THESIS

SPRINT INTERVAL TRAINING: THE INFLUENCE OF EXERCISE MODALITY

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ABSTRACT

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EXERCISE MODALITY

Sprint interval training (SIT), whether performed on a cycle ergometer or non-motorized treadmill, enhances exercise capacity and evokes favorable metabolic and cardiopulmonary adaptations. However, despite known differences between cycling and running, the influence of exercise modality on the adaptive response to SIT has not been directly addressed. Additionally, the effect of SIT on the angiogenic factors, pigment epithelial-derived factor (PEDF) and vascular endothelial growth factor (VEGF), has not been well characterized.

PURPOSE: To examine the influence of exercise modality on the adaptive response to SIT, we compared the effects of SIT performed on one of three different exercise machines: non-motorized treadmill, cycle ergometer, or plyometrics platform. Additionally, we sought to characterize the changes in circulating and skeletal muscle PEDF and VEGF following three weeks of SIT.

METHODS: Twenty-seven healthy, sedentary or recreationally active adults (age: 23 ± 5 years; body mass index: 25.7 ± 4.7 kg m^{-2}; VO_2peak: 36.7 ± 6.1 ml kg^{-1} min^{-1} (mean ± SE)) completed nine sessions of repeated (four to eight) 30-s bouts of maximal exercise on a non-motorized treadmill (RUN), cycle ergometer (CYC), or plyometrics platform (JMP) over 21 days. Prior to and following completion of SIT, peak oxygen uptake (VO_2peak) and time to exhaustion at 80%

VO_2peak were measured. Additionally, blood and skeletal muscle was sampled prior to and following completion of SIT to measure PEDF and VEGF.

RESULTS: Three weeks of SIT increased time to exhaustion (40.0 min ± 3.2 *vs.*51.3 ± 5.5 min, $P = 0.006$). The interaction with exercise modality did not achieve statistical significance ($P = 0.08$), however, it appears that time to exhaustion increased in the RUN (43.2 ± 5.2 *vs.* 57.4 ± 9.2 min) and CYC (41.7 ± 6.1 *vs.* 62.3 ± 11.6 min) groups, but not the JMP group (35.5 ± 5.6 *vs.* 35.0 ± 4.9 min). Circulating and skeletal muscle VEGF and PEDF were not altered by three weeks of SIT ($P > 0.05$).

DISCUSSION: Independent of exercise modality, three weeks of SIT improves endurance exercise capacity and does not alter circulating or skeletal muscle PEDF or VEGF.

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CHAPTER I

LITERATURE REVIEW

Physical Inactivity

Regular participation in physical activity has been known to promote health for over 2,000 years, having been advocated by such ancient scholars as Hippocrates, Plato, and Galen, among others [1-3]. However, it was not until a series of epidemiological studies conducted by Jerry Morris and colleagues that the foundation for an association between low levels of physical activity and increased risk for a number of non-communicable diseases was established [1]. The original observation from this group, published in 1953, was that the more active ticket-inspectors of London's double-decker buses had a lower incidence of coronary heart disease compared with their more sedentary driver peers [4]. Since 1953, a positive dose-response relationship has been shown to exist between physical activity and a number of non-communicable diseases, including cardiovascular disease, stroke, hypertension, colon cancer, type 2 diabetes, and osteoporosis [5]. Moreover, physical inactivity was estimated to have been responsible for 5.3 million of the 57 million deaths in 2008 [6] and was identified as the fourth leading risk factor for non-communicable diseases by the World Health Organization in 2009 [7].

The American College of Sports Medicine (ACSM) currently recommends that adults engage in at least 30 minutes per week of moderate intensity physical activity, 20 minutes per week of vigorous intensity physical activity, or some combination of moderate and vigorous intensities to accumulate 500-1,000 metabolic equivalent (MET) min week^{-1} [8]. Despite these and other similar recommendations, data taken from 122 countries have shown that 31.1% of

adults do not achieve at least 600 MET min week^{-1} [9]. Numerous individual and environmental factors have been suggested to explain the prevalence of physical inactivity [10], however, perceived lack of time has been repeatedly cited as a self-reported barrier to participation in physical activity [11-13]. Given the detrimental effects of physical inactivity, as well as the common perception of limited time as a barrier to physical activity participation, the development of a time-efficient mode of physical activity may represent a means for reducing mortality from non-communicable diseases around the world. Indeed, it has been estimated that a 25% reduction in physical inactivity could reduce worldwide mortality by 1.3 million deaths every year [6].

Sprint Interval Training

The introduction of sprint interval training (SIT) by Woldermar Gerschler over 70 years ago to augment athletic performance resulted in world-record athletic performances [14]. More recently, in research spearheaded by Martin Gibala and colleagues, SIT has been applied to both healthy and diseased populations to improve not only athletic performance, but also a number of physiological and health-related markers [15]. Sprint interval training may be defined as repeated bouts of high intensity exercise, with the most commonly employed SIT protocol consisting of repeated bouts of 30-s maximal efforts performed on a cycle ergometer, separated by four minutes of recovery [15]. This high intensity, short duration form of exercise has been repeatedly shown to evoke a number of favorable metabolic and cardiopulmonary adaptations that are typically associated with endurance training, sometimes in as little as two weeks [16-24].

The reduced time commitment of SIT compared with endurance training speaks to the potency of this form of exercise. Compared to six weeks of endurance training modeled after the

physical activity guidelines of ACSM, six weeks of SIT produced comparable adaptations, despite an appreciable shorter weekly training time (~1.5 hours vs. 4.5 hours) and a smaller weekly training volume (~225 kJ per week vs. 2250 kJ per week) [22]. Moreover, some evidence suggests that SIT may be more enjoyable than endurance training, as subjects rated a sprint interval running program higher than a continuous running program on the Physical Activity Enjoyment Scale [25]. The capacity for SIT to elicit favorable adaptations with minimal time requirements, coupled with its potential for being more enjoyable than endurance training, may make this form of exercise a practical component of healthy, active living.

An all-encompassing, fully comprehensive review of SIT is beyond the scope of this document. This review will be more focused. First, the role of intensity in eliciting the adaptive response to SIT will be explored. Second, the effects of chronic SIT will be outlined, with specific attention given to exercise capacity, metabolic adaptations, cardiopulmonary adaptations, alterations in oxidative stress, and changes in body composition. Finally, the role of exercise modality will be examined, both by looking at studies of sprint interval running, as well as by describing some of the known differences between cycling and running.

Intensity

The high intensity and therefore high levels of motor unit activation associated with SIT might account for its ability to elicit similar adaptations as high volume endurance training. In particular, the recruitment and adaptation of type II muscle fibers has been proposed to explain the potency of SIT [14, 26]. Type II, fast twitch, muscle fibers have been shown capable of responding to exercise training with oxidative adaptations [27-30]. Differences in the maximal activities of oxidative enzymes between type I and type II muscle fibers are smaller in trained

compared with untrained individuals. [27, 28], and detraining increases these differences between fiber types [27]. High intensity training may be necessary to elicit oxidative adaptations in type II muscle fibers. Seven to eight weeks of interval training at ~101% of pre-training maximal oxygen consumption (VO_2max), but not continuous training at ~79% of pre-training VO_2max, was capable of increasing the maximal activity of succinate dehydrogenase in type II muscle fibers [29]. An animal study provides further evidence for the role of intensity in determining the location of oxidative adaptations [30]. Rats trained at 20 m min^{-1} increased cytochrome c concentration in slow-twitch red skeletal muscle, but not in fast-twitch white skeletal muscle. Furthermore, increasing treadmill speed above 30 m min^{-1} increased the adaptive response to training in fast-twitch white fibers, but not in slow-twitch red fibers. These data suggest that type II muscle fibers have a threshold intensity below which no adaptations will occur, and that these fibers may also have a higher ceiling for oxidative adaptations.

Changes in metabolite concentrations following SIT likely play a role in the adaptive response and reflect the high intensity of this primarily anaerobic form of exercise. A single bout of SIT, consisting of 30-s of maximal cycling, depleted phosphocreatine levels to 16.9% of pre-exercise values [31]. Blood lactate increased to ~9 mmol L^{-1}, pH dropped to 6.69, and muscle glycogen decreased from 327.5 to 228.3 mmol kg dry $muscle^{-1}$. Evidence suggests that SIT also poses a significant challenge to the aerobic energy system [31-33]. For example, a single bout of SIT increased heart rate and oxygen consumption to values above 80% of estimated maximal values [33]. Repeated bouts of SIT may increase the demand placed on the aerobic energy system, as phosphocreatine levels were almost completely depleted ten seconds into the second bout of SIT, and oxygen consumption increased from 2.68 L min^{-1} during the first bout to 3.17 L

min^{-1} during the second bout [31]. Collectively, these data suggest that SIT may pose an appreciable challenge to both anaerobic and aerobic energy systems.

Exercise Capacity

Sprint interval training has been repeatedly shown to be capable of augmenting both anaerobic and endurance work capacity in previously untrained individuals. In light of its high intensity and short duration, it is perhaps unsurprising that two to seven weeks of SIT increases peak and mean power output during a single 30-s maximal cycling bout [22, 34, 35]. Six weeks of SIT increased peak and mean power by 17% and 7%, respectively [22]. These increases were comparable to those produced by six weeks of endurance training, although endurance training did not affect mean power.

Contrary to the principle of specificity, SIT augments endurance performance. Six or more weeks of SIT has been shown to increase peak oxygen uptake (VO_2peak) in both relative and absolute terms [21-24, 34, 36], although four weeks or less of SIT may only be sufficient to increase VO_2peak in overweight and obese males and females [17, 19, 20, 37]. Performance of 50, 250, and 750 kJ time trials improved by 4.1%, 9.6%, and 10.1%, respectively, following short-term SIT [16, 17]. Improvements in 50 and 750 kJ time trial performance were equivalent to the magnitude of improvement elicited by two weeks of endurance training [16]. Two to seven weeks of SIT also prolonged time to exhaustion while cycling at 80% and 130% VO_2peak by 100% and 21%, respectively [19, 34]. Taken together, these data demonstrate the potency of SIT for enhancing endurance exercise capacity.

Metabolic Adaptations

Improvements in exercise capacity with SIT may be partially attributed to augmented skeletal muscle metabolic capacity, as reflected by enhancements in anaerobic and aerobic enzymes. Six to seven weeks of SIT increased the maximal activities of enzymes involved in both glycolytic (aldolase, hexokinase, phosphofructokinase) and oxidative (citrate synthase, succinate dehydrogenase, malate dehydrogenase) energy pathways [35, 36]. Short-term SIT, conducted over the course of two weeks, also increased citrate synthase maximal activity [19], and increased the activity of active pyruvate dehydrogenase during submaximal exercise [17]. Maximal activity of cytochrome c oxidase (COX) and protein content of COX subunits II and IV increased to an equivalent magnitude following either short-term SIT or endurance training [16]. Adaptations to COX may occur uniformly across muscle fiber types, as six weeks of SIT increased COX protein content equally in both type I and II muscle fibers [24]. Animal studies support the capacity of SIT to elicit these adaptations, as high intensity exercise increased citrate synthase [38, 39] and COX [30] maximal activities to a similar extent as long duration exercise in rats.

Enhanced lipid metabolism represents another metabolic adaptation to SIT. Maximal activity of hydroxyacyl-CoA dehydrogenase (HAD) increased to a similar magnitude following either SIT or endurance training [22, 35], but not after short-term SIT [17]. A short-term SIT protocol consisting of repeated bouts of cycling at 90% VO_2peak increased HAD maximal activity, suggesting a minimal volume necessary for this adaptation to occur [40]. A study in rats supports also the role of HAD maximal activity in the adaptive response to high intensity exercise [38]. Short-term, high-volume SIT, also increased plasma membrane associated binding protein ($FABP_{pm}$) content [40], but six weeks of traditional SIT did not increase protein content

of either $FABP_{pm}$ or fatty acid translocase [41]. Adaptations in lipid metabolism likely contribute to shifts in substrate utilization at rest and during exercise. Both a single session [42], and short-term SIT [20] decreased respiratory exchange ratio at rest in a fasted state in overweight and obese males. Intramuscular triglyceride breakdown during 60 minutes of cycling at 65% of pre-training VO_2peak also increased following six weeks of SIT in type I muscle fibers [24].

Lactate, glycogen, and hydrogen ion metabolism also improve following SIT. Increases in muscle glycogen content are equivalent following either short-term SIT or endurance training [16]. Reductions in glycogenolytic flux during exercise following SIT may be due to tighter coupling of pyruvate production with pyruvate oxidation [17, 22]. Increased content of monocarboxylate transporters 1 and 4 likely contributes to reductions in lactate accumulation during exercise after SIT [17, 34, 41]. Additionally, short-term SIT or endurance training has been shown to enhance muscle buffering capacity, with no difference between groups [16].

Sprint interval training has also been shown capable of improving insulin sensitivity. Six weeks of SIT reduced Matsuda insulin sensitivity index and area under the curve for both insulin and glucose tolerance tests [23, 24]. Short-term SIT also increased glucose infusion rate during a hyperinsulemic-euglycemic clamp [18]. Enhanced insulin sensitivity may be partially due to glucose transporter type 4 content, which was increased after six weeks of SIT in humans [41], and after eight days of high intensity intermittent swimming in rats [39].

Peroxisome-proliferator activated receptor γ coactivator (PGC)-1α, known to regulate mitochondrial biogenesis in skeletal muscle, has been suggested to be responsible for the enhanced skeletal muscle oxidative capacity following SIT [15, 43]. The abundance of PGC-1α mRNA is influenced by intensity [44], and a single session of SIT increased expression of PGC-1α and phosphorylation of its upstream activators, 5'-adenosine monophosphate-activated

protein kinase (AMPK) and p38 mitogen-activated protein kinase (MAPK) [45]. Six weeks of SIT also increased PGC-1α protein content to a similar extent as endurance training [22].

Cardiopulmonary Adaptations

Sprint interval training also results in favorable cardiopulmonary adaptations. Six weeks of SIT lowered resting heart rate, diastolic blood pressure, and mean arterial pressure, all to a comparable magnitude as endurance training [23]. However, another study found no change in resting heart rate or blood pressure following six weeks of SIT [21]. Reduced resting systolic blood pressure has been observed in overweight and obese males following short-term SIT [20], with no effect of a single session [42]. Cardiopulomary responses to exercise improve following SIT in the same fashion as that observed following endurance training. Exercising heart rate at a matched workload is reduced after four to six weeks of SIT [21, 22, 24, 37]. Six weeks of SIT reduced exercising heart rate on average by 14 beats min^{-1} while cycling at ~65% pre-training VO_2peak, and to a comparable magnitude as endurance training [24]. An average decrease of 8 beats min^{-1} was compensated by a 9.7 mL $beat^{-1}$ increase in stroke volume after four weeks of SIT in overweight and obese females [37]. The increase in estimated plasma volume (86 mL) following SIT did not attain statistical significance, suggesting that increases in left ventricular chamber size or improvements in cardiac muscle contractility may have occurred.

Improvements in vascular health accompany SIT. The stiffening and thickening of the intima and media of large arteries is a predictor of risk for cardiovascular disease [46, 47]. Six weeks of SIT increased popliteal artery distensibility to a similar extent as endurance training [21]. Carotid artery distensibility did not change in either group, leading the authors to speculate that changes in the peripheral vasculature precede central adaptations. However, a more recent

study reported a reduction in central artery stiffness following six weeks of SIT [23]. Endothelial dysfunction is another predictor of cardiovascular disease [48, 49]. Popliteal endothelial function, as measured by flow-mediated dilation, improved following six weeks of SIT [21]. An enhanced vasodilatory response to acetylcholine was observed in the type 2 diabetic Otsuka Long-Evans Tokushima Fatty (OLETF) rat following either twelve weeks of sprint or endurance training [50]. Endothelial nitric oxide synthase (eNOS) content increased to a greater extent following SIT than endurance training in both rat aorta [51] and human skeletal muscle microvasculature [23]. Increased eNOS content was hypothesized to be beneficial due to greater nitric oxide production upon stimulation, resulting in greater vasodilation.

Sprint interval training appears to stimulate angiogenesis, although the data on humans are limited. Six weeks of SIT and endurance training increased the capillary to muscle fiber ratio on an individual-fiber basis by 27% and 32%, respectively, with no difference between groups [23]. Studies in animals have observed angiogenesis following various protocols of high intensity exercise [50, 52-54]. A combined sprint and endurance training regime in rats increased capillary to muscle fiber ratio, capillary numerical density, capillary surface area density, and capillary volume density throughout the gastrocnemius, with the greatest improvements found in the white gastrocnemius [52]. Eight to ten weeks of sprint training in rats increased the capillary to muscle fiber ratio in mixed and white sections of the gastrocnemius, but not in the red gastrocnemius [52]. Endurance training in rats did not increase capillarization in the white gastrocnemius [53]. Taken together, these data demonstrate the angiogenic adaptive capacity of type II muscle fibers, as well as suggest that the location of angiogenesis is dependent upon muscle fiber activation. However, a more recent study has shown no increase in arteriolar density in white or red gastrocnemius after ten weeks of sprint training in rats [54].

Vascular Endothelial Growth Factor

The angiogenic factors responsible for increased capillarization following SIT remain to be identified, however, some evidence supports a role for vascular endothelial growth factor (VEGF) [55]. A potent mitogen produced and stored by endothelial and skeletal muscle cells, VEGF mediates angiogenesis in skeletal muscle [56]. Selective deletion of VEGF in the skeletal muscle of mice decreased capillary to muscle fiber ratio by 64% [57]. Vascular endothelial growth factor functions by stimulating endothelial and skeletal muscle cell proliferation and migration, enhancing capillary permeability, and promoting endothelial cell survival and differentiation [58]. These effects of VEGF are mediated by VEGF receptor (VEGFR)-1 and VEGFR-2, expressed primarily in endothelial cells [59, 60].

Vascular endothelial growth factor has been implicated in exercise-induced angiogenesis [55]. Expression of VEGF in rat skeletal muscle is increased following electrical stimulation and acute exercise [61, 62]. Muscle contraction also increases VEGF release from skeletal muscle into the interstitial space [63-66]. Dialysate taken from the interstitial space of exercising muscle induces proliferation of cultured endothelial cells to a greater extent than dialysate taken from resting or recovering muscle [64]. Circulating VEGF has also been shown to increase after either wrist flexion exercise [67], or 60 minutes of cycling at 50% maximal power output in both endurance trained and sedentary individuals [68]. Additionally, ten days of endurance training increased basal VEGF expression and protein content in human skeletal muscle [69].

Two studies to date have examined the influence of SIT on VEGF [70, 71]. However, angiogenesis does appear to be intensity-dependent, as studies of training at 70-80% of VO_2max, but not 45% of VO_2max, have been shown to increase skeletal muscle capillarization [72-74]. Consistent with this hypothesis, a single session of SIT increased circulating VEGF, but 60

minutes of cycling at 50% VO_2peak did not [70]. In contrast, another study found that eight bouts of 60-s cycling at ~117% of VO_2max increased interstitial VEGF protein content, but this increase was 60% less than that observed following 60 minutes of cycling at ~65% of VO_2max [71]. Four weeks of this SIT protocol did not further increase muscle capillarization in individuals preconditioned with four weeks of endurance training.

Pigment Epithelium-Derived Factor

Angiogenesis following SIT may also be determined by alterations in anti-angiogenic factors such as pigment epithelium-derived factor (PEDF) [75]. First identified in human retinal epithelial cells, PEDF belongs to the family of serine protease inhibitors, despite its inability to function as a protease inhibitor [76, 77]. The effects of this adipokine are pleiotropic, influencing oxidative stress, inflammation, angiogenesis, and insulin sensitivity [78]. Adipose tissue is capable of secreting PEDF in abundance [79]. Secretion of PEDF from adipose tissue may be enhanced by insulin, or attenuated by hypoxia or troglitazone. Circulating levels of PEDF are elevated in individuals with metabolic syndrome [80, 81] and type 2 diabetes [82, 83]. Positive associations with the homeostasis model of insulin resistance [84, 85], and an inverse association with glucose infusion rate during a hyperinsulinemic-euglycemic clamp [18] have also been demonstrated to exist with circulating levels of PEDF.

Pigment epithelium-derived factor appears to have a direct role in the impairments in insulin sensitivity associated with obesity [86]. Acute administration of recombinant PEDF in mice impaired insulin signaling via a pro-inflammatory pathway. Moreover, chronic PEDF administration increased adipose tissue lipolysis, resulting in increased accumulation of lipid intermediates in skeletal muscle and impaired insulin sensitivity. Neutralizing PEDF in obese

mice improved insulin sensitivity. This study demonstrates one pathway by which excessive adiposity alters the adipose secretory profile and influences skeletal muscle function. Such crosstalk between adipose tissue and skeletal muscle has been hypothesized to exist [78, 87, 88]. In particular, some evidence suggests that excessive adiposity impairs the adaptations to exercise training, as a high-fat diet attenuated the hypertrophic response of skeletal muscle to loading in mice [89]. Excessive adiposity has also been suggested to attenuate the adaptations to exercise training through the anti-angiogenic actions of PEDF [78]. Indeed, PEDF inhibits angiogenesis through reductions in endothelial cell migration, even in the presence of such angiogenic factors as platelet-derived growth factor, lysophosphatidic acid, interleukin-8, acidic fibroblastic growth factor, and VEGF [75]. Pigment epithelial-derived factor may inhibit angiogenesis by inducing apoptosis of endothelial cells [90], reducing expression of VEGF [91], and interfering with the interaction between VEGF and its receptors, VEGFR-1 [92] and VEGFR-2 [93].

The ratio of VEGF to PEDF is believed to determine angiogenesis, and the relationship between these two antagonistic factors has been studied extensively in ocular diseases [94]. Retinal cells have been shown to increase VEGF secretion and decrease PEDF secretion in response to hypoxia [75]. Hypoxia induces retinal neovascularization in rats, and the time course of neovascularization coincides with changes in the VEGF:PEDF ratio, such that the highest ratio corresponds with the greatest rates of neovascularization [95]. No data exist on how this ratio influences angiogenesis in skeletal muscle, however, the angiogenic response to acute exercise is attenuated in diabetic mice compared with controls [96]. It has been speculated that the anti-angiogenic actions of PEDF contribute to this attenuated angiogenic response in diabetic mice [78], as individuals with type 2 diabetes have elevated levels of circulating PEDF [82, 83].

Exercise studies in humans have shown no change in circulating PEDF following either twelve weeks of aerobic exercise [97], or short-term SIT [18].

Oxidative Stress

The influence of SIT on oxidative stress has not been well characterized [98]. When cellular production of pro-oxidants surpasses the capacity of the system to render them inactive via endogenous antioxidant defenses and exogenously-derived antioxidants, oxidative stress is said to occur [99]. Oxidative stress causes cellular damage that is reflected by the modification of proteins, lipids, and nucleic acids. This condition may contribute to the aging process, as well as the development of age-related chronic diseases, including cardiovascular disease, diabetes, atherosclerosis, Alzheimer's disease, Parkinson's disease, rheumatoid arthritis, and diseases of the motor neuron, among others [99-101]. Oxidative stress has also been shown to be positively associated with obesity [102-104], and inversely associated with perceived physical fitness and exercise frequency [105].

Acute aerobic exercise results in a transient increase in oxidative stress. Exhaustive aerobic exercise increased free radical production, as measured by electron resonance, two- to three-fold in rat skeletal muscle and liver [106]. Concomitant increases in markers of cellular damage, including the loss of sarcoplasmic reticulum and endoplasmic reticulum integrity, as well as increases in malondialdehyde (MDA), a marker of lipid peroxidatition, were also found. Studies in humans support the animal data, demonstrating increases in free radical production, MDA, and lipid hydroperoxides in plasma and skeletal muscle following exhaustive aerobic exercise [107, 108]. Consumption of the antioxidant ascorbic acid attenuates the exercise-induced increase in free radical production, MDA, and lipid hydroperoxides [109, 110].

Acute anaerobic exercise is also believed to increase oxidative stress [98, 111]. Free radical production in an exercising isolated muscle bed was shown to be intensity-dependent [112], and increased resistance applied to the flywheel of a cycle ergometer during a 30-s sprint resulted in increased lipid peroxidation [113]. However, repeated ten second sprints did not increase plasma MDA or protein carbonyls [114], and a maximal jumping protocol did not change plasma MDA levels [115]. Sprint interval training has been shown capable of inducing oxidative stress, as a single bout of SIT increased lipid radical production and decreased glutathione levels [116].

The transient increase in oxidative stress following acute exercise may signal the body to enhance antioxidant defense systems to better withstand future oxidative challenges [117, 118]. Low-level peroxide administration and chronic aerobic exercise increased the resistance of rat cardiac cells to oxidative stress [119, 120]. Chronic aerobic exercise has also been shown to attenuate the increase in markers of oxidative stress following acute exercise [121]. Enhanced endogenous antioxidant enzyme capacity might explain the increased resistance to oxidative stress, as increases in the maximal activity of free radical scavenging catalase [121] and superoxide dismutase [122] have been reported following endurance training in rats. Resting activities of superoxide dismutase, glutathione peroxidase, glutathione reductase, and manganese superoxide dismutase have also been shown to be elevated in trained compared to untrained humans [115].

Fewer studies have reported on the influence of chronic SIT and other forms of high intensity exercise on oxidative stress. Adaptations to antioxidant enzymes may be intensity independent [122], and six weeks of SIT consisting of repeated 10-s bouts did not change the activity of any antioxidant enzymes [123]. However, a seventh week where training occurred

every day resulted in an increase in enzyme activity of glutathione peroxidase and glutathione reductase, suggesting that the increased volume was necessary to elicit these adaptations. Six weeks of sprint training in rats increased total glutathione content in skeletal muscle, as well as glutathione peroxidase, glutathione-S transferase, and glutathione reductase in both skeletal muscle and heart [124].

Oxidized low-density lipoprotein (OxLDL) represents another circulating marker of oxidative stress [125]. Low-density lipoproteins are major carriers of cholesterol in the body and may undergo oxidative modification [126]. Increased levels of circulating oxidized-LDL are associated with age and increased risk for diabetes, metabolic syndrome, and coronary artery disease [127-131]. Oxidized LDL also accelerate atherosclerosis by inducing monocyte migration through the endothelium [132].

Exercise training appears to evoke similar responses in OxLDL as other markers of oxidative stress. Acute exercise increased OxLDL in an intensity-dependent fashion, as exercise at 60% and 80%, but not 40% of VO_2max increased OxLDL [132]. Oxidized LDL appears to respond to chronic exercise training as well. Cross-sectional studies have found OxLDL to be inversely associated with physical activity status and aerobic capacity [130, 133, 134], and veteran endurance athletes have lower levels of OxLDL compared to controls [135]. A ten month exercise program decreased OxLDL in sedentary males and females by 23% [136]. To date, no data on the influence of SIT on OxLDL have been reported.

Body Composition

Information regarding the influence of SIT on body composition is limited. Several studies reported no change in body mass or body mass index following six weeks of SIT [20-24].

However, these measures are not capable of detecting changes in fat free mass, fat mass, and bone mineral content. Other SIT studies have shown increases in fat free mass [24], and decreases in fat mass [137], to the same extent as endurance training, despite no significant change in body mass. Moreover, short-term SIT decreased both hip and waist circumference without any change in body mass in overweight and obese males [20]. The only information pertinent to the influence of SIT on total bone mass comes from a sprint interval running study, where no change was observed after twelve weeks [138].

Animal studies on the influence of SIT on body composition have been somewhat equivocal. Eight to ten days of low intensity swimming reduced body weight and epididymal adipose tissue weight in male Sprague-Dawley rats compared to controls, but high intensity swimming did not [38, 39]. In contrast, ten to twelve weeks of treadmill sprint training in both male Sprague-Dawley rats and the type 2 diabetic OLETF rat reduced body weight and body fat percentage compared to controls [50, 54]. Differences in exercise modality or program duration might explain the inconsistencies between studies.

The capacity for SIT to influence body composition may be due in part to its ability to increase exercise post-exercise oxygen consumption (EPOC). A single session of SIT involving seven bouts of 30-s cycling at 120% VO_2max separated by 15-s of rest resulted in an average energy expenditure of 77 kcal, however, this protocol resulted in a greater EPOC in the 180 min following exercise [139, 140]. Moreover, a single session of SIT was shown to result in a similar 24 hour oxygen consumption as a continuous endurance exercise session [141]. These values were calculated based on eight separate 30 minute sessions of gas collection, spread out across the day following exercise. These data suggest that increased EPOC after a SIT session may explain the changes in fat mass observed following this low volume form of exercise.

Sprint Interval Running

Numerous studies have reported the performance enhancing effects of sprint interval running performed on either the treadmill or the track in a variety of athletes, including runners, cross-country skiers, soccer players, tennis players, wrestlers, judoka, lacrosse players, and handball players, among others [142-149]. Sprint interval running, just as SIT performed on a cycle ergometer, elicits improvements in performance, as well as physiological and health-related markers in previously untrained individuals [137, 138, 150, 151]. However, in contrast to SIT performed on the cycle ergometer, the protocols employed by these studies are more variable, with only a few consisting of repeated bouts of 30-s maximal efforts [137, 150].

Sprint interval running studies employing repeated bouts of 30-s maximal efforts result in similar adaptations as endurance training. Six weeks of sprint interval running improved 2,000 m time trial performance by 4.6%, increased top speed during a 30-s effort by 1 km h^{-1}, increased relative VO_2max by 11.5%, decreased fat mass by 12.4%, and increased fat free mass by 1% [137]. These adaptations were comparable to those elicited by endurance training. However, only the endurance trained group experienced increases in cardiac output. Eight weeks of sprint interval running also been reduced area under the curve during an oral glucose tolerance test, improved HOMA β-cell index, reduced LDL cholesterol, and reduced total cholesterol [150].

Alternative sprint interval running protocols have also proven effective. Twelve weeks of sprint interval running consisting of two minute long intervals of near-maximal efforts improved VO_2max to a greater extent than an endurance training program [138]. This sprint interval running protocol also improved plasma glucose following a two hour oral glucose tolerance test to a similar extent as the endurance trained group. In contrast, endurance training decreased resting heart rate, reduced body fat percentage, and reduced total cholesterol (TC) to high density

lipoprotein cholesterol (HDL-C) ratio, adaptations which were not observed in the SIT group. However, sprint interval running has been shown to increase HDL-C and reduce TC/HDL-C ratio, despite no change in TC [151].

Sprint interval running has also been shown to evoke favorable adaptations in obese children [152]. Twelve weeks of sprint interval running, consisting of 60-s bouts at 100% peak velocity, resulted in comparable improvements in absolute and relative VO_2peak, insulinemia, homeostasis model of insulin resistance, and body mass index as endurance training. Sprint interval running also decreased body weight by 2.6%, whereas the body weight reduction following endurance training did not attain statistical significance.

The mechanism underlying the adaptations to sprint interval running may be the same as that suggested to operate under SIT performed on a cycle ergometer. A single session of sprint interval running, consisting of three minute bouts at 90% VO_2max, increased phosphorylation of AMPK and MAPK [153]. Increases in PGC-1α expression were also shown, suggesting the activation of mitochondrial biogenesis pathways.

Exercise Modality

Numerous studies of running or cycling SIT have demonstrated the potency of this form of exercise to elicit adaptations analogous to those following endurance training. However, no study has directly compared the effects of SIT following either a cycling or running protocol. Although running and cycling both involve lower limb movement with primary recruitment of the quadriceps and plantar flexors [154-156], and have been shown to have a linear force-velocity relationship [157], differences exist between these two exercise modalities. For example, cycling involves only concentric muscle contractions, whereas running has both

concentric and eccentric components [154]. The eccentric component of running likely contributes to its greater delta efficiency compared to cycling [154]. Maximal oxygen consumption tests performed on a cycle ergometer may result in values as much as 11% lower than those performed on a treadmill in untrained subjects [158, 159]. However, trained cyclists do not experience this same decrease in VO_2max performed on a cycle ergometer, suggesting the importance of familiarity with exercise modality [160, 161].

A greater oxygen consumption (VO_2) slow component has been observed during cycling compared to running [162, 163]. The VO_2 response during the transition from rest to constant-load exercise below lactate threshold includes a cardiodynamic phase (phase I), and an approximate monoexponential rise in VO_2 (phase II) to reach a new steady state (phase III) [164]. However, at intensities above lactate threshold, an additional component of VO_2 causes VO_2 to rise above the predicted value. This VO_2 slow component is believed to originate in the working muscle, as 86% of the increase in pulmonary VO_2 was accounted for by an increase in leg VO_2 [165]. The VO_2 slow component may be due to increased recruitment of type II muscle fibers, which are less efficient than type I muscle fibers [166] and have been shown to be active at intensities associated with the VO_2 slow component [167]. Additionally, a negative correlation was shown to exist between the percentage of slow twitch fibers in the vastus lateralis and the magnitude of the VO_2 slow component during cycling [168]. These and other differences between cycling and running could potentially influence the adaptive response to SIT.

Statement of the Problem

Sprint interval training, whether performed on a cycle ergometer or non-motorized treadmill, has been repeatedly shown to be a time-efficient means for producing favorable physiological adaptations. However, the influence of exercise modality on the adaptations to SIT has not been studied, despite known differences between cycling and running. Therefore, we sought to compare the effects of three weeks of SIT performed on a cycle ergometer, non-motorized treadmill, or plyometrics platform.

Additionally, SIT has been shown to stimulate angiogenesis [23]. Angiogenesis represents an important adaptation to exercise training, as it determines nutrient delivery to and by-product removal from the working tissue. The VEGF:PEDF ratio is believed to regulate angiogenesis [94], and elevated levels of PEDF have been suggested to impair VEGF-mediated angiogenesis following exercise training [78]. However, data on how these two angiogenic factors respond to SIT is limited. Therefore, we sought to quantify changes in circulating and skeletal muscle VEGF and PEDF following SIT.

Hypotheses

Three weeks of SIT will improve endurance exercise capacity, with no differences between exercise modalities.

The VEGF:PEDF ratio in the circulation will increase following three weeks of SIT.

Changes in the VEGF:PEDF ratio in the circulation will be positively associated with improvements in endurance exercise capacity.

Specific Aims

1) To compare the effects of three weeks of SIT when performed on three different exercise machines (cycle ergometer; non-motorized treadmill; plyometrics platform) on endurance exercise capacity.

2) To quantify the changes in circulating and skeletal muscle VEGF and PEDF following three weeks of SIT.

3) To compare changes in circulating and skeletal muscle VEGF and PEDF to changes in endurance exercise capacity.

CHAPTER II

THE MANUSCRIPT[1]

INTRODUCTION

Sprint interval training (SIT) enhances endurance exercise capacity and elicits numerous favorable metabolic and cardiopulmonary adaptations [16-19, 21, 22, 41, 137, 150]. These adaptations are comparable to those following endurance training, despite a considerably shorter training time and smaller training volume [22]. Cycling has been the most common exercise modality employed by studies of SIT [16-19, 21, 22, 41], however, more recent work has demonstrated the efficacy of running protocols [137, 150]. Although both involve recruitment of the lower limb musculature, differences in muscle contraction characteristics [154], delta efficiency [154], and oxygen consumption (VO_2) slow component [162, 163] between cycling and running may influence the adaptive response to SIT. Despite the known differences between exercise modalities, no study to date has compared the adaptations to SIT following either a running or cycling protocol.

Angiogenesis is one component of the adaptive response to SIT [23]. The angiogenic factors responsible for the observed angiogenesis following SIT have not been fully described, however, evidence supports a role for vascular endothelial growth factor (VEGF) [70]. Produced and stored by endothelial and skeletal muscle cells, VEGF mediates angiogenesis in skeletal muscle [56]. Muscle contraction stimulates the release of VEGF from skeletal muscle into the

[1] Gregory R. Giordano, Rebecca L. Scalzo, Garrett L. Peltonen, Scott E. Binns, Anna L. Klochak, Hunter L. Paris, Melani M. Schweder, Kyle E. Sevitz, Steve E. Szallar, Lacey M. Wood, Raoul F. Reiser II, Christopher Bell. *Sprint Interval Training: The Influence of Exercise Modality*.

interstitial space [56, 64-66], and acute exercise, including SIT, increases circulating VEGF [67, 68, 70]. Ten days of endurance training has been shown capable of increasing resting VEGF expression and protein content in human skeletal muscle [69], but no data on the effects of chronic SIT on VEGF have been presented.

The anti-angiogenic factor, pigment epithelial-derived factor (PEDF), may also influence angiogenesis following SIT. Pigment epithelial-derived factor may impair angiogenesis by inducing apoptosis of endothelial cells [90], reducing expression of VEGF [91], and interfering with the interaction between VEGF and its receptors, VEGF receptor (VEGFR)-1 [92] and VEGFR-2 [93]. Adipose tissue has been shown capable of secreting PEDF in abundance [79], leading to the hypothesis that excessive adipose tissue may impair the angiogenic response to exercise training through secretion of PEDF [78]. Support for this hypothesis comes from an animal study, which demonstrated a blunted angiogenic response to acute exercise in diabetic mice compared to control mice [96]. This blunted angiogenic response was speculated to be due to elevated PEDF in the diabetic mice [78], as circulating PEDF is elevated in individuals with diabetes [82, 83]. Exercise training studies have not shown any changes in PEDF [18, 97], however, no study has measured PEDF and VEGF concurrently.

The purpose of this study was twofold. First, we sought to examine the influence of exercise modality on the adaptive response to SIT by comparing the effects of three weeks of SIT performed on one of three different exercise machines: cycle ergometer, non-motorized treadmill, or plyometrics platform. Second, we sought to characterize the changes in circulating and skeletal muscle VEGF and PEDF following three weeks of SIT.

METHODS

Subjects

Twenty-seven healthy adults were studied (9 males and 18 females). Recruitment criteria consisted of: being between the ages of 18 and 40 years, not pregnant, free from overt disease, non-smokers or had not smoked in the previous two years, weight stable (±2 kg) for one year, normotensive (< 140/90 mmHg), free from any recurring injury that would limit performance of vigorous exercise, and not taking any medications that would confound the interpretation of the data. The subjects were classified as healthy based on a medical history questionnaire. The experimental protocol conformed to the standards set by the *Declaration of Helsinki* of 1975, as revised in 1983, and was approved by the Institutional Review Board at Colorado State University. The nature, purpose, and risks of the study were explained to each subject before written informed consent was obtained.

Experimental Design

Subjects were randomly assigned to one of three SIT modalities: cycling (CYC), running (RUN), or repetitive vertical jumping (JMP). Subjects completed three weeks of SIT, sandwiched by assessment of peak oxygen uptake (VO_2peak) and time to exhaustion at 80% VO_2peak, and muscle and blood collection.

Pre-experimental Procedures

Prior to performing any experimental trials, all subjects completed habituation trials for the VO_2peak and time to exhaustion at 80% VO_2peak tests to become familiar with testing procedures. Verbal encouragement was provided by investigators during performance of all exercise trials.

An incremental exercise test to exhaustion was used to determine VO_2peak. All subjects were outfitted with a heart rate monitor (Polar) and a two-way non-rebreathing mouthpiece, valve, and headgear apparatus (Hans-Rudolph, St. Louis, MO). The CYC group performed this test on a cycle ergometer (Velotron Dynafit Pro, RacerMate, Inc., Seattle, WA, USA; Lode Excalibur, Groningen Netherlands), and the test consisted of a 20-35 Watts min^{-1} continuous ramp protocol from 0 Watts. The test was terminated once pedal cadence fell below 40 rpm. The RUN and JMP groups performed this test on a motorized treadmill (MedTrack ST65, Quinton, Bothell WA). Subjects self-selected a speed that remained constant throughout the test, and grade was increased by 2% every two minutes. The test was terminated once the subjects needed to hold onto the bars of the treadmill to remain on the moving belt. Heart-rate was measured for all subjects at rest in the exercise position and every two minutes during the test. Rating of perceived exertion (Borg) was measured every two minutes during the test. Ventilation and the gas composition of expired gases were measured by a metabolic cart (Parvo TrueOne 2400 Metabolic Measurement System, Parvo Medics, Sandy UT). Peak oxygen uptake was calculated as the mean of the four highest consecutive 15-s average VO_2 values. Maximal respiratory exchange ratio (RER) was calculated by averaging the four corresponding RER values.

The CYC group cycled on a cycle ergometer (Velotron; Lode), and the RUN and JMP groups ran on a motorized treadmill (Quinton) to exhaustion at a workload designed to elicit

~80% VO_2peak. Test termination criteria were the same as during the VO_2peak test. Subjects were blinded to all temporal information. Ventilation and gas composition of expired gases were measured (Parvo) and averaged over the six to ten minute period of exercise.

Experimental Protocol

The experimental protocol consisted of 1) baseline testing, 2) a three week SIT intervention, and 3) post-testing.

Baseline Testing

For all subjects, baseline testing consisted of anthropometrical and body composition measurements, a VO_2peak test, a time to exhaustion test at ~80% VO_2peak, as well as blood and muscle collection. Body height was measured to the nearest millimeter and body weight to the nearest 100 grams using a stadiometer and beam scale (Detecto, Webb City, MO, USA). Body mass index was calculated as body mass height^{-2} (kg m^{-2}). Dual-energy X-ray absorptiometry (Hologic, Discovery W, QDR Series, Bedford, MA, USA) was used to measure fat mass, fat free mass, and bone mineral content. The average of three waist measurements was determined as measured at the narrowest section of the trunk, with the subject standing during normal ventilation.

Baseline VO_2peak and time to exhaustion tests were performed on separate days, as already described. Forty-eight hours following the final baseline exercise test, subjects reported to the lab in the morning after a twelve hour fast and 24 hour abstention from exercise for blood collection and a muscle biopsy. Venous blood samples were collected from an antecubital or dorsal hand vein with a butterfly cannula (Blood Collection Set, BD, Franklin Lakes, NJ, USA).

Approximately 20 mL was preserved with K3 ethlenediaminetetraacetic acid (Vacuette EDTA tubes – Non-ridged [pull cap], Greiner Bio-One North America, Inc., Monroe, NC, USA), and approximately 20 mL was collected in a tube containing a silica clot activator, polymer gel, silicone-coated interior (Vacutainer SST Tube with Silica Clot Activator, Polymer Gel, Silicone-Coated Interior, BD, Franklin Lakes, NJ, USA). For the biopsy procedure, a local anaesthetic (1% lidocaine) was administered to the lateral portion of one thigh before a small incision was made through the skin and underlying fascia. A muscle biopsy (50-150 mg) was then obtained from the vastus lateralis using a Bergstrom needle.

Sprint Interval Training

Three weeks of SIT was performed by all subjects. Exercise sessions for each group consisted of repeated bouts of 30-s maximal efforts, separated by four minutes of active recovery. The CYC group cycled against a load equivalent to 0.075 kg kg body mass^{-1} on a cycle ergometer (Velotron). The RUN group ran on a non-motorized treadmill (Force, Woodway, Waukesha, WI, USA) against a load equivalent to 0.075 kg kg body mass^{-1}. The JMP group performed repetitive vertical jumping on a plyometrics platform (Pneubounder, Plyo Systems, http://www.plyosystems.com) at a predetermined absolute resistance. Active recovery was performed on a cycle ergometer (Velotron) for the CYC group and on a motorized treadmill (Quinton) for both RUN and JMP groups. The number of bouts performed during each session progressed from four to eight over the three week period for each group. The final session consisted of four bouts for comparison with the first session, and was performed at least 48 hours following the previous session.

Post-Testing

Forty-eight hours following the final training session, all subjects reported to the lab for a second muscle biopsy and blood collection. A time to exhaustion test at the same workload as the baseline test was performed 24 hours following the muscle biopsy and blood collection, and a VO_2peak test was performed 24 hours following the time to exhaustion test. The post-testing procedures for the muscle biopsy, blood collection, and exercise tests were identical to the baseline testing procedures.

Dietary and Physical Activity Controls

Subjects were asked to maintain their current dietary and physical activity habits during participation in the study. In addition to their normal diet, a standardized meal (Ensure and Power Bar) was provided to subjects to be consumed two hours prior to time to exhaustion tests.

Blood Analysis

Samples preserved with K3 ethlenediaminetetraacetic acid were collected in chilled tubes, placed on ice immediately following collection, and centrifuged at -4°C and 3,600 rpm for ten minutes within 60 minutes of collection to isolate plasma. Samples collected in silica clot activator, polymer gel, silicone-coated interior plasma tubes, were collected and maintained at room temperature for approximately 30 minutes after collection. Samples were then centrifuged at 4°C and 3,600 rpm for ten minutes within 60 minutes of collection to isolate serum. All samples were stored at -80°C until analysis.

Enzyme-linked immunosorbent assays (ELISA) were used to measure concentrations of C-peptide, adiponectin, and pigment-epithelial derived factor (all Millipore Corporation,

Billerica, MA, USA), insulin (ALPCO Diagnostics, Salem, NH, USA), VEGF (R&D Systems, Inc., Minneapolis, MN), C-reactive protein (R&D Systems, Inc., Minneapolis, MN), and oxidized low density lipoprotein (OxLDL; ALPCO Diagnostics, Salem, NH, USA). Glucose concentration was analyzed using an automated device (2300 STAT Plus Glucose Lactate Analyzer, YSI Inc., Yellow Springs, OH, USA).

Skeletal Muscle Analysis

Immediately following collection, each muscle biopsy sample was frozen by plunging the biopsy needle into liquid nitrogen. The sample was then homogenized and spun to acquire fractions. Skeletal muscle was analyzed for PEDF and VEGF protein content, adjusted for actin, via standard Western blotting procedures.

Statistical Analysis

Analysis of variance (ANOVA) with repeated measures was used to examine changes from baseline to post intervention within groups. Newman-Keuls post hoc analysis was performed to determine the location of significance when differences were detected. The level of statistical significance was set at $P < 0.05$. Data are reported as mean ±SE.

RESULTS

Sprint Interval Training

Twenty-seven subjects were prescribed 55 sprints over three weeks. Five subjects reported feelings of nausea, one of light-headedness, and two of muscle soreness during SIT. Three subjects required an extended rest period (> 4 min) between bouts in a given session due to nausea. One subject was unable to complete the prescribed number of sprints in a session due to nausea, and the remaining sprints were completed in a subsequent session. One subject suffered a knee sprain during SIT, causing one session to be postponed. Despite these potential adverse effects, all subjects completed 55 sprints over the three week period.

Subject Characteristics

Subjects were young (23 ± 5 years), slightly overweight (25.7 ± 4.7 kg m^{-2}), and of low to average aerobic capacity (36.7 ± 6.1 ml kg^{-1} min^{-1}). Selected physical subject characteristics organized by training group are presented in **Table 1**. No differences were found between training groups for any parameter ($P > 0.07$).

Anthropometry and Dual-Energy X-ray Absorptiometry

Sprint interval training did not alter body composition ($P > 0.05$; **Table 2**). An interaction with exercise modality was detected between groups for bone mineral content ($P = 0.04$) and bone mineral density ($P = 0.008$). Post-hoc analysis revealed the RUN group increased bone mineral content by ~2% (2.26 ± 0.12 *vs*. 2.30 ± 0.13 kg, $P = 0.02$; **Table 3**) and bone mineral density by ~1%, although the effect on bone mineral density did not achieve statistical

significance (1.07 ± 0.38 *vs*. 1.08 ± 0.38 g cm^{-2}, $P = 0.06$). Bone mineral density was decreased in the JMP group by ~2% (1.12 ± 0.37 *vs*. 1.10 ± 0.37 g cm^{-2}, $P = 0.009$)

Time to Exhaustion

Sprint interval training increased time to exhaustion at ~80% VO_{2peak} by ~29% (40.0 min ± 3.2 *vs*.51.3 ± 5.5 min, $P = 0.006$; **Figure 1**). The interaction of exercise modality did not achieve statistical significance, however, a trend toward a difference in improvement in time to exhaustion was found ($P = 0.08$). Time to exhaustion appears to have been improved in the RUN (43.2 ± 5.2 *vs.* 57.4 ± 9.2 min) and CYC (41.7 ± 6.1 *vs.* 62.3 ± 11.6 min) groups, but not in the JMP (35.5 ± 5.6 *vs.* 35.0 ± 4.9 min) group.

Peak Oxygen Uptake

Sprint interval training increased absolute VO_2peak, although the effect did not achieve statistical significance (2.76 ± 0.13 *vs.* 2.80 ± 0.14 L min^{-1}, $P = 0.09$; **Figure 2**), but VO_2peak relative to body mass did not change (36.7 ± 6.1 *vs.* 36.8 ± 1.2 mL kg^{-1} min^{-1}, $P = 0.85$). No interaction with exercise modality was detected for absolute ($P = 0.39$) or relative VO_2peak ($P = 0.81$). Maximum heart rate achieved during the graded exercise test was not statistically different between pre- and post-tests (187 ± 2 *vs.* 186 ± 1 beats min^{-1}, $P = 0.22$), indicating that changes in VO_2peak were not to be due to differences in maximum heart rate achieved.

Blood Parameters

Sprint interval training did not alter circulating glucose, insulin, adiponectin, C-peptide, or C-reactive protein ($P > 0.05$; **Table 4**). The homeostasis model assessment: insulin resistance

(HOMA-IR), was unaffected by SIT (1.39 ± 0.29 *vs.* 1.70 ±0.46, $P = 0.47$). Sprint interval training decreased OxLDL by ~12% (73.50 ± 19.24 *vs.* 64.91 ± 16.24 pg mL^{-1}, $P = 0.05$; **Figure 3**). No interaction with exercise modality was detected for OxLDL ($P = 0.74$). The change in OxLDL was related to the change in time to exhaustion (r = -0.47, $P = 0.03$; **Figure 4**) and the change in VO_2peak relative to body mass (r = 0.62, $P = 0.03$).

Sprint interval training did not alter circulating PEDF (39.31 ± 4.16 *vs.* 44.83 ± 7.22 ng mL^{-1}, $P = 0.48$; **Figure 5**) VEGF (240.12 ± 51.68 *vs*. 244.76 ± 49.25 pg mL^{-1}, $P = 0.86$), or the VEGF:PEDF ratio (6.23 ± 1.52 *vs*. 7.07 ± 1.41, $P = 0.45$). Pre-SIT PEDF and VEGF were related (r = 0.48, $P = 0.03$), and the change in PEDF was related to the change in VEGF (r = -.52, $P = 0.02$). The change in the VEGF:PEDF ratio was not related to the change in time to exhaustion (r = -0.02, $P = 0.92$), the change in absolute VO_2peak (r = -0.27, $P = 0.19$), or the change in VO_2peak relative to body mass (r = -0.03, $P = 0.21$).

Skeletal Muscle Parameters

Sprint interval training did not alter skeletal muscle VEGF (41.62 ± 20.05 AU *vs.* 43.97 ± 20.45, $P = 0.22$; **Figure 6**) or PEDF (54.52 ± 22.12 *vs.* 56.02 ± 23.33, $P = 0.52$). Pre-SIT PEDF and VEGF were related (r = .45, $P = 0.04$), and the change in PEDF was related to the change in VEGF (r = .47, $P = 0.03$).

DISCUSSION

The primary findings of this study are: (1) three weeks of SIT increased time to exhaustion at 80% VO_2peak. The interaction of exercise modality with time to exhaustion did not achieve statistical significance, however, it appears as though the RUN and CYC groups increased time to exhaustion, whereas the JMP group did not; (2) three weeks of SIT did not alter circulating or skeletal muscle PEDF or VEGF (3) and exercise modality had significant effects on bone mineral content and bone mineral density. We also report for the first time that three weeks of SIT decreased circulating OxLDL.

Running and cycling SIT protocols have repeatedly proven to be time-efficient means for enhancing endurance exercise capacity, as reflected by time to exhaustion or time-trial performance [16, 17, 19, 34, 137]. These improvements in endurance performance are likely due to metabolic and cardiopulmonary adaptations [15]. Increased maximal activities of the oxidative enzymes citrate synthase [19, 35, 36], succinate dehydrogenase [36], malate dehydrogenase [36], and cytochrome c oxidase [17] have been observed following SIT. Sprint interval training has also been shown to increase basal glycogen content in skeletal muscle [16], decrease glycolytic flux [17, 22] and lactate accumulation [17, 34, 41] during exercise, and improve skeletal muscle buffering capacity [16]. Cardiopulmonary adaptations to SIT that may contribute to enhanced endurance performance include reduced exercising heart rate [21, 22, 24, 37], increased stroke volume [37], decreased arterial stiffness [21, 23], improved endothelial function [21], and increased skeletal muscle capillarization [23].

Consistent with previous studies, we observed an increase in time to exhaustion at 80% VO_2peak following three weeks of SIT. We are the first to compare the performance

improvement following three weeks of SIT performed on one of three exercise machines: cycle ergometer, non-motorized treadmill, or plyometrics platform. The interaction of exercise modality with the improvement in time to exhaustion did not achieve statistical significance, however, it appears that run and cycle SIT resulted in improved performance, but jump SIT did not. The lack of improvement following jump SIT may be due in part to the resistance provided by the plyometrics platform. Run and cycle SIT was performed against a resistance of 0.075 kg kg body mass^{-1}, but jump SIT was performed against a predetermined absolute resistance. This absolute resistance may not have been sufficient to stimulate type II muscle fiber recruitment in all subjects, which are believed to underlie the potency of SIT [14]. Additionally, run and cycle SIT involved training and performing exercise tests on the same or similar exercise machines, whereas jump SIT involved training on a plyometrics platform and performing exercise tests on a motorized treadmill. Training specificity is known to influence performance outcomes [169-174], so the jump SIT group may have been able to improve performance had the test been completed on the plyometrics platform. However, the nature of the plyometrics platform made conducting a time to exhaustion test on this machine unfeasible.

Sprint interval training interventions lasting six weeks or longer have shown increases in VO_2peak in both relative and absolute terms in previously untrained individuals [21-24, 34, 36], but SIT interventions of four weeks or less may only be sufficient to increase VO_2peak in overweight and obese males and females [17, 19, 20, 37]. We report an increase in absolute VO_2peak following three weeks of SIT, although this increase did not achieve statistical significance. In support of these data, our group has previously observed an increase in relative VO_2max following three weeks of run SIT [MANUSCRIPT IN PREPARATION]. Our data may

provide additional insight into the intervention length required to increase VO_2peak through SIT in previously untrained individuals.

The majority of studies have reported no change in body mass or BMI following SIT [20-24]. Despite no change in body mass, other studies of six weeks of SIT have reported increases in fat-free mass [24] and decreases in fat mass [137]. Twelve weeks of a high intensity cycling protocol decreased body mass, increased fat free mass, and decreased fat mass in overweight young males. Additionally, our group has observed increases in fat free mass, decreases in fat mass, and decreases in body fat % following three weeks of run SIT, despite no change in body mass [MANUSCRIPT IN PREPARATION]. In the present study, we observed no changes in body composition following three weeks of SIT, possibly due to the length of our intervention. We did observe a significant interaction of exercise modality with bone mineral content and bone mineral density. Post-hoc analysis revealed that run SIT increased bone mineral content and bone mineral density, although the change in bone mineral density did not achieve statistical significance. Cross-sectional studies have documented increased bone mineral density in runners compared to sedentary controls [175, 176]. Sprinters, in particular, have increased bone mineral density when compared to medium- and long-distance runners, possibly due to the high levels of force involved in this type of running [177]. The only SIT study to report on bone mineral content found no change following twelve weeks of a running protocol [138]. This same study reported that twelve weeks of strength training increased bone mineral content by ~2% or 0.06 kg, which was similar to the increase we observed (~2% or 0.04 kg). However, the rapid nature of the increase we observed was surprising, and additional studies should be conducted to determine if these results can be replicated.

Low-density lipoproteins (LDL) are major carriers of cholesterol in the circulation and may undergo oxidative modification [126]. Thus, OxLDL is a marker of oxidative stress [125] which has been positively associated with age [131] and increased risk for diabetes [127], metabolic syndrome [128, 130], and coronary artery disease [129]. Also, OxLDL accelerate atherosclerosis by inducing monocyte migration through the endothelium [132]. Cross-sectional studies have reported decreased levels of OxLDL in veteran endurance athletes compared to controls [135], as well as inverse associations of OxLDL with physical activity status and aerobic capacity [130, 133, 134]. A ten month program of primarily walking exercise has been shown to decrease OxLDL in sedentary males and females [136]. We are the first to report that three weeks of SIT decreased OxLDL. This decrease in OxLDL may be due to decreased total LDL [136, 150], increased LDL antioxidant potential [136], or reduced concentration of the denser subfractions of LDL, which are more susceptible to oxidation [136, 178].

Vascular endothelial growth factor is produced and stored by endothelial and skeletal muscle cells, and is known to mediate angiogenesis in skeletal muscle [56]. Acute exercise stimulates the release of VEGF from skeletal muscle into the interstitial space, where it may enter the circulation [67, 68, 70]. The data on the effect of chronic exercise training on circulating VEGF have been equivocal. A cross-sectional study found no difference in circulating VEGF between trained and untrained individuals [68]. Ten days of endurance training decreased circulating VEGF [69]. Altitude training in competitive swimmers increased circulating VEGF, however, these levels returned to baseline after one month at sea level, despite no change in training load [179]. One week of Austrian Special Forces Training resulted in no change in circulating VEGF [180]. Circulating VEGF is elevated in individuals with hypertension [181], dyslipidemia [182], atherosclerosis [183], and congestive heart failure [184].

We observed no change in circulating VEGF following three weeks of SIT. Circulating VEGF in endurance trained males was measured to be ~221 pg mL^{-1} [68]. The average of circulating VEGF in our subjects was ~245 pg mL^{-1} at baseline, suggesting that the lack of change we found may be due in part to circulating VEGF levels already being close to optimal levels. Additional studies should be performed to clarify the role of this circulating angiogenic factor on the adaptive response to exercise training.

Pigment epithelium-derived factor is an adipokine with insulin desensitizing [86] and anti-angiogenic [75, 90-93] effects. Studies of sprint interval training have documented decreases in fat mass [137], increases in insulin sensitivity [18], and increases in skeletal muscle capillarization [23]. Therefore, it seems plausible that SIT would decrease circulating PEDF. However, we observed no change in circulating PEDF following three weeks of SIT. We have previously reported no change in circulating PEDF following three weeks of SIT [18]. Twelve weeks of aerobic exercise training increased glucose infusion rate during a hyperinsulinemic-euglycemic clamp without a change in BMI, but also did not alter circulating PEDF [97]. A study of diet-induced weight loss has observed a decrease in circulating PEDF [185], which may suggest the necessity of weight loss to decrease circulating PEDF.

The ratio of PEDF and VEGF is believed to determine angiogenesis [94]. A rodent study of hypoxia-induced retinal neovascularization has shown that the highest VEGF:PEDF ratio corresponds with the greatest rates of neovascularization [95]. We observed no change in this ratio of circulating VEGF:PEDF, possibly due the length of our intervention. Six weeks of SIT has been shown to be sufficient to stimulate angiogenesis [23], but it is not known whether this adaptation occurs within three weeks. Additional studies should be conducted to examine the relationship of this ratio with measures of angiogenesis. We did not detect any relationship

between change in this ratio and improvements in exercise capacity, suggesting that manipulation of this ratio is not necessary to enhance performance. We do report significant associations between VEGF and PEDF in the circulation and in skeletal muscle, which suggests the presence of regulatory mechanisms to exist between these two antagonistic factors.

Previous studies have documented the adaptations to both run [137, 150] and cycle [16, 17, 19, 21, 22, 35, 36, 41] SIT. We report for the first time on the adaptations to a novel modality, jump SIT. We are also the first to directly compare the adaptations to SIT performed on three different exercise machines. With the exception of markers of bone health, it appears as though exercise modality does not influence the adaptive response to SIT. No significant differences were observed between the improvements in time to exhaustion or VO_2peak following three weeks of SIT. These data may carry an important health message, as SIT confers beneficial effects, regardless of the exercise modality employed. Whereas cycling requires specific equipment, running is cheap and accessible, requiring only a pair of running shoes and adequate space.

In summary, three weeks of SIT increased time to exhaustion at 80% VO_2peak and increased absolute VO_2peak, although the increase in absolute VO_2peak did not achieve statistical significance. Three weeks of SIT did not alter circulating or skeletal muscle PEDF or VEGF. Decreased OxLDL was observed following three weeks of SIT. Excepting bone mineral content and bone mineral density, we observed no significant interactions of exercise modality with adaptations to SIT.

TABLES

TABLE 1. Selected physical subject characteristics by training group. Data are mean ± S.E.M. RUN, run sprint interval training. JMP, jump sprint interval training. CYC, cycle sprint interval training. BMI, body mass index. VO_2peak, peak oxygen uptake. HR_{peak}, peak heart rate. RER_{peak}, peak respiratory ratio.

	RUN	**JMP**	**CYC**	***P* value**
Male/female	3/6	2/7	4/5	-
Age (years)	22 ± 1	23 ± 2	23 ± 1	0.92
Height (m)	1.69 ± 0.02	1.70 ± 0.03	1.72 ± 0.03	0.74
Body mass (kg)	79.5 ± 5.0	73.5 ± 5.4	73.2 ± 5.4	0.64
BMI (kg m^{-2})	26.8 ± 1.3	25.7 ± 2.1	24.7 ± 1.4	0.66
% Body fat	35.1 ± 1.2	34.1 ± 2.0	29.8 ± 2.2	0.12
Fat mass (kg)	26.9 ± 1.7	24.8 ± 2.6	21.1 ± 1.7	0.15
Fat-free mass (kg)	47.7 ± 3.2	45.2 ± 3.3	48.5 ± 4.6	0.82
VO_2peak (L min^{-1})	2.99 ± 0.23	2.59 ± 0.15	2.69 ± 0.29	0.47
VO_2peak (ml kg^{-1} min^{-1})	38.1 ± 1.6	36.1 ± 2.7	36.0 ± 1.8	0.71
HR_{peak} (beats min^{-1})	190 ± 3	190 ± 3	184 ± 2	0.19
RER_{peak}	1.07 ± 0.01	1.03 ± 0.02	1.11 ± 0.03	0.07

TABLE 2. Influence of three weeks of sprint interval training on selected anthropometrics and dual-energy X-ray absorptiometry data. Data are mean ± S.E.M. RUN, run sprint interval training. JMP, jump sprint interval training. CYC, cycle sprint interval training. BMI, body mass index.

	RUN		JMP		CYC	
	PRE	**POST**	**PRE**	**POST**	**PRE**	**POST**
Body mass (kg)	79.5 ± 5.0	78.7 ± 4.8	73.5 ± 5.4	74.2 ± 5.4	73.2 ± 5.4	72.6 ± 5.3
BMI (kg m^{-2})	26.8 ± 1.3	27.1 ± 1.3	25.7 ± 2.1	25.9 ± 2.1	24.7 ± 1.4	24.6 ± 1.4
Waist (cm)	82.1 ± 3.7	83.1 ± 3.7	81.4 ± 4.8	79.6 ± 4.6	80.7 ± 4.9	79.2 ± 4.3
% Body fat	35.1 ± 1.2	36.3 ± 1.0	34.1 ± 2.0	34.6 ± 1.9	29.8 ± 2.2	29.9 ± 2.4
Fat mass (kg)	26.9 ± 1.7	28.04 ± 2.0	24.8 ± 2.6	25.6 ± 3.0	21.1 ± 1.7	21.1 ± 1.9
Fat-free mass (kg)	47.7 ± 3.2	46.9 ± 3.2	45.2 ± 3.3	45.1 ± 2.8	48.5 ± 4.6	48.1 ± 4.5

TABLE 3. Influence of three weeks of sprint interval training on bone mineral content and bone mineral density. Data are mean ± S.E.M. RUN, run sprint interval training. JMP, jump sprint interval training. CYC, cycle sprint interval training. BMC, bone mineral content. BMD, bone mineral density. * denotes main effect of training. † denotes interaction with exercise modality.

	RUN		JMP		CYC	
	PRE	**POST**	**PRE**	**POST**	**PRE**	**POST**
Bone mineral content (kg)†	2.26 ± 0.12	2.30 ± 0.13*	2.32 ± 0.14	2.34 ± 0.14	2.43 ± 0.16	2.41 ± 0.16
Bone mineral density (g cm^{-2})†	1.07 ± 0.03	1.08 ± 0.03	1.12 ± 0.03	1.10 ± 0.03*	1.15 ± 0.03	1.14 ± 0.03

TABLE 4. Influence of three weeks of sprint interval training on selected blood parameters. Data are mean ± S.E.M.

	RUN		JMP		CYC	
	PRE	**POST**	**PRE**	**POST**	**PRE**	**POST**
Glucose (mg dL^{-1})	86.54 ± 1.72	85.07 ± 1.28	85.99 ± 2.07	84.70 ± 1.90	86.25 ± 1.99	89.14 ± 3.37
Insulin (µU mL^{-1})	8.09 ± 3.02	6.01 ± 1.27	6.60 ± 1.51	6.57 ± 1.60	3.99 ± 0.39	10.21 ± 5.21
Adiponectin (ng mL^{-1})	20.80 ± 4.37	25.20 ± 4.59	19.00 ± 3.45	20.45 ± 4.15	12.05 ± 2.00	12.46 ± 3.29
C-peptide (ng mL^{-1})	2.71 ± 0.30	2.60 ± 0.30	3.30 ± 0.53	2.25 ± 0.47	2.96 ± 0.59	3.19 ± 0.82
C-reactive protein (ng mL^{-1})	1887 ± 52	2359 ± 704	1568 ± 759	2234 ± 870	1488 ± 612	940 ± 263

FIGURES

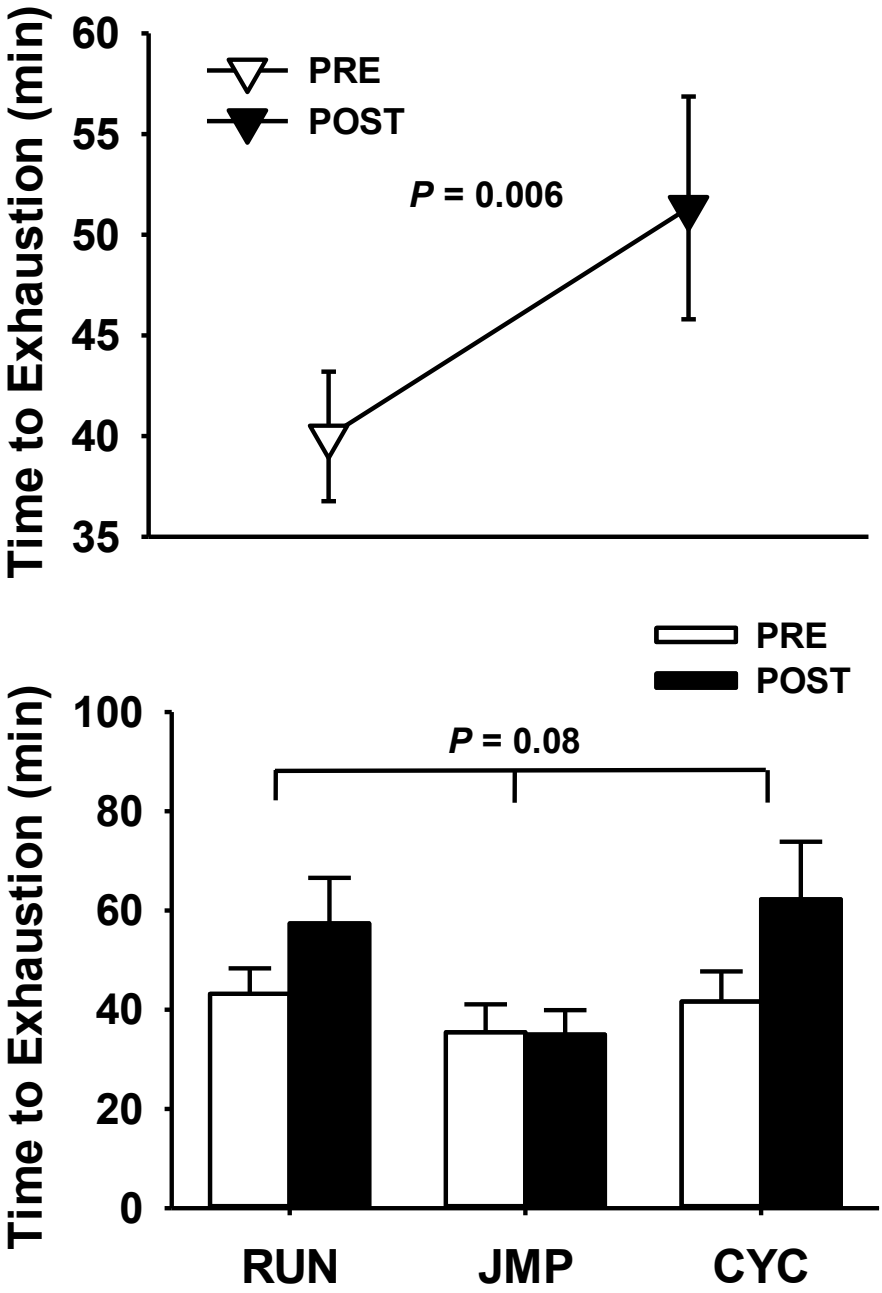

FIGURE 1. Increased time to exhaustion following three weeks of sprint interval training (P = 0.006). Trend toward difference in improvement in time to exhaustion between sprint interval training groups (P = 0.08). Sprint interval training appears to have increased time to exhaustion

in run and cycle sprint interval training groups, but not in jump sprint interval training group. Data are mean ± S.E.M. RUN, run sprint interval training. JMP, jump sprint interval training. CYC, cycle sprint interval training.

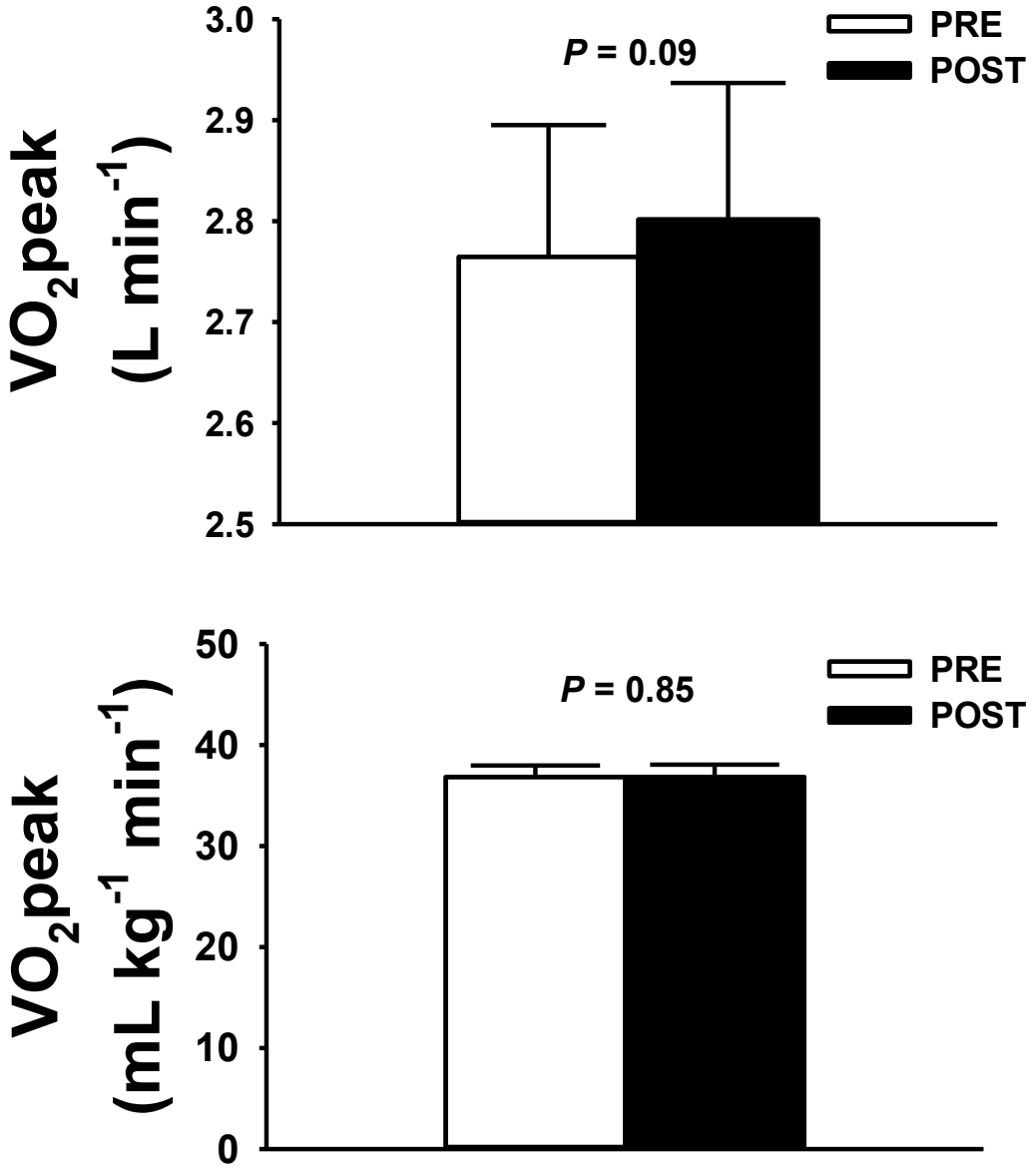

FIGURE 2. Three weeks of sprint interval training increased absolute peak oxygen uptake, although this increase did not achieve statistical significance ($P = 0.09$). No change in relative peak oxygen uptake following three weeks of sprint interval training ($P = 0.85$). Data are mean ± S.E.M. VO_2peak, peak oxygen uptake.

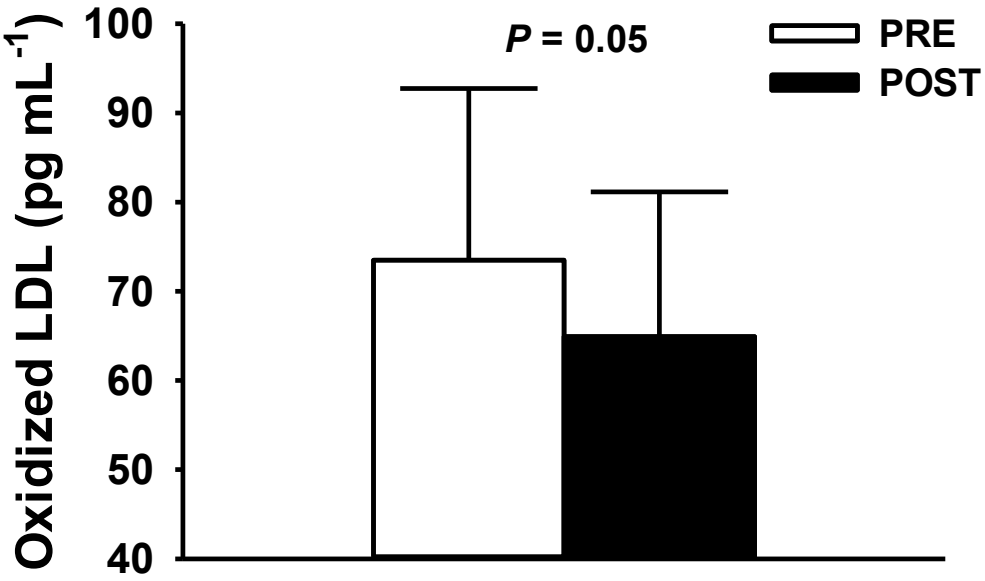

FIGURE 3. Decreased plasma oxidized low-density lipoprotein following three weeks of sprint interval training ($P = 0.05$). Data are mean ± S.E.M. LDL, low-density lipoprotein.

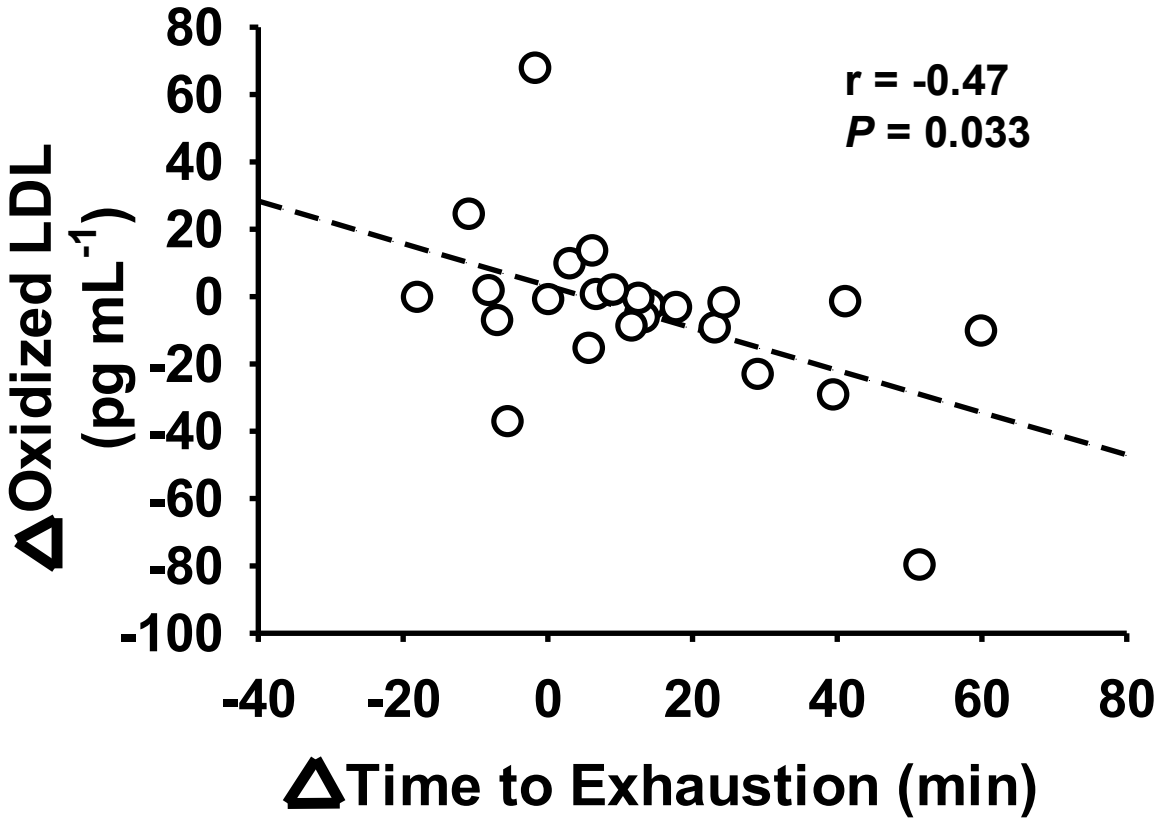

FIGURE 4. Significant association between the change in plasma oxidized low-density lipoprotein and the change in time to exhaustion (r = -0.51, *P* = 0.013) following three weeks of sprint interval training. LDL, low-density lipoprotein.

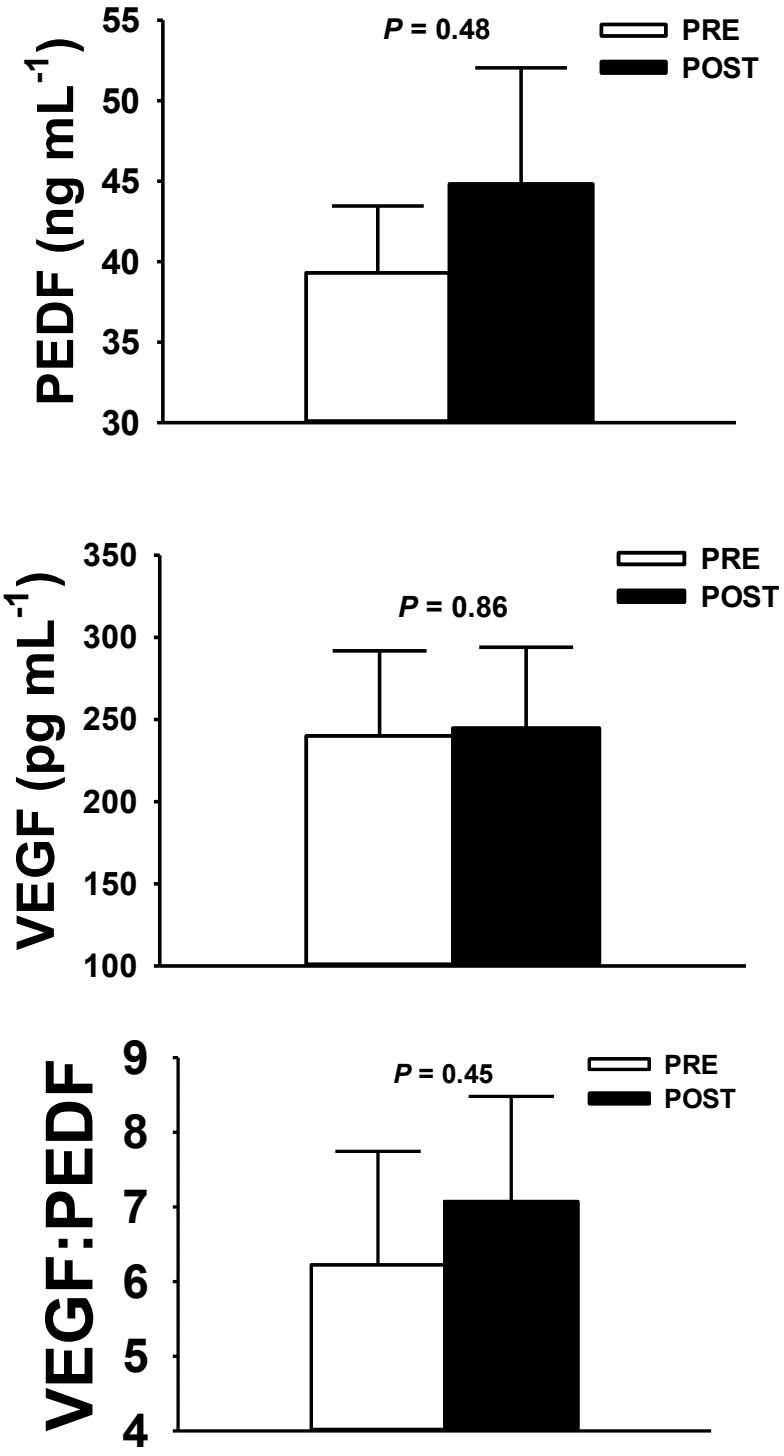

FIGURE 5. No change in plasma pigment epithelium-derived factor ($P = 0.48$), vascular endothelial growth factor ($P = 0.86$), or the VEGF:PEDF ratio ($P = 0.45$) following three weeks of sprint interval training. Data are mean ± S.E.M. PEDF, pigment epithelium-derived factor. VEGF, vascular endothelial growth factor.

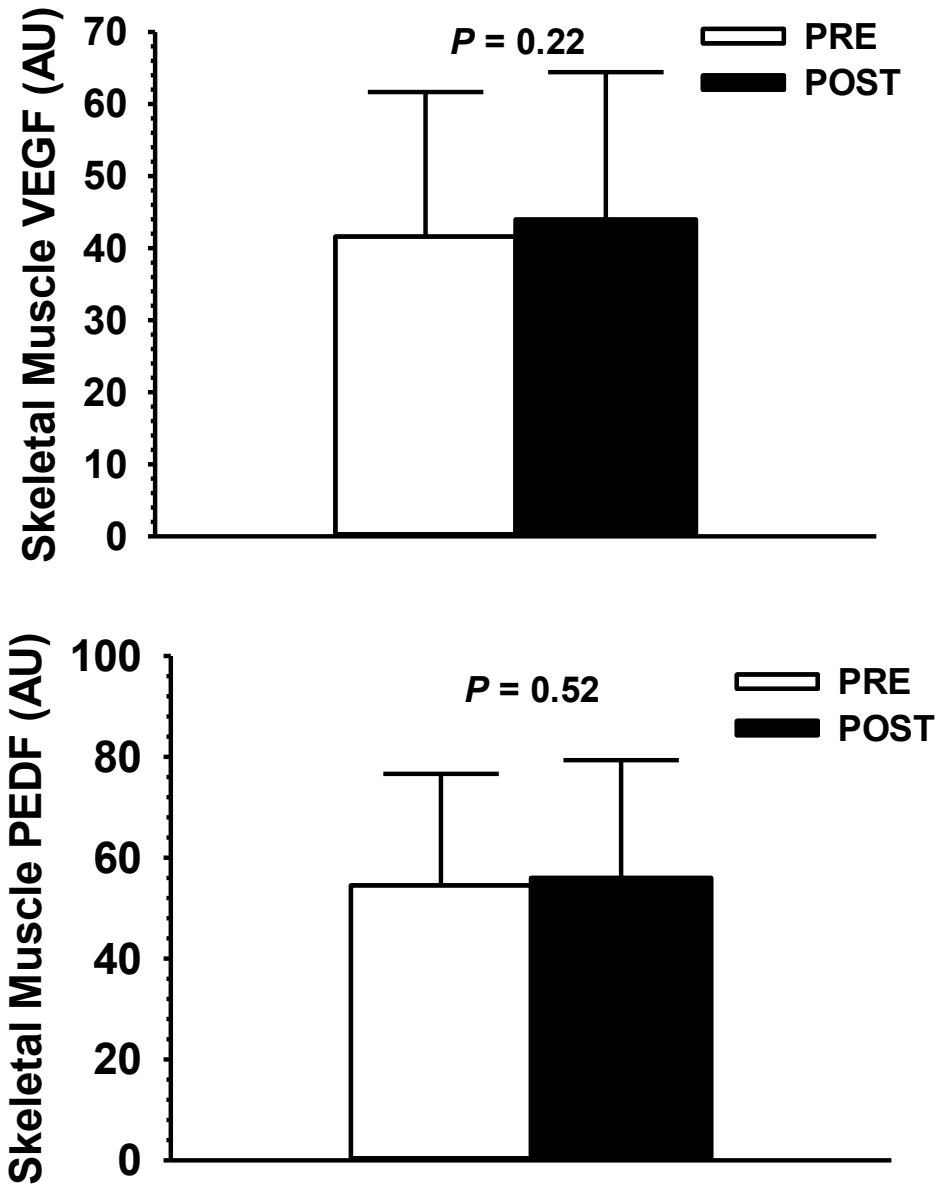

FIGURE 6. No change in skeletal muscle VEGF ($P = 0.22$) or PEDF ($P = 0.52$) following three weeks of sprint interval training. Data are mean ± S.E.M. PEDF, pigment epithelium-derived factor. VEGF, vascular endothelial growth factor. AU, arbitrary units.

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APPENDIX

CONSENT FORM

Consent to Participate in a Research Study
Colorado State University

TITLE OF STUDY:
Short-Term Sprint Interval Training: Influence of Exercise Modality

PRINCIPAL INVESTIGATOR:
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WHY AM I BEING INVITED TO TAKE PART IN THIS RESEARCH?
You are an adult man or woman aged between 18 and 40 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 undergraduate students.

WHAT IS THE PURPOSE OF THIS STUDY?
The purpose of the study is to compare the influence of sprint-interval training when performed on different exercise machines (treadmill vs. exercise bike vs. jumping machine). You will be randomly assigned to complete 3 weeks of exercise training on one of these machines.

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). The HPCRL has emergency supplies including a medicine trolley equipped with heart machines.

This whole research project will take place over a period of approximately one year. You will be asked to be involved for approximately 2 months. The total time of your participation is approximately 15 hours.

WHAT WILL I BE ASKED TO DO?

After completing some initial tests you will be asked to complete 3 weeks of very high-intensity exercise training on either a treadmill, an exercise bike, or a jump machine. After exercise training you will repeat many of the initial tests. 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

You will be asked to perform a vigorous exercise test. 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. Depending on your age, a physician may 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 stress test. You will be asked to ride an exercise bike, run/walk on a treadmill or jump on a jumping machine until you are too tired to continue. It will become more and more difficult to keep exercising. While you are riding/walking/running/jumping 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. This test will be performed three times; it lasts roughly 1 hour.

Exercise Test

You will be asked to ride an exercise bike or run on a treadmill ride or jump on a machine without stopping until you are exhausted and cannot continue. The goal of this test is that you perform the exercise for as long as possible. 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. This test will be performed three times; it lasts roughly 1.5 hours.

Heart Function During Exercise

During some of the exercise tests we will also measure the function of your heart. You will be asked to wear a nose clip (something that stops you breathing through your nose) and asked to breathe a special gas that we will provide. The gas contains a normal (21%; same as room air) amount of oxygen, a small amount (5%) of helium, a very small amount (0.5 %) of acetylene, and a close to normal amount (73.5 %) of nitrogen. Each measure of heart function will last approximately 1 minute.

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 circumference of your waist and hip using a tape measure. This test will be performed twice; it lasts approximately 15 minutes.

Standing Vertical Jump
After a brief warm-up (consisting of a few practice jumps and a little stretching) you will be asked to jump as high as you can from a standing start. The starting position will consist of feet shoulder-width apart and pointing forwards. Arms will hang loosely by your sides. You will then be asked to bend your knees, swing your arms, and jump as high as you can, straight up in the air. To allow us to measure the height of your jump, you will be asked to tap one of many plastic arms that are attached to a pole. You will be asked to repeat this jump five times, with ample time allowed for recovery between jumps. This test will be performed twice; it last approximately 10 minutes.

Blood Collection
We will be taking blood from you on two 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 insulin and glucose (blood sugar). Your blood will be taken from veins in your arms or hands using needles and hollow plastic tubes called catheters. Your blood will not be kept for other research purposes.

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., or Benjamin Miller, Ph.D., professors in the Department of Health & Exercise Science, or Matthew Robinson, a PhD student in the same department, will perform these procedures; they have performed these procedures on over 800 research volunteers. This test will be performed

twice; each test lasts approximately 1 hour. Your muscle will not be kept for other research purposes.

Sprint Interval Exercise Training
You will be asked to report to the lab on 9 separate occasions, each visit separated by 1-2 days. During each visit you will be asked to perform between 4 and 8 bouts of cycle, running or jumping exercise. The cycling exercise will be performed on a stationary cycle ergometer. The Running on a treadmill, and the jumping on a new piece of equipment called a Pneubounder (for more information visit: http://plyosystems.com/). You will be asked to perform 4 sprints during the first training visit, 5 sprints during the second training visit, 6 sprints during the third and fourth training visits, 7 sprints during the fifth and sixth training visits, 8 sprints during the seventh and eight training visits, and 4 sprints during the ninth (final) training visit. 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 40 years.
2) You are pregnant.
3) You are a nursing mother.
4) You smoke or have smoked during the previous two years.
5) You are not free of overt disease as assessed by medical history, physical examination, ECG and blood pressure at rest and during incremental exercise.
6) Your participation has not been approved by a physician or by a senior member of the research team.
7) You are taking medications that would confound interpretation of the results of the studies.
8) You are participating in another research study that may confound interpretation of the results of this study.
9) You are unable or unwilling to perform repeated vigorous 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 has emergency supplies including a medicine trolley equipped with heart machines. The investigator has a great deal of experience with all of the procedures. Some of the procedures for which you are being asked to volunteer have a number of associated risks:

<u>All Exercise Tests and Sprint Training</u>
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. Exercise can make you feel very tired. Sprint training might make you feel dizzy or

queasy; you may faint or vomit. Further, sprint training is likely to induce considerable muscle soreness and increase the risk of minor musculoskeletal injuries (sprains and strains).

Heart Function Tests

These tests are very safe and have been used without incident to measure heart function in many "at-risk" adults including pregnant women during labor, intensive care patients, and patients with cardiopulmonary and chronic respiratory diseases. Wearing a mouthpiece and nose-clip can sometimes cause dryness in the mouth and mild discomfort. You may notice a "funny"/strange taste while breathing the pre-mixed gas, or you may not notice anything.

Body Composition

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 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.**

Blood Collection

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

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. Benjamin Miller, Ph.D., or 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 Novocaine (Lidocaine).

Standing Vertical Jump

Potential risks include damage (e.g. sprains) to muscles, tendons and/or ligaments resulting from jumping, as well as a risk of falling and/or landing incorrectly. An awkward landing could result in an ankle sprain. The likelihood of these risks occurring is small as most people are familiar with performing a vertical jump, and you will be allowed to practice before we make our measurements.

ARE THERE ANY BENEFITS FROM TAKING PART IN THIS STUDY?
There are no direct benefits in participating, however you will receive a copy of your results and information pertinent to your body composition (i.e. height and weight), and metabolic and cardiovascular risk factors. Specifically, in blood we will measure concentrations of insulin, glucose, norepinephrine, epinephrine, inflammatory markers (e.g. c-reactive protein), and markers of oxidative stress (e.g. isoprostanes). You will be provided with a copy of your DEXA scan that you may wish to have interpreted by a medically qualified professional. You may also become fitter and experience improved physiological function. The overall benefit for conducting the research will be the knowledge that the favorable responses to sprint training are not limited to one kind of exercise

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?
Other than transport to and from the lab, your participation should incur no costs.

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 if you become pregnant or if you miss an excessive number of appointments.

WILL I RECEIVE ANY COMPENSATION FOR TAKING PART IN THIS STUDY
On completion of the study, for experiments that involve blood and muscle sampling and breathing acetylene you will be paid $15/hour. This is $90 in total.

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. Participants should check with their insurance as to their coverage in the event of a research 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 970-491-3495 or physiology@cahs.colostate.edu. If you have any questions about your rights as a volunteer in this research, contact Janell Barker, 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 _8_ 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