance and T2D (84).

Thiazolidinediones attenuate insulin resistance by lowering blood glucose levels through binding to and activation of peroxisome proliferator-activated receptor gamma (PPARγ). PPARγ is a nuclear regulating protein involved in the transcriptions of genes involved in glucose and lipid metabolism. This group of agents are believed to be the most efficient long-term treatment for insulin resistance because of the associated β-cell preservation and lower rates of CVD seen with treatment of Thiazolidinediones (90).

Concern regarding the use of oral anti-diabetic agents includes the fact that most patients need multiple pharmacological interventions to maintain glucose regulation (90). After three years from diagnosis, 50% of subjects needed multiple pharmacological interventions to keep fasting blood glucose $\leq$ 140 mg/dL and by nine years, 75% of subjects needed multiple agents to maintain these levels (90). In addition to the necessity for taking numerous agents, the effectively prescribed doses must increase over time as hyperglycemia leads to a progressive loss of functional β-cells. At the point where β-cells no longer produce and secrete physiological effective amounts of insulin, additional intervention by means of exogenous insulin administration is necessary (90). Pharmacological intervention is an efficient short-term treatment in the attenuation of hyperglycemia. However, the effectiveness of any single or combined anti-diabetic agents declines with time. In addition, anti-diabetic agents are not aimed to target all suspected components thought to underlie the mechanisms behind insulin resistance, but are only formulated to modify single aspects of the pathology. Unlike pharmacological treatments, exercise as a therapy for T2D has been shown to modulate numerous components thought to cause insulin resistance. In addition, exercise is notably less expensive and has none of the adverse side effects seen with oral anti-diabetic agents.

Influence of Exercise on Insulin Resistance and MetS

In addition to the risk factors associated with MetS, poor physical fitness is one of the strongest indicators in the development of insulin resistance and T2D (42, 56, 132). Physical activity is defined as any movement of skeletal muscle that results in energy expenditure, whereas exercise is defined as planned, structured and repetitive physical

activity with the purpose of enhancing one or more of the components of physical fitness (26). When compared to medication and nutritional interventions, exercise is one of, if not the most efficient method to improve insulin sensitivity (29, 83). In obese, middle-aged type 2 diabetics, short-term exercise improved insulin sensitivity by ~50% (101). The relationship between physical fitness and insulin resistance is extensively researched because many of the suspected causes of insulin resistance are modified with concomitant changes in physical fitness.

Moderate to high levels of cardiorespiratory fitness are tightly associated with lower MetS risk factors whereas low physical fitness is a key, independent predictor of adult mortality (10, 99). The Diabetes Prevention Program observed a 41% decrease in the development of MetS following a ~3 year lifestyle intervention program including improved diet and increases in physical activity (35, 102). A 16% increase in cardiorespiratory fitness following a 20 week endurance training intervention (The HERITAGE Family Study) was seen concomitantly with a 30.5% decrease in the prevalence of MetS (81). Although effective, continuous aerobic exercise can be difficult to maintain for individuals with MetS. Higher intensity interval exercise is a more easily attainable form of exercise for persons with MetS and elicits similar adaptations to lower intensity, long duration training (73). To date, weight loss and exercise are the most efficient modes of treatment for MetS, particularly by increasing insulin sensitivity in cells (29, 37, 92, 109, 122).

Whole body glucose and fatty acid utilization improves in T2D patients who participate in regular physical activity (137). Glucose homeostasis can improve through habitual participation in exercise, even without weight loss (12). Moderate exercise can

reduce fasting plasma glucose levels, increase whole-body glucose metabolism and decrease insulin resistance in as short as six weeks (29).

When compared to a sedentary control, the incidence of the development of T2D decreased by 58% in at risk individuals who consumed a hypocaloric/low fat diet while participating in 150 min/week of moderate physical activity for ~3 years (35). The HERITAGE Family Study showed a 10% increase in insulin sensitivity, including significant improvements in whole-body glucose uptake, following a 20-week endurance training program involving 316 women and 280 men of varying ethnicities (12). A one metabolic equivalent (MET) (3.5 $mlO_2 \cdot min^{-1} \cdot kg^{-1}$) lower cardiorespiratory fitness level was correlated to an increased fasting plasma glucose and increase of T2D risk factors by 20% and 28%, respectively (37). MET is applied as a functional means of conveying the intensity and energy expenditure of physical activities in a way comparable among persons of different weight. Compared to unfit persons, individuals who participate in regular exercise experience a lower insulin response to a given glucose load, suggesting higher sensitivity to insulin than unfit individuals (61). Despite the decreased insulin response, these individuals do not experience blunted whole-body glucose uptake (61).

Several studies have illustrated increases in insulin sensitivity and insulin-stimulated glucose uptake by skeletal muscle in healthy, obese and diabetic persons following endurance training (17, 31, 32, 71, 72, 110). Short-term resistance training may also improve insulin sensitivity and glucose uptake (131).

Numerous physiologic adaptations occur by way of exercise, which lead to augmented insulin sensitivity. Improvements in whole-body glucose utilization following exercise are partly attributed to increases in GLUT-4 (91, 106, 127). Increases

in GLUT-4 expression (mRNA and protein) are associated with improvements in glucose uptake (88, 89). Furthermore, exercise training increases both mRNA and protein content of GLUT-4 in skeletal muscle cells (30, 82). A high fat diet-induced 20% decrease in skeletal muscle GLUT-4 content was normalized and ultimately increased by 50% following endurance training (94). Glucose uptake is necessary to replenish the depleted muscle glycogen content resulting from exertion. Some believe increases in muscle GLUT-4 as a result of endurance training are due to increases in glycogen build up during early exercise recovery and the amount of muscle glycogen supercompensation (54). An increased glycogen pool provides substrate for immediate skeletal muscle utilization.

Improvements in GLUT-4 content and activity are likely associated with enhanced insulin signaling via increased insulin receptor substrate-1 (IRS-1) and IRS-1-associated phosphoinositide 3-kinase (PI3K) activity following exercise training (71, 85). However, other studies suggest that increased GLUT-4 translocation may not be a result of increased insulin sensitivity, but the insulin-independent GLUT-4 translocation caused by the repetitive skeletal muscle contraction during exercise (29, 101, 111). Exercise-induced increases in glucose uptake through insulin-independent mechanisms although effective, may not be as efficient as insulin-dependent mechanisms because research shows that glucose uptake into the cell is 20-30 times greater in the presence of insulin than without (11). Skeletal muscle IRS-1 and IRS-2 phosphorylation increased 1.8 and 1.5 fold in response to insulin, respectively, after 6 weeks of aerobic exercise (95). In this model, IRS-1 and IRS-2-associated PI3K phosphorylation increased 2.3 and 1.9 fold, respectively (95). Increases in PI3K and IRS-1 activity through exercise are applicable

because of the low activity of these proteins in insulin resistant persons and individuals with T2D (9, 53). Increased activity and protein content of additional insulin signaling cascade proteins, Akt and Akt substrate, AS160, also augments insulin sensitivity in trained individuals (34, 47). A single bout of endurance exercise increased phosphorylation of Akt by 1.8 fold and AS160, by 2 fold (33). Reduced Akt and AS160 activity caused by a high fat diet was normalized following 4 weeks of aerobic training (94).

5' AMP-activate protein kinase (AMPK) is commonly known as the body's master regulator of metabolism. AMPK plays a regulatory role in glucose uptake, insulin signaling proteins, fatty acid oxidation and mitochondrial biogenesis. Increased GLUT-4 content may be associated with training-induced increases in AMPK, as AMPK signaling upregulates GLUT-4 protein content in skeletal muscle (18, 66). Compared to an untrained control, endurance trained muscle experiences up to 94% and 49% increases in AMPK activity associated with the α-1 and α-2 AMPK isoforms, respectively (46).

As little as 7-10 days of low-moderate aerobic training (2 hours·day^{-1} at 65% VO_{2max}) can increase skeletal muscle mitochondrial activity, which has also been associated with improved insulin sensitivity (121). Increases in mitochondrial enzymes and biogenesis following exercise are partially attributable to increases in AMPK activity (134). Changes in peroxisome proliferator-activated receptor gamma coactivator 1 alpha (PGC-1α) also play a major role in exercise-induced increases in mitochondrial biogenesis. Increased lipid oxidation following 4 weeks of exercise was associated with a 30% increase in PGC-1α expression (94). Short-term intense interval training increases PGC-1α expression 2-fold via upregulation of AMPK and p38 signaling protein activity

(50). Six weeks of endurance training increased mRNA content of mitochondrial biogenesis-associated proteins PGC-1α and peroxisome proliferator-activated receptor-alpha (PPAR-α) by 2.7 and 2.2-fold, respectively (116). Four weeks of one-legged knee extensor exercise training resulted in a 10->40-fold increase in PGC-1α transcription, a 7-10-fold increase in PGC-1α mRNA content and a 2-fold increase in PPAR-α activity (105). Another mitochondrial transcription factor, nuclear respiratory factor-1 (NRF-1) is positively correlated with glucose utilization in skeletal muscle (6). Exercise augments NRF-1 expression in muscle (4). Chronic exercise characteristically increases mitochondrial biogenesis-associated proteins such as NRF-1, PPAR-α and PGC-1α (74). Enhancements in mitochondrial function and biogenesis have been established through observed increases in mitochondrial density and oxidative enzymes following endurance training (65, 68, 69).

The majority of previous research suggests that exercise intensity may be the most influential factor in determining changes in glycolytic and mitochondrial enzymes following exercise training. Collectively, low-moderate intensity training is associated with increases in aerobic enzymes and high intensity training is linked with rises in anaerobic enzymes. Endurance training, such as long distance running, generates a minimal effect on anaerobic metabolism (65). Significant proliferation of glycolytic enzyme activity is typically seen following high intensity training, such as SIT (25, 76, 112). Lipid oxidation and aerobic enzyme activity is greatest during and following low-moderate intensity training (70). The accumulation of lipid species in sedentary individuals leads to impaired insulin signaling and insulin resistance (16, 58). Low-moderate intensity exercise normalizes and improves impairments via alterations in:

substrate utilization, enzyme content and activity, insulin signaling and gene transcription (16). Current research of exercise intensity and metabolism is discovering improvements in aerobic metabolism by means of high intensity exercises.

When plasma glucose and insulin are maintained at constant physiologic concentrations using the hyperinsulinemic euglycemic clamp procedure, insulin stimulated glucose disposal increases in trained individuals (24, 61). Although exercise augments insulin sensitivity, inactivity has an opposite effect of similar magnitude. Individuals with an elevated capacity for physical exertion have higher insulin sensitivity than sedentary persons, but insulin action decreases even in trained individuals following as little as a week of inactivity (61). The insulin sensitizing effects of a single bout of exercise only persist up to 48 hours following the bout (43, 75, 135). As little as 6-8 days of detraining can abrogate the long-term training-induced enhancement of insulin sensitivity in skeletal muscle (31, 115). Ten days of inactivity in fit individuals resulted in a 23% reduction in the glucose disposal rate during the final 30 minutes of a hyperinsulinemic euglycemic clamp (83). This suggests that people must stay physically active on a daily basis to maintain an efficient state of insulin action and plasma glucose homeostasis.

Disagreements persist regarding the volume and intensity necessary to elicit a change in insulin sensitivity. One study observed that significant increases in insulin sensitivity were seen only with high intensity exercise ($\geq$ 70 % $VO_{2\ peak}$) (78), while another concluded that moderate intensity exercise was most efficient to elicit improvements in insulin sensitivity (78, 97). Recently, one study observed that the duration of training is more powerful in augmenting insulin sensitivity than the intensity

of training (71). Not only is there disagreement as to what volume and intensity is best for improving insulin action, the best modality of exercise to elicit these changes is also controversial.

Conclusively, regular light-moderate endurance training improves insulin sensitivity. However, attempts to engage subjects with T2D in regular exercise training have been disappointing (13, 120). It has recently been suggested that multiple, short duration, higher intensity endurance training bouts are more effective in augmenting insulin sensitivity than a single, long duration, lower intensity endurance training bout, over time (40). Findings such as this propose that intensity may in fact be more influential on metabolic health than duration. Moreover, time is one of, if not the most commonly used excuses when individuals do not adhere to a training intervention (120). Because of this, it is necessary to explore alternative, time-efficient, training strategies to attenuate MetS complications, particularly insulin resistance.

Sprint Interval Training

Interval training has been used by athletes for many years as a means to improve performance, particularly power. SIT was introduced 70 years ago by Woldemar Gerschler as a means to rapidly improve athletic explosiveness (27). Sprint interval training is defined as brief recurring sessions of high intensity exercise. SIT may be beneficial to all individuals as it causes numerous beneficial metabolic changes in human skeletal muscle (114). Martin J. Gibala and colleagues lead the way in constructing a foundation for new experiments and ideas to determine the benefits of SIT. Gibala and

others have identified numerous metabolic adaptations resulting from SIT that are typically associated with traditional endurance training (19-22, 96, 114).

Literature from the 70's and earlier suggested that SIT caused little or no changes in mitochondrial enzyme activity (63, 64, 117). These studies suggested that low-volume high-intensity interval training does not put enough stress on the body's aerobic systems to elicit changes in aerobic metabolism. However, it was determined that glycolytic enzymes do increase with high-intensity interval training. Recent novel findings and method modifications have given way to new research and discoveries. These breakthroughs have produced ideas opposed to those stated in the 70's and prior. In fact, enzymes of all three energy systems show signs of adaptation following SIT (114).

Similar to endurance training, improvements in carbohydrate oxidation, lipid oxidation and muscle metabolic capacity have recently been observed in training protocols of 2-7 week SIT studies (20, 21). Seven weeks of SIT elicited improvements in both glycolytic enzyme and oxidative enzyme activity. Compared to baseline, hexokinase and phosphofructokinase activity increased 56% and 49%, respectively. Moreover, increases in citrate synthase (36%), succinate dehydrogenase (29%) and malate dehydrogenase (65%) also occurred (96). Similar adaptations of aerobic enzymes occur with as little as two weeks of SIT. Two weeks of SIT lead to increases of 38% and 60% in citrate synthase and beta-hydroxyacyl-CoA dehydrogenase (β-HAD), respectively (103, 113). Six sessions of SIT over two weeks elicits increases in resting muscle glycogen and citrate synthase activity of 26% and 38%, respectively (22). Another study showed a significant increase in maximal citrate synthase activity of 11% following only

two weeks of SIT (22). Tighter coupling of glycogenolytic flux and pyruvate oxidation during sub maximal exercise ensues following 2 weeks of SIT (20).

Based on observations of increased aerobic enzymes following SIT, many researchers have looked at more specific markers of mitochondria activity and biogenesis following short- and long-term SIT. Cytochrome c oxidase subunit IV increased (35%) after one week of SIT and remained elevated after 6 weeks (19). Maximal activity and protein content of both cytochrome c oxidase subunits II and IV increased following as little as six sessions of SIT (49). Skeletal muscle biopsies taken three hours following a single session of SIT showed a two-fold increase in mitochondrial biogenesis marker, PGC-1α mRNA expression (50). In addition, phosphorylation of AMPK and p38 MAPK mitochondrial signaling proteins increased immediately following four single bouts of SIT (50). These results indicate SIT as an efficient stressor to increase an individual's aerobic potential.

Historically, findings suggested the only way to improve an individual's cardiorespiratory performance and aerobic capacity was through endurance training. However, impressive findings following as little as six sessions of SIT over two weeks show not only significant increases in glycolytic enzyme activity, resting skeletal muscle metabolites and muscle oxidative capacity, but also endurance and anaerobic work capacity (19, 20, 22, 103, 113). Two weeks of SIT augmented cycle endurance capacity 81%-169% compared to baseline (22). Seven weeks of SIT resulted in significant increases in both mean power output and VO_{2max} (baseline: 3.73 ± 0.13 l/min vs. post-SIT: 4.01 ± 0.08 l/min) (96). Increases in aerobic performance were demonstrated following 2 weeks of SIT by decreasing the amount of time to complete a 250-kJ time

trial by 9.6% (20). Resting muscle glycogen increases up to 50% following 2-7 weeks of SIT (20, 113). Decreases in skeletal muscle glycogen degradation, lactate accumulation and nonoxidative ATP generation following SIT suggest an improved reliance on aerobic metabolism during low to moderate intensity exercise (57).

Attenuated cellular lactate accumulation in high-intensity trained muscle is likely attributable to increases in lactate/H^+ transporters, MCT1, MCT4 and Na^+/H^+ exchanger (77, 104). SIT-induced improvements in aerobic capacity may be partly attributable to decreases in glycogenolysis, anaerobic ATP production and lactate/H^+ accumulation during submaximal exercise (20, 57). These decreases alongside the increases in mitochondria biogenesis signaling proteins suggest augmented aerobic function resulting from high intensity anaerobic exercise. Increases in aerobic capacity have been achieved with concomitant skeletal muscle buffering capacity increases of 7.6% following short-term SIT (49).

Improved overall aerobic metabolism resulting from SIT is clinically relevant because of the tight correlation between improvements in cardiorespiratory fitness and augmented insulin sensitivity (128, 137). Measurable SIT-induced metabolic improvements, similar to those previously thought to only occur via endurance training, have set the stage for contrasting and comparing two very diverse exercise modalities.

Sprint Interval Training Compared to Endurance Training

Exercise has long been identified as “aerobic” or “anaerobic”, with high volume, low to moderate-intensity work associated with increased mitochondria density and oxidative capacity and low-volume, high-intensity work usually associated with increased

skeletal muscle mass (5). Nevertheless, greater improvements in glucose tolerance occur with multiple, short duration moderate-intensity endurance training sessions·day^{-1} than with a single, long duration moderate-intensity endurance training session·day^{-1}, over five weeks (40). This finding proposes that multiple bouts of short interval exercise may play a larger role in modifications in glucose utilization than previously thought. Hence, a SIT exercise training model may be as equally efficient as endurance training in not only improving performance-related, but also health-related physiologic changes. "All out" high intensity interval training elicits similar metabolic changes to traditional endurance training (49, 117).

Sprint interval training is able to increase performance during tasks that rely primarily on aerobic energy production (20, 22). SIT may improve muscle buffering capacity more effectively than endurance training as demonstrated by subjects' ability to perform repeated sprints (5-6 seconds·30 seconds^{-1}) following 5 weeks of both types of training (39). Higher repeated sprints ability occurred in the high-intensity trained group despite no differences between groups in resting ATP concentrations, post exercise lactate levels or lactate threshold (39). Six weeks of SIT and endurance training produced lower levels of glycogenolysis during exercise with no difference between groups (19). Improvements in 50 and 750kJ time trials and cycle endurance capacity were seen following 2 weeks of SIT and endurance training, with no difference between groups (49). SIT, an anaerobic training routine, improves aerobic capacity to the same extent as endurance training, indicating similar functional improvements in carbohydrate metabolism, lipid metabolism, improved metabolic control during exercise and mitochondrial biogenesis.

Surprisingly, variations in SIT-induced oxidative ability and mitochondrial capacity are parallel to those generated following 6-10 days of prolonged, moderate-intensity exercise (121, 136). Increases in maximal cytochrome c oxidase activity was observed in both endurance training and SIT trained groups with no difference between groups (49). High intensity swimming in rats, consisting of 8 days of <5 minutes·day^{-1}, increased citrate synthase activity to levels provoked by 6 hours of daily, low-intensity training (126). Mitochondrial markers of carbohydrate oxidation, pyruvate dehydrogenase, and lipid oxidation, β-HAD and citrate synthase, were augmented in SIT and endurance training groups following 6 weeks of training with no differences between groups (19). PGC-1α content also increased similarly in endurance training and SIT groups subsequent to 6 weeks of training (19). These novel findings disregard prior knowledge that considered aerobic exercise as the only way to improve aerobic metabolism.

In studies where SIT is matched to endurance training by exercise mode, training frequency and training duration, similar metabolic adaptations occur in ~10% of the total training time commitment (2.5 hours vs. 10.5 hours·2 weeks^{-1}) (49). Over two weeks, total SIT volume was ~10% that of endurance training volume (~630 vs. ~6500 kJ) (49). During six weeks, actual time spent training with SIT versus endurance training was 10 minutes·week^{-1} compared to 4.5 hours·week^{-1} (21). These conclusions signify the remarkable time and volume contrasts between two very different exercise modalities. However, only one of these is currently recognized as treatment for augmenting the complications associated with MetS.

Statement of the Problem

The several physiologic improvements accompanying SIT establish it as a metabolically efficient mode of exercise. When compared to endurance exercise the same changes occur despite the fact that the training volume and time are up to 90% lower with SIT (21). Considering that limited time and excessive training volume are the most commonly expressed excuses underlying people's lack of physical activity, time efficient modes of exercise are needed to attenuate the country's biggest health epidemic (13, 120). If individuals were aware they are able to experience the same health improvements in one-tenth the time, they may be more driven to become physically active.

The role SIT plays in insulin sensitivity is still relatively unclear. Presently, only one study has determined how short-term SIT affects insulin sensitivity (7). However, this study examined only general whole-body glucose tolerance and not whole-body insulin sensitivity. To determine an individual's insulin sensitivity, it is necessary to determine the body's glucose reaction to a given amount of insulin. This is achieved by determining how much glucose is needed to maintain an unchanging plasma glucose concentration in response to a given amount of insulin. Many of the physiologic changes seen with endurance training are correlated to insulin sensitivity. Similar adaptations by way of SIT indicate it as a more practical intervention in the treatment of insulin resistance.

Hypothesis

Two weeks of high-intensity sprint-interval training will increase insulin sensitivity in young adult humans.

Specific Aims

1) To compare insulin sensitivity, prior to and 72 hours following completion of short-term sprint interval training using the hyperinsulinemic euglycemic clamp technique.

2) To quantify the effect of a single bout of sprint interval training on insulin sensitivity.

3) To determine day-to-day variability in insulin sensitivity following a period of inactivity.

CHAPTER II

THE MANUSCRIPT

SHORT-TERM SPRINT-INTERVAL TRAINING IMPROVES INSULIN SENSITIVITY IN YOUNG ADULT HUMANS

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ABSTRACT

Habitual endurance exercise has been shown repeatedly to increase insulin sensitivity, one of the principal determinants of blood glucose control. Many adults however, choose not to participate in this type of exercise, citing insufficient time as a perceived obstacle. A recent study has described improved blood glucose control post-consumption of a glucose beverage following 2-weeks of sprint-interval training (SIT), implying that SIT may increase insulin sensitivity. PURPOSE: Using the gold standard measure, the hyperinsulinemic euglycemic clamp technique, we investigated the hypothesis that SIT will increase insulin sensitivity. METHODS: 12 healthy, sedentary or recreationally active adults (age: 27 ± 3 yr; body mass index: 26.2 ± 1.4 kg/m^2; VO_{2peak}: 36.0 ± 3.3 ml/kg/min (mean ± SE)) completed 6 sessions of repeated (4 to 7) 30-second bouts of extremely high-intensity cycle ergometer exercise (i.e. a Wingate protocol) over 14 days. Prior to and 72 hours following completion of SIT the glucose infusion rate (GIR) required to maintain a blood glucose concentration of 90 mg/dL during a standardized infusion of insulin was determined. In order to quantify the effect of the most recent SIT bout on insulin sensitivity, GIR was determined in 7 adults (25 ± 1 yr; 25.7 ± 1.4 kg/m^2; 37.1 ± 4.3 ml/kg/min) prior to and 72 hours following a single bout of SIT. Finally, in order to establish the day-to-day variability in GIR, 9 adults (23 ± 2 yr; 26.8 ± 1.6 kg/m^2, 33.3 ± 2.2 ml/kg/min) served as a sedentary control. RESULTS: Compared with baseline, insulin sensitivity was increased following short-term SIT (GIR: 6.2 ± 0.7 *vs.* 8.0 ± 0.8 mg/kg/min; $P = 0.02$) but was unchanged following a single bout of SIT (9.7 ± 1.3 *vs.* 10.7 ± 1.4; $P = 0.43$) or a period of inactivity (7.9 ± 0.9 *vs.* 8.3 ± 1.0; $P = 0.38$). Regardless of intervention, blood glucose concentration at the end of

the hyperinsulinemic euglycemic clamp, body mass and fasting blood glucose concentration remained unchanged (all $P > 0.2$), with the exception of a small increase in fasting glucose following a single bout of SIT (73.6 ± 1.8 *vs*. 75.8 ± 1.3 mg/dL; $P = 0.01$). CONCLUSION: These data, collected using the gold standard hyperinsulinemic euglycemic clamp technique, suggest that short-term SIT is a viable alternative to endurance training as a strategy to improve insulin sensitivity.

INTRODUCTION

Internationally, humans are experiencing life threatening diseases related to lifestyle behaviors. A lack of physical activity and excessive eating habits are the driving forces behind these health problems. Rapid increases in cardiovascular and metabolic disease morbidity and mortality are the primary concerns associated with these behaviors (36). Metabolic syndrome (MetS) is the combination of six risk factors leading to cardiovascular disease (CVD) and metabolic impairments in humans (55).

Insulin resistance associated with MetS significantly increases an individual's risk of developing type 2 diabetes mellitus (T2D) (14, 55, 92, 109, 122). Weight loss and exercise have been shown to improve insulin resistance and attenuate complications associated with T2D (14, 92, 109, 122).

There is considerable research focused on ways to treat and prevent insulin resistance and T2D with various types of exercise. Habitual endurance exercise has been repeatedly shown to increase insulin sensitivity, the principal determinant of blood glucose control (23). Many adults however, choose not to participate in endurance training, citing insufficient time as the perceived obstacle (13, 120). Research comparing

low volume, high intensity sprint interval training (SIT) to high volume, low-moderate intensity endurance training has established similar physiologic adaptations, despite an extreme disparity in the intensity and volume of exertion (20, 21, 96, 114).

The implementation of SIT to induce physiologic improvements similar to those seen following endurance training is a novel research idea. A recent study has described improved blood glucose control post-consumption of a glucose beverage following 2-weeks of SIT, implying that SIT may increase insulin sensitivity (7). The aforementioned study does not directly measure insulin-stimulated glucose uptake following a short-term SIT intervention. It is necessary to establish if short-term SIT will augment glucose uptake via increases in insulin sensitivity. To date, no studies have used the hyperinsulinemic euglycemic clamp procedure to measure changes in insulin sensitivity following SIT.

Hypothesis

Two weeks of high-intensity sprint-interval training will increase insulin sensitivity in young adult humans.

Specific Aims

1) To compare insulin sensitivity, prior to and 72 hours following completion of short-term sprint interval training using the hyperinsulinemic euglycemic clamp technique.
2) To quantify the effect of a single bout of sprint interval training on insulin sensitivity.
3) To determine day-to-day variability in insulin sensitivity following a period of inactivity.

METHODS

Subjects and Experimental Design

Twelve healthy, sedentary or recreationally active adults were recruited (4 men and 8 women) (**Table 1**). None of the subjects were engaged in structured exercise at time of recruitment. At time of recruitment no subjects used tobacco or had any overt diseases. Subjects completed an insulin sensitivity test prior to and following short-term (2 weeks) high intensity SIT. Insulin sensitivity was determined using a hyperinsulinemic euglycemic clamp. This procedure consisted of determining the glucose infusion rate (GIR) needed to maintain a blood glucose concentration of 90mg/dL during a constant intravenous infusion of insulin. After routine medical and fitness screening, the subjects were informed of the procedures to be employed in the study and their associated risks. All subjects provided written, informed consent for the experimental procedures. The experimental protocol was approved by the Colorado State University Institutional Review Board.

Pre-experimental procedures

Prior to baseline measurements, all subjects underwent physical fitness and anthropometric assessment. Subjects' first visit included: 1) Maximal rate of oxygen uptake ($VO_{2\ max}$); 2) dual energy x-ray absorptiometry (DEXA); 3) anthropometric measurements (body mass index and waist-hip ratio). 1) $VO_{2\ max}$ was determined via an incremental exercise test to exhaustion on a treadmill (Quinton Q65 Series 90) or cycle ergometer (Lode Excalibur, Groningen Netherlands) test. Subjects were outfitted with a standard blood pressure cuff, Polar® heart rate monitor, and a Hans-Rudolph® (St.

Louis, MO) two-way non-rebreathing mouthpiece, valve, and headgear apparatus. The cycle ergometer maximal test consisted of 20-30 Watts·min^{-1} continuous ramp protocol. The test was terminated when pedal cadence dropped below 40 rpm. The treadmill maximal test consisted of a constant selected speed by the subject and increases in grade of 2% every 2 minutes. At the point of volitional fatigue, the test was terminated. Heart rate and blood pressure were measured during rest in supine, sitting and standing positions and every two minutes throughout the maximal test. During the exercise test, gas composition of expired gases was analyzed using a metabolic cart (Parvo TrueOne 2400 Metabolic Measurement System, Sandy, UT; gas- and flow-calibrated daily) and breath-by-breath ventilation (L/min) was established using a pneumotachometer (Hans Rudolph® Model 3813). Metabolic measurement software assessed absolute (L/min) and relative (mL/kg/min) VO_{2max} and maximal respiratory exchange ratio (RER_{max}). 2) Total fat mass, bone mineral composition and fat free mass was analyzed using DEXA (DEXA; Hologic Discovery-W™ with QDR™ for Windows software, Bedford, MA). 3) Height, weight, waist and hip circumference of each subject in the standing position was documented. The average of three waist measurements made at the umbilicus with the subject standing relaxed, during normal respiration, was determined. The average of three hip measurements was determined as measured where girth is the largest over the buttocks.

Experimental Protocol

The experimental protocol consisted of *1*) baseline testing; *2*) a 2-week SIT intervention; and *3*) post testing, as described further below (**Fig. 1**).

Sprint Interval Training. The two week SIT protocol was executed as previously described by Gibala et al (22). Short-term SIT consisted of six sessions spread out over 14 days. Each training session consisted of 4-7 repeated 30-s "all-out" efforts on a Monark® cycle ergometer (Monark Ergomedic 874 E, Monark, Sweden) against a resistance equivalent to 0.075 kg·kg body mass^{-1} (i.e., a Wingate test). Immediately preceding each repetition, subjects were instructed to pedal $\geq$ 110 rpm against the ergometer's inertial resistance. Resistance equivalent to 7.5%of body mass (kg) was then applied to the cycle ergometer fly wheel and the subject pedaled as rapid as possible for 30 seconds. Between each repetition, the subjects remained on the bike for 4 minutes recovery, and either rested or cycled against the ergometer's inertial resistance at a low cadence (50 rpm), to prevent venous pooling and its associated nausea and light-headedness. Peak power, mean power and fatigue index for each repetition were determined using an online data acquisition system (Wingate software version 1.11, Lode). The six SIT sessions were separated by 1-2 days rest in between (e.g. Monday, Wednesday and Friday for 2 weeks). Training progression was accounted for by increasing the number of repetitions from four to seven over the first five sessions. The last session consisted of four repetitions for comparison to the first session.

Hyperinsulinemic Euglycemic Clamp. The hyperinsulinemic euglycemic clamp technique as described previously (28) was performed on all subjects at baseline and 72 hours following the last bout of exercise. Subjects refrained from any alcohol consumption 24 hours prior to the experiment and any exercise 72 hours prior to the experiment. All subjects reported to Colorado State University's Human Performance and Clinical Research Laboratory in the morning, following an overnight fast. For

infusion of glucose and insulin (Harvard Pumps, Harvard Apparatus, Holliston, MA) a cannula was inserted in a forearm vein at the antecubital fossae. The opposite lower arm was wrapped in a heating pad and heated throughout the procedure and a dorsal hand vein was cannulated for arterialized blood sampling. A primed, continuous infusion of insulin was administered during the hyperinsulinemic euglycemic clamp. The priming dose, starting at a rate of 127.6 $mU \cdot m^2$ body surface area$\cdot min^{-1}$ and lowering to a fixed rate of 40.0 $mU \cdot m^2$ body surface area, was administered to begin the procedure. Initial glucose infusion at time point 4 minutes was set at 2.0 $mg \cdot kg^{-1} \cdot min^{-1}$. Throughout the remainder of the procedure (170 minutes), insulin was continuously infused at a rate of 40 $mU \cdot m^{-2} \cdot min^{-1}$, because endogenous glucose release during this level of insulinemia is insignificant (100). Blood glucose concentration was measured every 5 minutes after the start of continuous insulin infusion (YSI 2300 Stat Plus, Yellow Springs, OH). The rate of a 20% dextrose (Baxter Corporation, Deerfield, IL) infusion was adjusted based on the negative feedback principle to maintain a plasma glucose concentration of 90mg/dL. The time interval from 150-180 minutes was considered "steady-state" and the average glucose infusion rate ($mg \cdot kg^{-1} \cdot min^{-1}$) during this period was calculated as an expression of whole-body insulin-stimulated glucose uptake (ISGU). Steady-state was achieved when fluctuations in blood glucose concentration were no more than 5% between blood draws.

Metabolic Flexibility. Indirect calorimetry was utilized to determine the subjects' ability to adjust the level of lipid and carbohydrate oxidation necessary to meet metabolic demand based on fuel availability. Metabolic flexibility is determined as the magnitude of increase in respiratory exchange ratio measured at baseline and during the last 5-

minutes of the hyperinsulinemic euglycemic clamp. Average rate of oxygen consumption and rate of carbon dioxide production were determined via respiratory mass spectrometry equipment and software (MA Tech Services MGA 1100, St. Louis, MO). Averages were calculated from respired gases collected for 5-8 minutes at baseline and during the last 5-8 minutes of the steady-state period of the procedure.

Dietary and Physical Activity Controls

Subjects were asked to maintain diet and refrain from any outside, structured physical activity aside from daily living.

Control Groups

Sixteen subjects were recruited to identify the effects of a period of inactivity and of a single bout of exercise on insulin sensitivity. All subjects participated in the previously described screening and pre-experimental procedures.

To demonstrate that changes in insulin sensitivity were a result of the short term SIT training program, and not because of the acute effects of the final bout of SIT, seven subjects participated in a baseline hyperinsulinemic euglycemic clamp and a hyperinsulinemic euglycemic clamp 72 hours following a single bout (4 repetitions) of SIT (**Fig. 1**).

To ascertain that it was the short term SIT training that changed insulin sensitivity, and not day-to-day variability in insulin sensitivity, nine subjects participated in a baseline hyperinsulinemic euglycemic clamp and a hyperinsulinemic euglycemic clamp following two weeks of inactivity (**Fig. 1**).

Statistics

All data were analyzed using Statistica version 4.1 for Macintosh. Data across all groups were averaged and presented as mean ± SE. Analysis of variance (ANOVA) with repeated measures was used to determine any changes from baseline to post intervention within groups. If a significant value was determined, Neuman-Keuls post hoc analysis was used to determine where significance existed.

RESULTS

Subject Characteristics

Sprint interval training, single bout and sedentary participants were young, slightly overweight and relatively unfit (SIT: 29 ± 3 years, 26.2 ± 1.3 $kg \cdot m^{-2}$, 32.7 ± 2.1 ml $O_2 \cdot kg^{-1} \cdot min^{-1}$, Single bout: 25 ± 3, 25.3 ± 1.3, 37.1 ± 4.3, Sedentary: 23 ± 2, 26.8 ± 1.6, 36.0 ± 3.3) (**Table 1**).

Short-Term SIT Improves Whole-Body Insulin Stimulated Glucose Uptake

Compared with baseline, two weeks of SIT augmented insulin sensitivity as demonstrated by an increase in the glucose infusion rate necessary to maintain plasma glucose levels ~90 mg/dL during a steady state of hyperinsulinemia (GIR: 6.3 ± 0.6 *vs.* 8.0 ± 0.8 $mg \cdot kg^{-1} \cdot min^{-1}$; $P = 0.02$) (**Fig. 2**). This enhancement was not seen following a single bout of SIT (9.6 ± 1.1 *vs.* 10.3 ± 1.1; $P = 0.52$) or a period of inactivity (7.9 ± 0.9 *vs.* 8.3 ± 1.0; $P = 0.38$).

Blood Glucose Concentrations

Fasting blood glucose was not altered by SIT (80.0 ± 2.6 *vs.* 80.0 ± 2.6 mg/dL; *P* = 0.99) or by a period of inactivity (73.4 ± 1.5 *vs.* 75.1 ± 1.1; *P* = 0.87) (**Fig. 3**). Fasting blood glucose did however slightly increase in the single bout group (73.4 ± 1.5 *vs.* 75.1 ± 1.1; *P* = 0.03). Plasma glucose concentration during the final 30-minutes of the hyperinsulinemic euglycemic clamp was unaffected by any intervention and was not different between groups (**Fig. 4**; P>0.05).

Body Mass

Improvements in SIT-induced insulin sensitivity were seen without concomitant changes in body mass (75.9 ± 5.9 *vs.* 75.7 ± 5.5 kg; *P* = 0.62) (**Fig. 5**). Similarly, neither a single bout of SIT (69.2 ± 4.6 *vs.* 68.8 ± 4.6; *P* = 0.23) nor a period of inactivity (75.7 ± 5.0 *vs.* 75.7 ± 5.0; *P* = 0.98) elicited any changes in body mass.

Metabolic Flexibility

Metabolic flexibility was determined by comparing the change in respiratory exchange ratio from baseline to steady state during the baseline and post SIT hyperinsulinemic euglycemic clamps. Comparing baseline to post intervention clamps, there was no significant difference in the change in metabolic flexibility from resting to steady state hyperinsulinemia euglycemia following any of the three interventions (BASELINE: SIT; 0.80 ± 0.03 *vs* 0.95 ± 0.03 VCO_2/VO_2, Single bout; 0.77 ± 0.02 *vs* 0.97 ± 0.03, Sedentary; 0.82 ± 0.03 *vs* 0.93 ± 0.04, POST INTERVENTION: SIT; 0.77 ±

0.03 *vs* 0.96 ± 0.05, Single bout; 0.82 ± 0.01 *vs* 0.96 ± 0.03, Sedentary; 0.82 ± 0.02 *vs* 0.96 ± 0.02) (**Fig. 6**).

DISCUSSION

The major finding from the present study is that two weeks of SIT increased insulin sensitivity as demonstrated by an increase in the glucose infusion rate necessary to maintain a blood glucose concentration of 90 mg/dL during a constant administration of insulin (hyperinsulinemic euglycemic clamp). There were no significant day-to-day fluctuations in insulin sensitivity as demonstrated by the absence of a change in the glucose infusion rate during repeated hyperinsulinemic euglycemic clamps performed on sedentary individuals. Insulin sensitivity was unaffected in individuals performing a single bout of SIT, suggesting that the augmented insulin sensitivity observed with SIT was a training effect and not simply due to the acute effects of the final bout. All insulin sensitivity tests were performed 72 hours following the final SIT session or the single bout of SIT. This delay was necessary because of the established insulin-sensitizing effects of an acute bout of exercise, that persist ~48 hours (43, 135).

The effectiveness of short-term and chronic endurance exercise in augmenting insulin sensitivity is well established (62). However, to date, only one other study has considered the impact of SIT on whole body glucose control. In this study, responses to an oral glucose tolerance test (OGTT) prior to and following two weeks of SIT were reported (7). The improved clearance of glucose following oral glucose consumption in this study could be attributed to differences in glucose absorption by the gut, increased insulin release and/or insulin sensitivity. In the present study we have utilized the

hyperinsulinemic euglycemic clamp to directly determine insulin sensitivity. This technique is commonly recognized as the "gold standard" measurement of insulin sensitivity. The hyperinsulinemic euglycemic clamp was established in 1979 has been since used as the most accurate and direct measure of whole-body insulin sensitivity (28, 98). OGTT's are the most widely used measurement of insulin action because of the simplicity of their administration. However, numerous studies have identified confounding results with the use of OGTT's (98). In addition to opposing data, there are multiple indices used to calculate insulin action based on the data from a single OGTT and no "standard" index has been established for universal use (98). Following a six week endurance training program, no change in plasma glucose concentrations at any time point following a 75 gram oral glucose load compared to baseline were reported, while significant improvements in insulin-stimulated glucose uptake was observed during hyperinsulinemic euglycemic clamps (29). Another study noted extreme within subject variability using numerous OGTT's indices of insulin action (130). While time and labor intensive, hyperinsulinemic euglycemic clamps are the best way to directly measure insulin-stimulated whole-body glucose uptake during "steady state". Unlike in an OGTT, it is established that at "steady state" during a hyperinsulinemic euglycemic clamp, hepatic glucose production and pancreatic insulin production does not occur in healthy individuals (98). The absence of these variables allows for direct manipulation of insulin and plasma glucose concentrations and thus, determination of tissue-level insulin sensitivity. Our study was the first to concomitantly examine and contrast the effects of short-term SIT on insulin sensitivity, day-to-day variability in insulin sensitivity and the effects of a single bout of SIT on insulin action.

Sprint interval training is a time-efficient exercise intervention that produces appreciable increases in insulin sensitivity over a very short period of exercise, that is, only 16 minutes of cumulative exercise over two weeks. The majority of studies looking at changes in insulin sensitivity resulting from exercise have examined the effects of extended periods of endurance training, lasting anywhere from six weeks to as long as three years (12, 29, 35, 81, 102). The design of these long-term endurance training studies fails to establish whether or not insulin sensitivity can be augmented following only a few sessions of exercise. However, improvements in insulin sensitivity and its associated components have been observed following as little as one to two weeks of endurance exercise interventions (101, 121). Although insulin sensitivity increased in these short-term studies, these changes did not occur until as many as 5-10 hours·week^{-1} had been committed to the training regimen. This time commitment is considered by many as unattainable, as demonstrated by the poor adherence of insulin resistant individuals to endurance exercise programs (13, 120). The considerably lower time obligation needed to attain similar increases in insulin sensitivity as experienced with endurance exercise makes SIT a viable option for clinical treatment of impaired glucose regulation. The proposal of SIT as an intervention is strengthened by the fact that no subjects in this study were unable to complete the six sessions of SIT.

Improvements in insulin sensitivity are typically associated with concomitant weight loss (3). However, improvements in glucose homeostasis in the absence of weight loss have occurred following a long-term endurance training intervention (12). Our study is the first to establish improvements in insulin sensitivity in the absence of weight loss following short-term SIT as the modality of exercise. This observation

allows the elimination of weight fluctuation as one of the possible mechanisms behind SIT-induced decreases in insulin resistance.

Multiple studies have identified additional similar metabolic adaptations following two weeks of SIT compared to two weeks of traditional endurance training (21, 49). Many of the previously observed SIT-induced changes have occurred in variables that are closely related to changes in insulin sensitivity. As mentioned earlier, mitochondrial dysfunction and impaired oxidative capacity may be the underlying complication leading to and advancing insulin resistance and T2D. Mitochondrial biogenesis transcriptional co activator, PGC-1α, significantly increased following two weeks of SIT (21, 50). Mitochondrial enzymes, AMPK and p38MAPK, upstream of PGC-1α also increased following short-term SIT (50). Short-term SIT increased muscle oxidative capacity as demonstrated by increases in the maximal activity of cytochrome c oxidase, citrate synthase and β-HAD (20-22, 49). Increases in number of mitochondria and improved oxidative capacity improve the efficiency of substrate metabolism and can attenuate complications underlying insulin resistance. In our study, respiratory exchange ratio did not vary from the baseline hyperinsulinemic euglycemic clamp. This indicates that the improvement in insulin-stimulated glucose uptake was not a result of shift in substrate utilization from lipid to carbohydrate, but from an increase in tissue level insulin sensitivity. Changes in metabolic flexibility following short-term SIT can be examined in future studies by using tracers to determine whether glucose is being oxidized or stored during hyperinsulinemic euglycemic clamps. Additional research will likely clarify a combination of the aforementioned variables as the driving force behind SIT-induced improvements in insulin sensitivity.

Further examination of muscle biopsy tissue and/or blood plasma from the subjects in this study may reveal other possible explanations for the improvement in insulin sensitivity following short-term SIT. Changes in adipokines, leptin and adiponectin, have been shown to elicit changes in insulin sensitivity possibly by increasing rates of fatty acid oxidation which lead to a decrease in intramuscular triglyceride content and improved insulin sensitivity (38). Muscle biopsy data may reveal changes in the number and size of mitochondria and/or mitochondrial enzymes and transcription factors as the reason for improved insulin sensitivity.

A limitation to the applicability of these findings is the fact that SIT is a very difficult mode of exercise that may require supervision and/or coaching. Future research will determine if subjects will be able to complete a SIT exercise program without external administration. Although extremely labor intensive, all overweight, obese and unfit adults in this study were able to complete the short-term SIT intervention and thus improve insulin sensitivity. On average, our subjects were able to complete 94% of all SIT bouts over the two week intervention. Future long-term SIT studies will determine if fatigue, overtraining or lack of energy balance would alter the completion rates for a SIT exercise intervention that were observed in our study. The biomechanics of sprint interval training make it less stressful to the joints of obese persons than walking or running (44). Because of this, SIT, although extremely physically demanding, may be easier than endurance exercise for people with joint complications.

Sprint interval training should be considered as an effective treatment for insulin resistance. Given the history of poor adherence to endurance training interventions, SIT provides a reasonably time-efficient and attainable treatment to enhance insulin

sensitivity. Future research is needed to examine the success of SIT in a clinical setting. A short-term SIT intervention for T2D patients is necessary to ensure that similar improvements will occur in individuals who are extremely insulin resistant and to also establish that they will adhere to the intervention. A long-term SIT study will establish to what extent insulin sensitivity can be amplified and at what point the improvements plateau. Unfortunately the commonly used lifestyle interventions and pharmacological treatments for insulin resistance are not extremely effective on a long-term basis. Independently, or in combination with other treatments, sprint interval training can serve as an efficient treatment for patients suffering from insulin resistance and T2D.

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TABLE 1. Subject characteristics

	SIT	Single Bout	Sedentary Control
Sex	5 ♂, 7 ♀	1♂, 7 ♀	1 ♂, 8 ♀
Age, yr	29 ± 3	25 ± 3	23 ± 2
Mass, kg	75.8 ± 5.8	69.4± 4.6	75.4 ± 5.1
Height, cm	169 ± 3	165 ± 4	168 ± 3
Body Mass Index, kg/m^2	26.2 ± 1.3	25.3 ± 1.3	26.8 ± 1.6
Waist-to-hip ratio, waist cm/hip cm	0.87 ± 0.03	0.81 ± 0.03	0.88 ± 0.05
% Body Fat	29.6 ± 1.8	29.3 ± 2.7	31 ± 2.8
$VO_{2\ max}$, $ml \cdot kg^{-1} \cdot min^{-1}$	32.7 ± 2.1	37.1 ± 4.3	36.0 ± 3.3

Values are means ± SE for all subjects. $VO_{2\ max}$, maximal rate of oxygen consumption. RER_{max}, maximal respiratory exchange ratio.

FIGURE LEGENDS

Figure 1. Overview of study design. PRE, pre intervention. POST, post intervention. SIT, sprint interval training. (Figure format mimics short-term SIT figure created by Burgomaster et al. (22)).

Figure 2. Greater rate of intravenous glucose administration (P=0.02) required to maintain a circulating blood glucose concentration of ~ 90 mg/dL during final 30 minutes of hyperinsulinemic euglycemic clamp following two weeks of SIT. No change in rate of intravenous glucose administration ($P=0.43$) following a single bout of SIT. No change in rate of intravenous glucose administration ($P=0.38$) following two weeks of inactivity. Data: mean ± SE.

Figure 3. No change in fasting plasma glucose following short-term SIT or two weeks of inactivity. A slight increase in fasting plasma glucose following a single bout of SIT (P= 0.02). Data: mean ± SE.

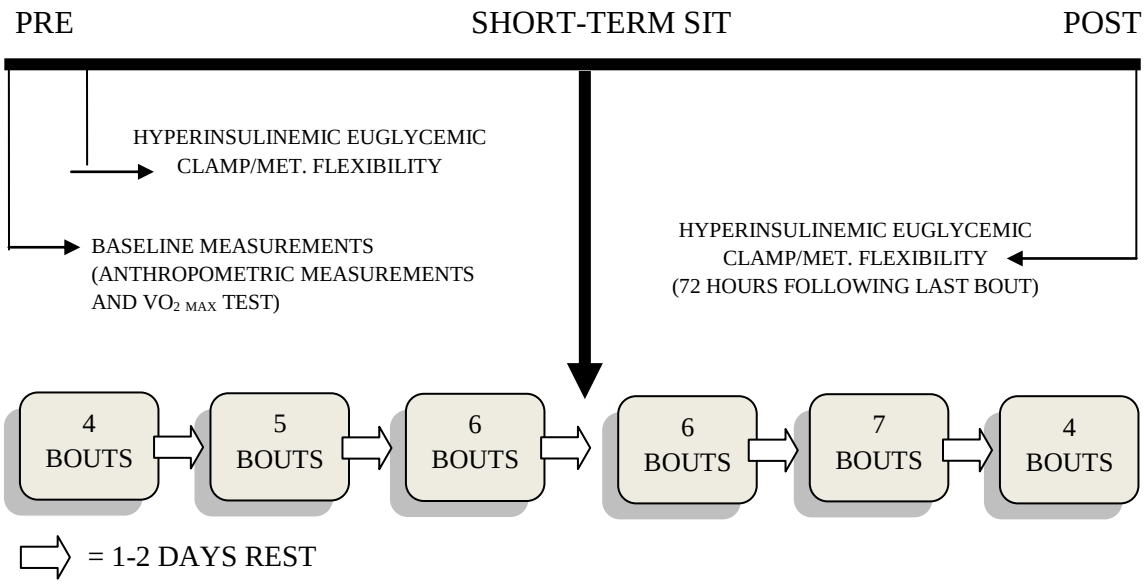
Figure 4. No change in plasma glucose at the conclusion of the clamp following short-term SIT, a single bout of SIT or two weeks of inactivity. Data: mean ± SE.

Figure 5. No change in body mass following short-term SIT, a single bout of SIT or two weeks of inactivity. Data: mean ± SE.

Figure 6. No change in metabolic flexibility following short-term SIT, a single bout of SIT or two weeks of inactivity, as shown by the lack of difference in delta respiratory exchange ratio from baseline to post intervention hyperinsulinemic euglycemic clamps. Data: mean ± SE.

FIGURE 1

SIT PROTOCOL



SINGLE SESSION/SEDENTARY CONTROL PROTOCOL

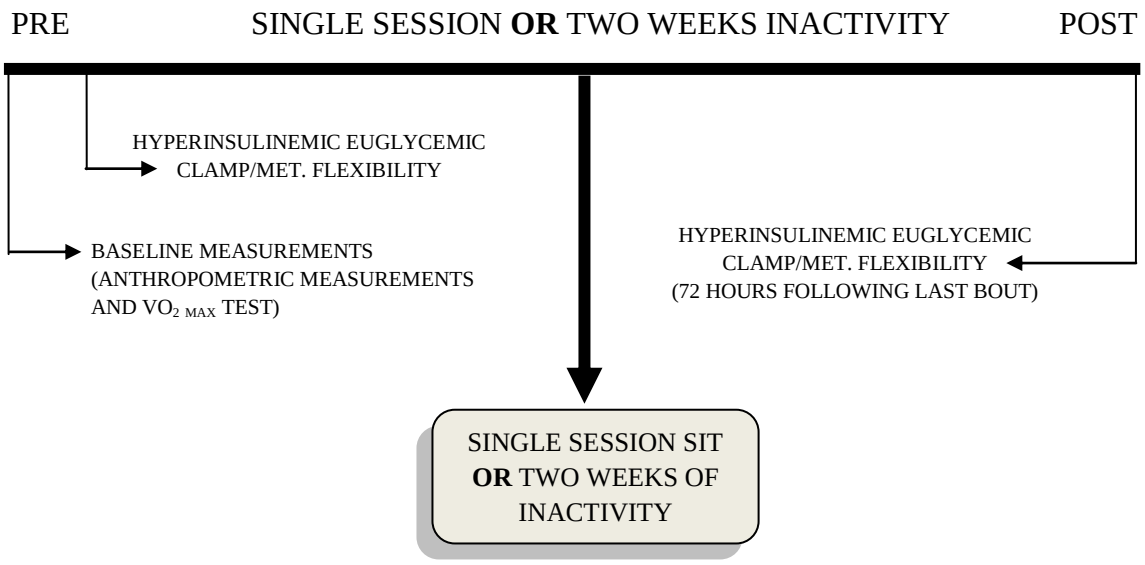


FIGURE 2

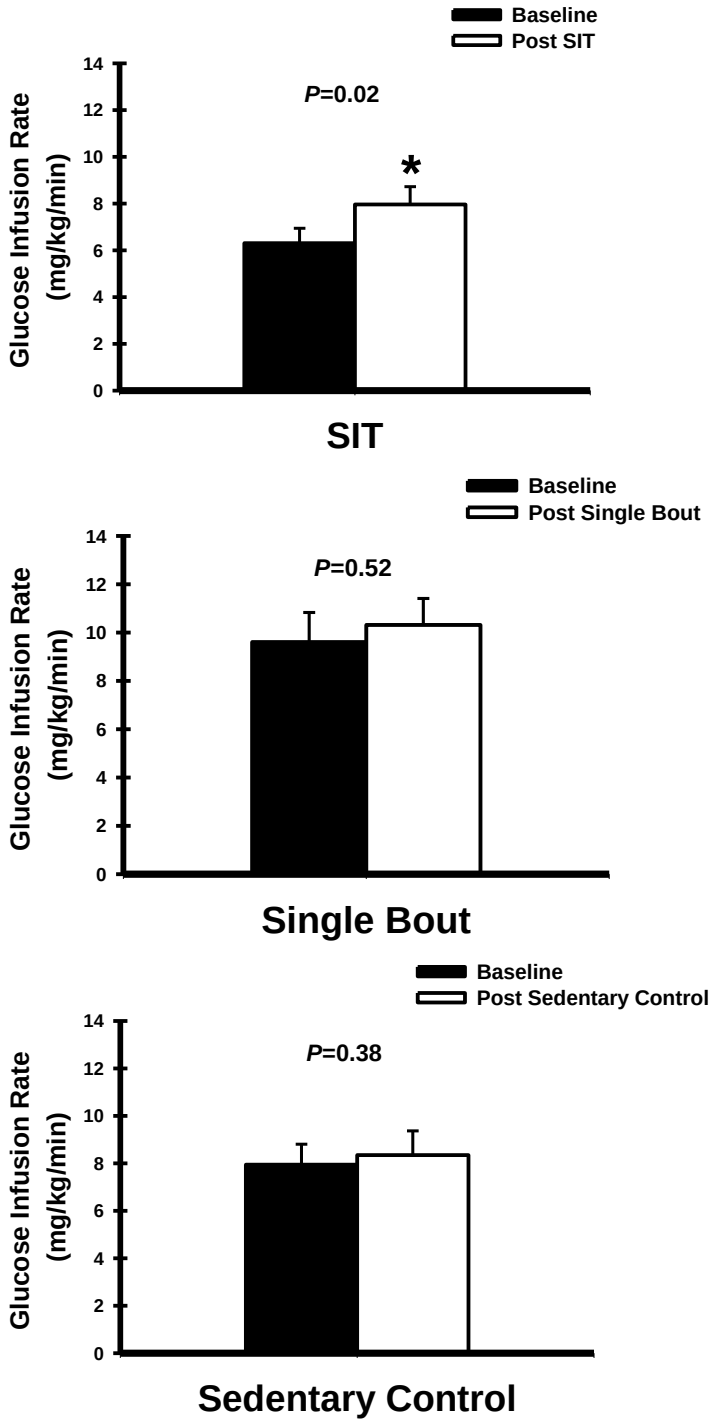

Baseline
Post SIT
P=0.02
*
Glucose Infusion Rate (mg/kg/min)
0
2
4
6
8
10
12
14
SIT
Baseline
Post Single Bout
P=0.52
Glucose Infusion Rate (mg/kg/min)
Single Bout
Baseline
Post Sedentary Control
P=0.38
Glucose Infusion Rate (mg/kg/min)
Sedentary Control

FIGURE 3

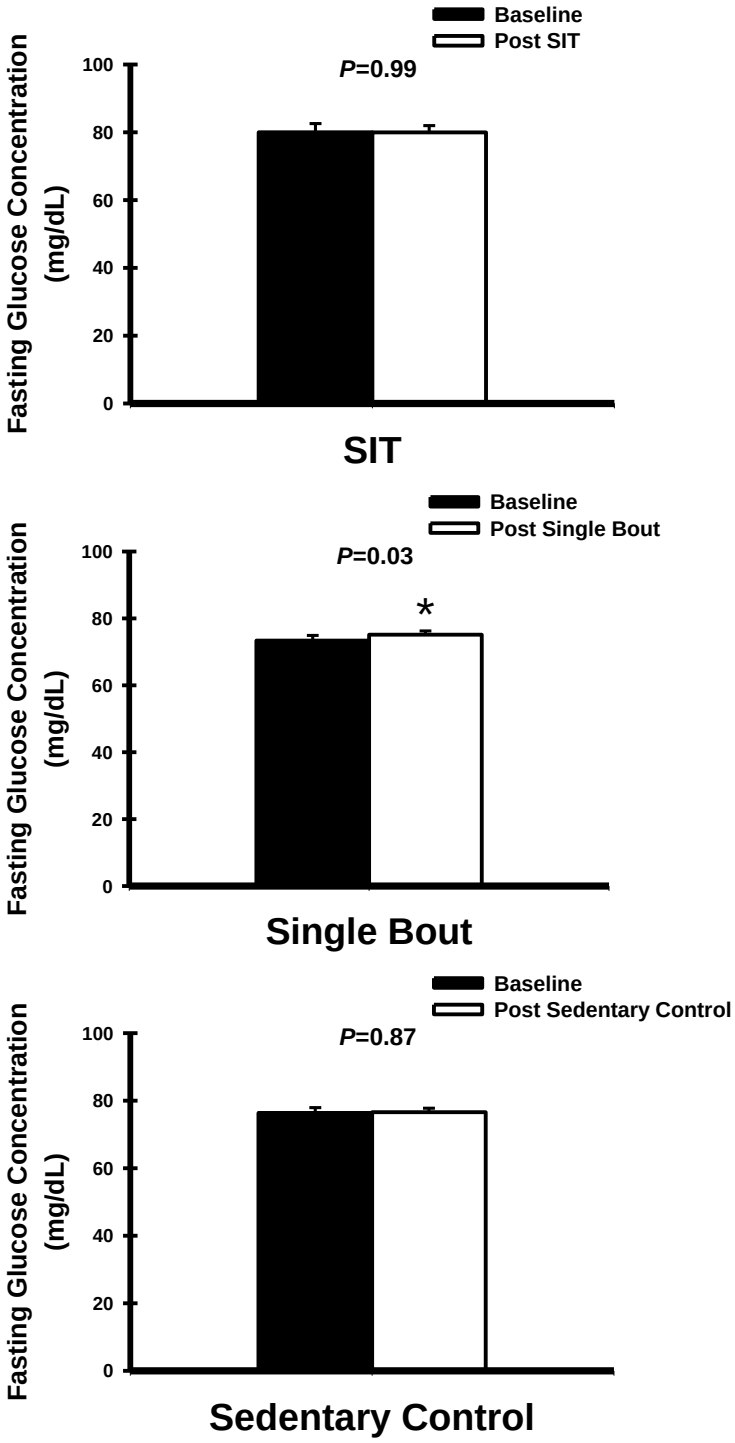

Baseline
Post SIT
P=0.99
Fasting Glucose Concentration (mg/dL)
100
80
60
40
20
0
SIT
Baseline
Post Single Bout
P=0.03
*
Fasting Glucose Concentration (mg/dL)
Single Bout
Baseline
Post Sedentary Control
P=0.87
Fasting Glucose Concentration (mg/dL)
Sedentary Control

FIGURE 4

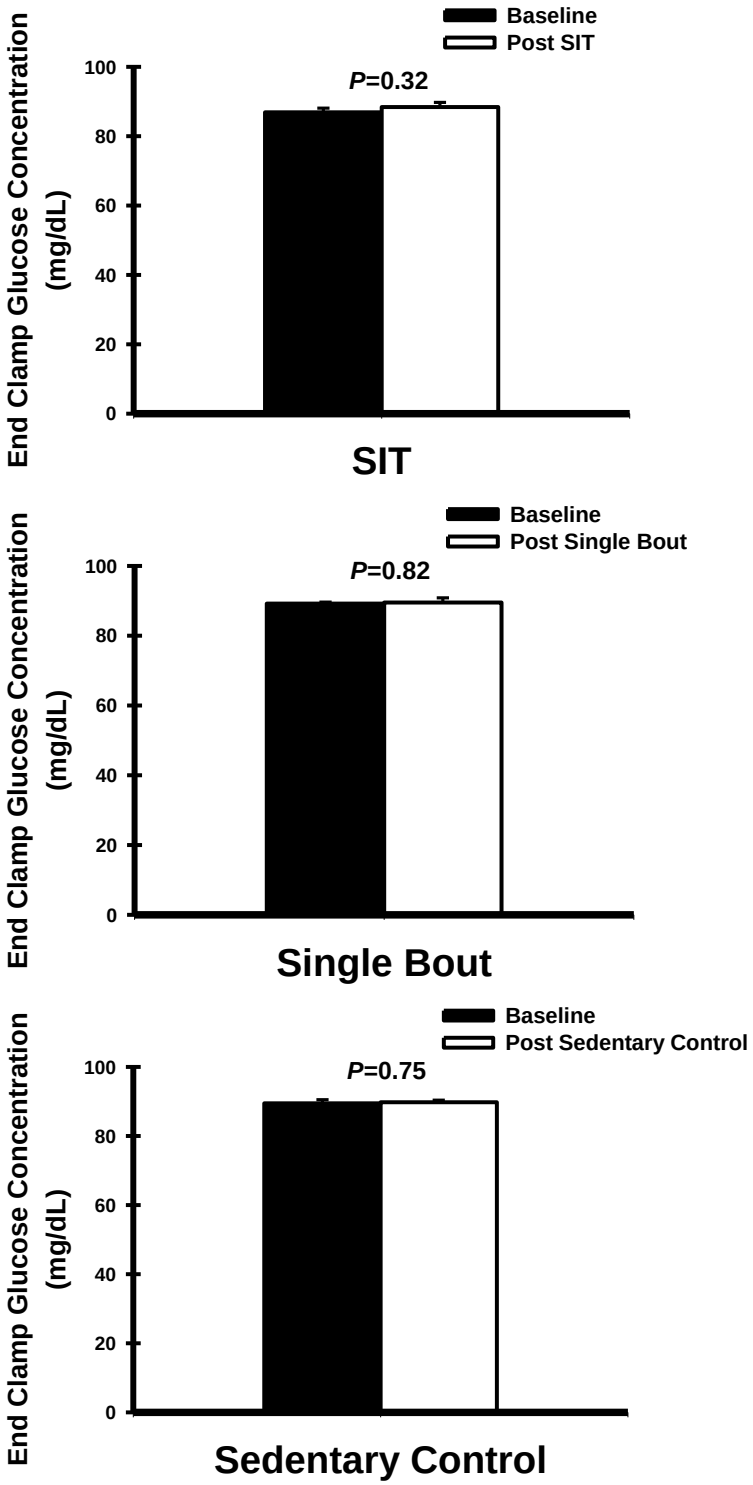

Baseline
Post SIT
P=0.32
End Clamp Glucose Concentration (mg/dL)
100
80
60
40
20
0
SIT
Baseline
Post Single Bout
P=0.82
End Clamp Glucose Concentration (mg/dL)
100
80
60
40
20
0
Single Bout
Baseline
Post Sedentary Control
P=0.75
End Clamp Glucose Concentration (mg/dL)
100
80
60
40
20
0
Sedentary Control

FIGURE 5

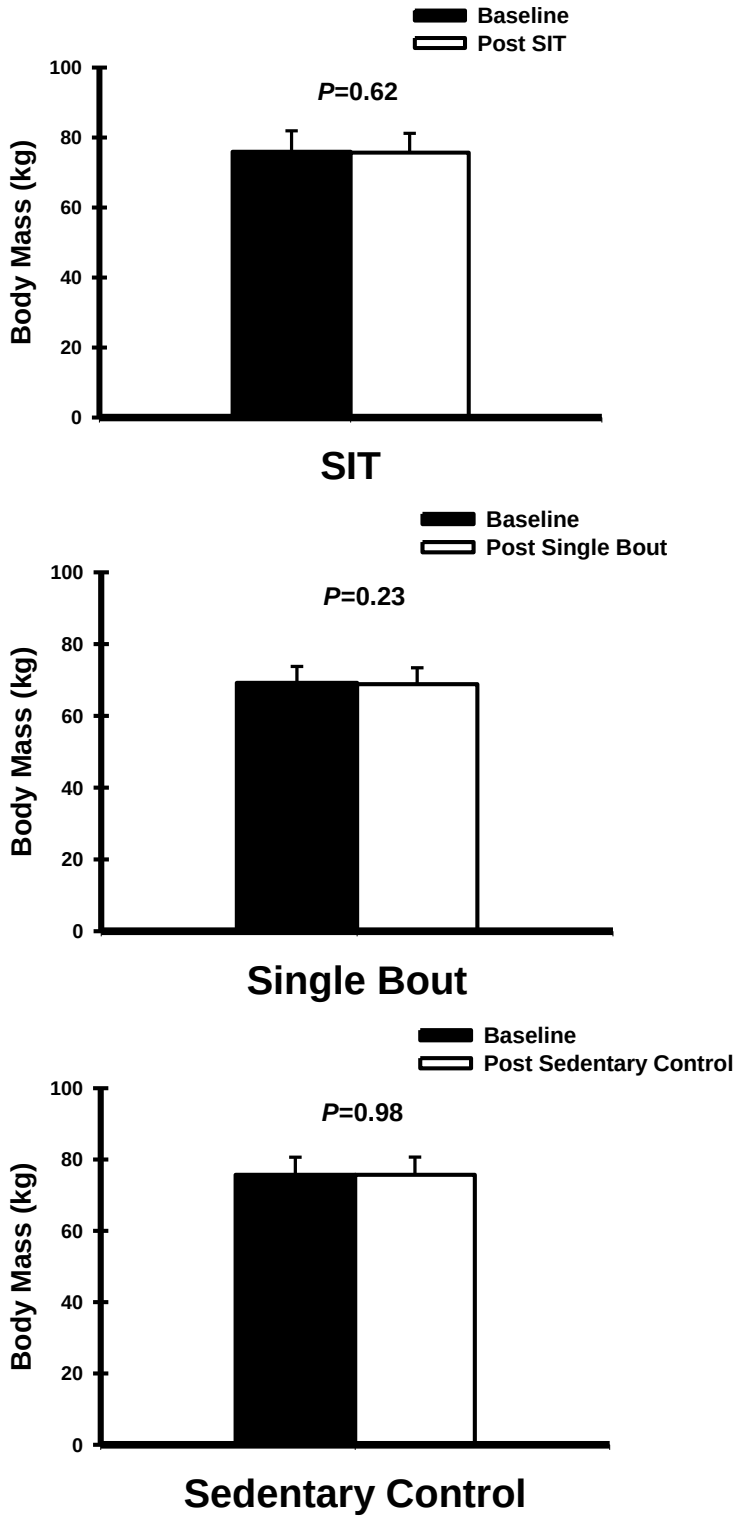
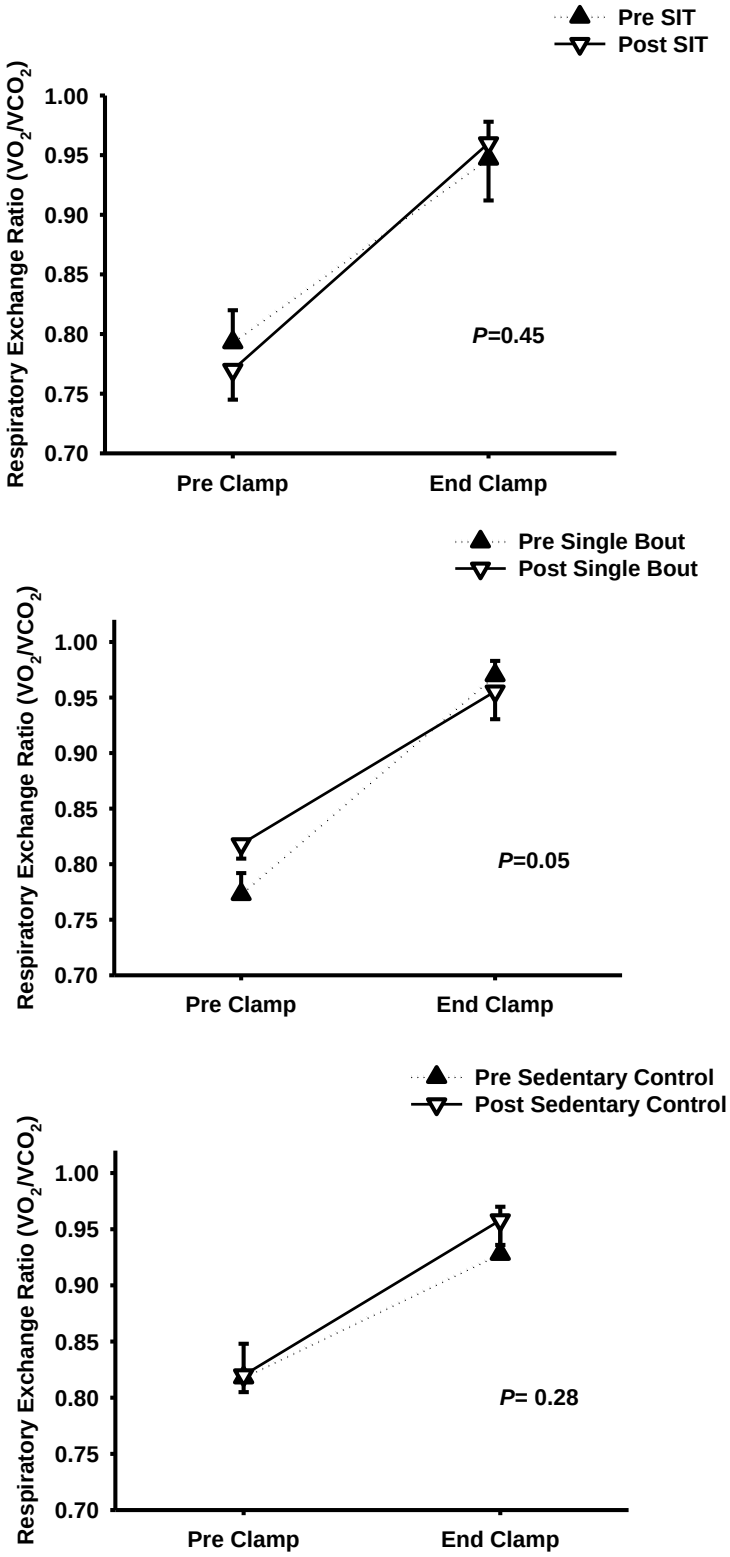

Baseline
Post SIT
P=0.62
Body Mass (kg)
100
80
60
40
20
0
SIT
Baseline
Post Single Bout
P=0.23
Body Mass (kg)
100
80
60
40
20
0
Single Bout
Baseline
Post Sedentary Control
P=0.98
Body Mass (kg)
100
80
60
40
20
0
Sedentary Control

FIGURE 6


Pre SIT
Post SIT
Respiratory Exchange Ratio (VO_2/VCO_2)
1.00
0.95
0.90
0.85
0.80
0.75
0.70
P=0.45
Pre Clamp
End Clamp
Pre Single Bout
Post Single Bout
Respiratory Exchange Ratio (VO_2/VCO_2)
1.00
0.95
0.90
0.85
0.80
0.75
0.70
P=0.05
Pre Clamp
End Clamp
Pre Sedentary Control
Post Sedentary Control
Respiratory Exchange Ratio (VO_2/VCO_2)
1.00
0.95
0.90
0.85
0.80
0.75
0.70
P= 0.28
Pre Clamp
End Clamp

APPENDIX A

Consent to Participate in a Research Study

Colorado State University

TITLE OF STUDY: Sprint Interval Training Increases Beta-Adrenergic Receptor Metabolic Responsiveness and Insulin Sensitivity

PRINCIPAL INVESTIGATOR: *Christopher Bell, Ph.D.*

Dept. of Health & Exercise Science

Colorado State University

Telephone: 970 491 7522

Email: cbell@cahs.colostate.edu

WHY AM I BEING INVITED TO TAKE PART IN THIS RESEARCH? *You are an adult man or woman aged between 18 and 79 years. You do not smoke. You are not pregnant.*

WHO IS DOING THE STUDY? *Christopher Bell, Ph.D., an assistant professor in the Department of Health and Exercise Science at Colorado State University will perform this research. He is being helped by trained graduate and under-graduate students and also by a medical doctor, Wyatt Voyles, M.D.*

WHAT IS THE PURPOSE OF THIS STUDY? *The purpose of the study is to answer the following questions: 1) Does sprint interval training improve the way in which the nervous system helps the body to burn calories? 2) Does sprint interval training improve how the body handles sugar?*

WHERE IS THE STUDY GOING TO TAKE PLACE AND HOW LONG WILL IT LAST? *All of the procedures (unless otherwise stated) will take place in the Human Performance Clinical Research laboratory (HPCRL) in the Department of Health & Exercise Science (Moby Complex). All of the procedures involving drug infusions will be supervised by a medical doctor. The HPCRL has an automated defibrillator with built in transcutaneous pacing and a "crash-cart" stocked with oxygen and emergency medications.*

WHAT WILL I BE ASKED TO DO? *Briefly, you will be asked to participate in a series of procedures before and after a vigorous program of exercise training, or a period of time (2 weeks) during which you will not make any significant changes to your lifestyle. An overview is attached that provides a time-table of the order of events. Below is a detailed description of all of the procedures; a member of the research team will fully explain each procedure and its duration.*

Exercise Stress Test

If you are a man older than 35, or a woman older than 40 years an exercise stress test will be performed on your first visit to the laboratory. 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. Wyatt Voyles, 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. 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.

Exhausting Exercise Test (or VO_{2max} test)

This test will tell us how fit you are and is very similar to the treadmill stress test. You will be asked to ride an exercise bike, until you are too tired to continue. It will become more and more difficult to push the pedals around. While you are riding we will measure your heart rate with an electrocardiogram (ECG). We will ask you to wear a nose clip (something that stops you breathing through your nose) and ask you to breathe through a mouthpiece. This will let us measure the gases you breathe in and out. These tests last roughly 1 hour.

Pregnancy Test

If you are female you will be required to have a sample of your urine tested for the presence of human chronic gonadotropin (HCG), a hormone that indicates whether you may be pregnant. This will require approximately 1 cup of your urine. If you are pregnant or the test indicates that you are pregnant you will not be able to participate in this study. (~10 minutes)

Blood Pressure

We will measure your blood pressure using a standard blood pressure cuff (the same as in a doctor's office). Blood pressure will be measured during all of the tests performed in the lab with the exception of body composition. There are no known risks associated with this procedure.

Body Composition

We will measure how much fat you have in your body using a test called dual energy x-ray absorptiometry (DEXA). The DEXA test requires you to lie quietly on a padded table while a small probe gives off low-level x-rays and sends them over your entire body. This test gives very accurate measurements of your body fat and bone mineral density. We will also measure the circumeference of your waist and hip using a tape measure. These tests last approximately 30 minutes; you will be asked to perform them on two occasions.

Calories Burnt at Rest

We will measure the amount of calories you burn at rest. This is called your resting metabolic rate. This test will take place while you are lying down on a bed. A plastic transparent bubble will be placed over your head and shoulders and room air will be pumped in through a pipe. We will measure the air that you breathe in and out. There are no known risks associated with this procedure. We will perform this test when injecting

drugs into your veins and during microneurography (see below). You will perform these tests twice; they last for approximately 4-hours.

Isoproterenol

Isoproterenol is a drug that will probably make your heart rate, blood pressure, and metabolic rate bigger. The drug will be injected into a vein in your arms or hands for roughly 90 minutes. The drug will be injected first in small amounts for 30 minutes, then in bigger amounts for the next 30 minutes, and in even bigger amounts for the final 30 minutes. During this time we will measure the calories you burn. We will also measure your heart rate and your blood pressure.

Microneurography

This test will measure the activity of your nerves while you rest on a bed. We will place a very thin needle just under the skin below your knee and record the electrical patterns of a nerve there. To help locate the area where we will need to place the needle, an electric stimulus will be used that may cause brief (1-2 seconds) feelings of "pins and needles" or a dull ache in your lower leg. When the needle is in the proper position, your foot will twitch by itself. The electric stimulus will then be turned off, and there will be no other feelings during the recording of your nerve activity. The stimulus could be used periodically for up to 1 hour, but not more than 1 hour.

Blood Collection

We will be taking blood from you on different days while you are taking part in our study. We will be taking less than the amount that is typically given when a person donates blood. Your blood will be tested for various things that are involved with your nerves, the amount of calories you burn at rest, and the drugs that we will be giving you. Your blood will be taken from veins in your arms or hands using needles and hollow plastic tubes called catheters.

Cutting Little Pieces of Muscle from Your Legs

This test is commonly called a muscle biopsy. During the muscle biopsy a drug (an anesthetic) will be injected into an area of your thigh to make it feel numb. A small incision (roughly 1/4 inch) will be made using a sharp sterile blade. A sterile probe will be inserted into your leg and a little piece of muscle (roughly the size of a sweet corn kernel) will be removed. Matthew Hickey, Ph.D., an Associate Professor in the Department of Health & Exercise Science will perform these procedures; he has performed these procedures on over 500 research volunteers.

Clamp

This is the name commonly given to the procedure formally know as the hyperinsulinemic euglycemic clamp. This procedure measures the ability of your body to handle sugar. We will inject sugar (glucose) into one of your veins and insulin (a naturally occurring substance produced by your body) into a different vein. We will continue to inject insulin and sugar into your veins to try to keep the concentration of sugar in your blood the same. We will measure this concentration every 5-minutes. This test lasts approximately 3 hours and we will ask you to perform it twice.

Food Questionnaire

You will be asked questions about the types and amounts of food you eat. We will estimate how many calories you eat per day, and how many of those calories come from fat, carbohydrate, and protein. There are no known risks associated with this procedure.

Activity Questionnaire

You will be asked questions about the types of activities you perform every day. Some of these questions will be about the activities you perform at work and during exercise. There are no known risks associated with this procedure.

Sprint Interval Exercise Training
You will be asked to report to the lab on 6 separate occasions, each visit separated by 1-2 days. During each visit you will be asked to perform between 4 and 7 bouts of exercise on a cycle ergometer. 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.

ARE THERE REASONS WHY I SHOULD NOT TAKE PART IN THIS STUDY?
You will not be allowed to participate in these studies for any of the following reasons:

1) You are not aged between 18 and 79 years.
2) You are pregnant.
3) You are a nursing mother.
4) You smoke or have smoked during the previous two years.
5) You have high blood pressure (greater than 145/90 mmHg).
6) You have asthma.
7) You are not free of overt disease as assessed by medical history, physical examination, ECG and blood pressure at rest and during incremental exercise.
8) You are taking systemic vasoactive drugs (i.e. drugs that affect blood pressure and/or heart function).
9) Your participation has not been approved by a physician, Dr. Wyatt Voyles, or by a senior member of the research team.
10) You are taking medications that would confound interpretation of the results of the studies.

11) You have a musculoskeletal injury that will be exacerbated by, or prevents you from performing, vigorous, high-intensity exercise.

WHAT ARE THE POSSIBLE RISKS AND DISCOMFORTS?

It is not possible to identify all potential risks in research procedures, but the researchers have taken reasonable safeguards to minimize any known and potential, but unknown, risks. The Human Performance Clinical Research Laboratory keeps an automated defibrillator with built in transcutaneous pacing and a "crash-cart" stocked with oxygen and emergency medications. A medical doctor will supervise all of the drug infusions. The investigators have a great deal of experience with all of the procedures. Some of the procedures you are being asked to volunteer for have a number of associated risks:

Treadmill Stress Test (Low Risk)

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). Exercise can make you tired and uncomfortable.

Exhausting Exercise Test (Low Risk)

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.

Body Composition (Low Risk)

There is a small amount of radiation exposure (0.05 mRem) associated with the DEXA test that is less than 1/20 of a typical chest x-ray. The more radiation you receive over the course of your life, then the greater the risk of having cancerous tumors or of inducing changes in genes. The changes in genes possibly could cause abnormalities or disease in your offspring. The radiation in this study is not expected to greatly increase these risks, but the exact increase in such risks is unclear. **Women who are or could be pregnant should receive no unnecessary radiation and should not participate in this study.**

Microneurography (Moderate Risk)

There is a small chance of a "pins and needles" feeling off-and-on for 1 to 7 days after the test (20% or less of all subjects); there is also a very small chance (less than 1% of all subjects) of an aching feeling in your leg off-and-on for a period of several weeks or months after the test.

Blood Collection (Moderate Risk)

When the needle goes into a vein, it may hurt for a short period of time (a few seconds). Also there may be minor discomfort of having the needle/plastic tube taped to your arm.

In about 1 in 10 cases, a small amount of bleeding will occur under the skin that will cause a bruise. The risk of forming a blood clot in the vein is about 1 in 100, and the risk of significant blood loss is 1 in 1,000. Additionally, there is a risk that you may faint while having blood collected or having the catheter inserted in your vein.

Muscle Biopsy (Moderate Risk)

During the procedure you may feel discomfort associated with the injection of the numbing drug (the anesthetic) but during the actual muscle removal the discomfort should be minimal. There is a risk that you may faint during the procedure. There is also a risk of muscle cramp, bleeding, of loss of feeling in your leg, and of damage to a skin (cutaneous) nerve. The risk of infection and bruising is extremely small if you follow the instructions for caring for the incision. A very small and minor scar will remain as a result of the incision, but may not be noticeable. Matthew Hickey, Ph.D. will perform these procedures under surgically clean conditions. Emergency medical equipment will be available. You will be screened prior to the procedure for history of allergic reactions to Novocain.

Clamp

The procedure involves placement of a catheter (hollow plastic needle) inside a vein thus the usual risks of blood collection apply (minor discomfort, bruising, fainting and blood clot (rare)). In addition there is a risk of hypoglycemia (low blood sugar); symptoms include hunger, nervousness and shakiness, perspiration, dizziness or light-headedness, sleepiness, confusion, difficulty speaking, and feeling anxious or weak. Although hypoglycemia can happen suddenly it can usually be treated very quickly by terminating the insulin infusion and continuing the glucose infusion, returning blood sugar concentration back to normal. To reduce the risk of hypoglycemia blood glucose concentration is measured every 5 minutes. If blood glucose concentration falls below 70 mg/dL insulin administration will be terminated and glucose infusion continued until normal concentration (70 – 100 mg/dL) is resumed; this usually occurs very quickly (~ 5 minutes).

Isoproterenol (Injection) (Higher Risk)

This drug will probably make your heart rate and blood pressure bigger. We will be measuring your heart rate and blood pressure very carefully when we are injecting this drug. There is a small risk of hypertension (high blood pressure), cardiac arrhythmias (unusual rhythm of heart), myocardial ischemia (decrease of oxygen to heart), and myocardial infarction (heart attack). The symptoms of blood pressure getting too big are dizziness, fainting, or in rare instances heart block. If your systolic blood pressure goes up by more than 35 mmHg, or if your heart rate goes up by more than 50 bpm we will stop the injection immediately.

WILL I BENEFIT FROM TAKING PART IN THIS STUDY?
If you are selected to participate in the sprint interval exercise training you may gain increases in skeletal muscle function and increases in aerobic fitness. All subjects will receive a copy of their results and information on body composition and metabolic and cardiovascular risk factors.

DO I HAVE TO TAKE PART IN THE STUDY?

Your participation in this research is voluntary. If you decide to participate in the study, you may withdraw your consent and stop participating at any time without penalty or loss of benefits to which you are otherwise entitled.

WHAT WILL IT COST ME TO PARTICIPATE?
There is no cost to you for participating except that associated with your transportation to our facilities.

WHO WILL SEE THE INFORMATION THAT I GIVE?
We will keep private all research records that identify you, to the extent allowed by 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 you 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.

CAN MY TAKING PART IN THE STUDY END EARLY?
Your participation in the study could end in the rare event of muscle strain, if you become pregnant, or if you miss an excessive number of appointments.

WILL I RECEIVE ANY COMPENSATION FOR TAKING PART IN THIS STUDY?
For experiments that involve blood and muscles, fine wire electrodes, and venous catheterization, you will be paid $15/hour.

WHAT HAPPENS IF I AM INJURED BECAUSE OF THE RESEARCH?

The Colorado Governmental Immunity Act determines and may limit Colorado State University's legal responsibility if an injury happens because of this study. Claims against the University must be filed within 180 days of the injury.

WHAT IF I HAVE QUESTIONS?

Before you decide whether to accept this invitation to take part in the study, please ask any questions that might come to mind now. Later, if you have questions about the study, you can contact the investigator, Christopher Bell, Ph.D. at 970 491 7522 or cbell@cahs.colostate.edu. If you have any questions about your rights as a volunteer in this research, contact Janell Meldrem, Human Research Administrator at 970-491-1655. We will give you a copy of this consent form to take with you.

WHAT ELSE DO I NEED TO KNOW?

Your signature acknowledges that you have read the information stated and willingly sign this consent form. Your signature also acknowledges that you have received, on the date signed, a copy of this document containing 7 pages.

_____ _____

Signature of person agreeing to take part in the study Date

_____

Printed name of person agreeing to take part in the study

_____ _____

Name of person providing information to participant Date

_____

Signature of Research Staff