

## ADAPTATIONS TO LOW-VOLUME INTERVAL EXERCISE TRAINING

PHYSIOLOGICAL AND HEALTH-RELATED  
ADAPTATIONS TO LOW-VOLUME INTERVAL  
EXERCISE TRAINING IN HUMANS

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### **LAY ABSTRACT**

This thesis examined physiological and health-related adaptations to interval training, which involves brief bouts of intense exercise interspersed with recovery periods. One protocol involved alternating 60-second hard and easy cycling efforts for 20 minutes; the other involved three, 20-second ‘all-out’ sprints interspersed with 2 minutes of recovery. Both protocols improved indices of cardiometabolic health in previously inactive adults who trained three times per week for 6 weeks, even though the amount of exercise performed was lower than typically recommended in public health guidelines. When the latter protocol was directly compared against traditional endurance training, the improvement in cardiometabolic health after 12 weeks was the same, despite a five-fold difference in the total amount of exercise performed. Our findings highlight the effectiveness of short bursts of high-intensity exercise for improving health. These results may appeal to individuals who cite “lack of time” as a barrier to exercise.

## **ABSTRACT**

This thesis sought to advance our understanding of the physiological and health-related adaptations to low-volume interval training. Three separate studies were conducted in previously sedentary adults who trained three times per week. High-intensity interval training (HIIT) involved ten, 60-second cycling efforts at an intensity that elicited ~90% of maximal heart rate, interspersed with 60 seconds of recovery, whereas sprint interval training (SIT) involved three, 20-second ‘all-out’ cycling efforts interspersed with 2 minutes of recovery. Both protocols involved a brief warm-up and cool-down, resulting in 25- and 10-minute sessions for HIIT and SIT, respectively. Peak oxygen uptake ( $\text{VO}_{2\text{peak}}$ ), skeletal muscle mitochondrial content as reflected by the maximal activity and protein content of mitochondrial enzymes, and glycemic control based on oral glucose tolerance tests (OGTTs), intravenous glucose tolerance tests (IVGTTs) or continuous glucose monitoring (CGM), were determined before and after training. Study 1 found that 6 weeks of HIIT in the fed or fasted state increased  $\text{VO}_{2\text{peak}}$  and mitochondrial content in women, but insulin sensitivity based on OGTTs was unchanged. Study 2 showed that 6 weeks of SIT increased  $\text{VO}_{2\text{peak}}$  and mitochondrial content in men and women, whereas mean 24-hour glucose based on CGM was reduced in men only. Study 3 directly compared 12 weeks of SIT to traditional moderate-intensity continuous training (MICT) in men. The two protocols elicited similar improvements in  $\text{VO}_{2\text{peak}}$ , mitochondrial content and insulin sensitivity based on IVGTTs, despite SIT involving a five-fold lower

exercise volume and time commitment. This work advances our understanding of the potency of brief, intense exercise training to induce physiological remodeling and improve cardiometabolic health. It also highlights potential sex-specific adaptations to interval training that warrant clarification. Further investigation into the mechanisms of physiological remodeling to HIIT and SIT is needed, as are large-scale randomized clinical trials that compare these protocols to MICT.

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## LIST OF ABBREVIATIONS

AMPK	5'AMP-activated protein kinase
AUC	area under the curve
$\beta$ -HAD	$\beta$ -hydroxy acyl CoA dehydrogenase
CGM	continuous glucose monitoring
COX	cytochrome c oxidase
CS	citrate synthase
CS <sub>I</sub>	insulin sensitivity index
CTL	control group
DBP	diastolic blood pressure
DEXA	dual x-ray absorptiometry
END	endurance training
FPG	fasting plasma glucose
FPI	fasting plasma insulin
GLUT4	glucose transporter 4
HIIT	high-intensity interval training
HOMA	homeostatic model assessment
HRmax	maximal heart rate
ISI	insulin sensitivity index
IVGTT	intravenous glucose tolerance test
K <sub>G</sub>	glucose disappearance rate
MAP	mean arterial pressure
MICT	moderate-intensity continuous training
M <sub>ISI</sub>	matsuda composite index
MPO	mean power output
OGTT	oral glucose tolerance test
p38 MAPK	p38 mitogen-activated protein kinase
PGC-1 $\alpha$	peroxisome proliferator-activated receptor gamma co-activator - 1 alpha
PPAR- $\delta$	peroxisome proliferator-activated receptor-delta
PPO	peak power output
RPE	rating of perceived exertion
SBP	systolic blood pressure
SDH	succinate dehydrogenase
S <sub>I</sub>	minimal model insulin sensitivity index
SIT	sprint interval training
VO <sub>2</sub> peak	peak oxygen consumption
Wmax	maximal workload

**PREFACE**  
**DECLARATION OF ACADEMIC ACHIEVEMENT**

**FORMAT AND ORGANIZATION OF THESIS**

This thesis is prepared in the “sandwich” format as outlined in the School of Graduate Studies’ Guide for the Preparation of Theses. It includes a general introduction, three independent studies prepared in journal article format, and an overall discussion. The candidate is the first author on all of the manuscripts. At the time of the thesis preparation, Chapters 2 and 3 were published in peer-reviewed journals and Chapter 4 was under peer-review.

## **CONTRIBUTION TO PAPERS WITH MULTIPLE AUTHORSHIP**

### **Chapter 2 (Study 1):**

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**CHAPTER 1:**  
**INTRODUCTION**

## 1.1. Introduction

Renewed interest in the topic of physiological and health-related adaptations to low-volume interval training has sparked a plethora of research over the past decade. Interval training refers to the concept of alternating periods of relatively intense exercise with periods of rest or low-intensity exercise for recovery. *Low-volume* interval training refers to training sessions that are relatively brief, consisting of  $\leq 10$  minutes of intense exercise within a ~25-minute time commitment including warm-up, cool-down and recovery periods between intervals. In an effort to standardize terminology, a recent classification scheme proposed that the term ‘high-intensity interval training’ (HIIT) be used to describe protocols involving target intensities between 80 and 100 % of maximal heart rate (i.e., ‘near-maximal’), and ‘sprint interval training’ (SIT) be used to describe protocols involving ‘all-out’ efforts, in which target intensities correspond to workloads greater than what is required to elicit 100 % of maximal oxygen uptake (i.e., ‘supramaximal’) (117). Using these definitions, an example of a low-volume SIT protocol is repeated Wingate tests, which typically involve four to six, 30-second ‘all-out’ cycle sprints, interspersed with 4 minutes of recovery. An example of low-volume HIIT is ten, 60-second cycling efforts at 90 % maximal heart rate, interspersed with 60 seconds of recovery. This intermittent exercise pattern is different than moderate-intensity continuous training (MICT) generally reflected in public health guidelines (Figure 1).

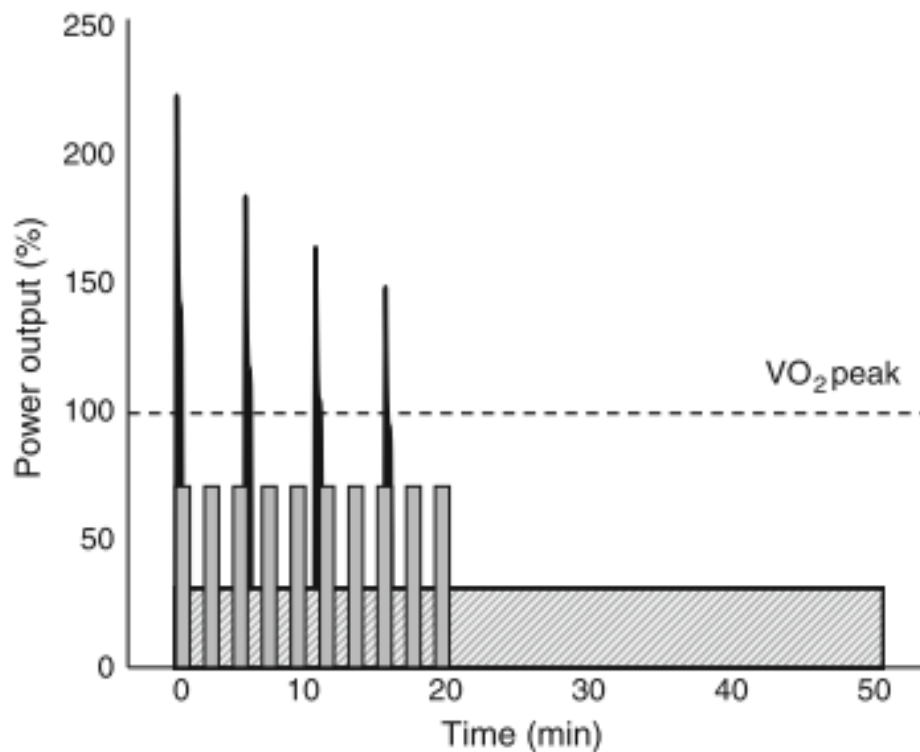


Figure 1. Example of SIT (4 x 30 second ‘all-out’ efforts; black bars), HIIT (10 x 60 second efforts at ~90 % HRmax; grey bars) and MICT (50 min at 70 % HRmax; hatched box). Taken with permission from Gibala et al. (2014) *Sports Medicine*. 44(2): 127-37.

At least over the short term (i.e. up to six weeks), low-volume interval training elicits physiological remodelling similar to MICT, despite a reduced total exercise volume and time commitment (35). For example, as little as six sessions of low-volume interval training over 2 weeks increased muscle oxidative capacity to the same extent as an endurance training protocol that required a three-fold greater time commitment and nine-fold higher training volume (15, 34). Several weeks of interval training also elicits favourable changes in markers of health status, including cardiorespiratory fitness (14) and glycemic control in both

healthy individuals (5, 51, 87) and those with type 2 diabetes (65). These findings are significant from a public health perspective, considering a “lack of time” remains a commonly cited barrier to regular exercise participation (17, 96, 113). These findings also challenge the notion of training specificity, and suggest that – at least over the short term – brief bursts of intense exercise (i.e., low-volume HIIT and SIT), induce similar adaptations as those elicited by prolonged, moderate-intensity muscular contractions.

The present thesis sought to advance our understanding of the physiological and health-related adaptations to low-volume interval training by addressing three major questions: 1) *does nutritional manipulation augment the adaptive response to low-volume HIIT*, 2) *what is the minimal dose of SIT required to improve cardiometabolic health*, and 3) *how do SIT-induced improvements in cardiometabolic health compare to those elicited by traditional MICT over the long term (i.e., 12 wk)*? Primary emphasis was placed on skeletal muscle mitochondrial content and markers of cardiometabolic health including cardiorespiratory fitness, insulin sensitivity and body composition. All studies involved 6- or 12-week exercise training interventions in previously sedentary adults.

## **1.2. Adaptations to low-volume interval training in humans**

### *1.2.1. Skeletal muscle*

One of the most well characterized adaptations to low-volume interval training is increased skeletal muscle mitochondrial content. As little as six sessions of SIT or HIIT over 2 weeks has been demonstrated to increase the maximal activity and/or protein content of mitochondrial enzymes including citrate synthase (CS) and cytochrome c oxidase (COX) (13, 15, 34, 51, 56, 67, 115). More recent evidence has revealed training-induced increases in mitochondrial respiratory capacity in permeabilized fibers (56, 115) and elevated in vivo oxidative capacity using phosphorus magnetic resonance spectroscopy (61). In addition to increased mitochondrial capacity, low-volume SIT and HIIT protocols lasting up to 6 weeks induce a number of other adaptations typically associated with MICT, including: increased resting muscle glycogen content (15, 67), a lower rate of glycogen utilization and lactate production during submaximal exercise (15), increased glucose transport capacity (12, 51, 65, 67), enhanced capacity for whole-body (14) and skeletal muscle lipid oxidation (14, 95), improved peripheral vascular structure and function (22, 84) and increased skeletal muscle microvascular content and enzyme activity (21, 22).

The molecular mechanisms underlying skeletal muscle remodeling following low-volume interval training appear to be mediated, at least in part, through similar molecular signaling pathways proposed to regulate the adaptive response to MICT (66). For example, acute activation of signaling pathways involved in mitochondrial biogenesis have been reported following a single session of SIT or HIIT, including phosphorylation of 5'AMP (adenosine-

monophosphate)-activated protein kinase (AMPK) (36, 66), p38 mitogen-activated protein kinase (p38 MAPK) (36, 66) and p53 (6, 28). Peroxisome-proliferator activated receptor  $\gamma$  coactivator (PGC)-1 $\alpha$ , which is regarded as the ‘master regulator’ of skeletal muscle mitochondrial biogenesis (125), is up-regulated following acute low-volume SIT and HIIT when measured 3 hours post exercise (28, 36, 66). Consistent with the response to MICT (124), PGC-1 $\alpha$  acutely translocates to the nucleus (66), which coincides with increased mRNA expression of several mitochondrial genes (28, 66). These transient and repeated pulses of gene expression in response to successive exercise bouts precede changes in skeletal muscle protein content and enzymatic activity (74) and have been proposed to regulate mitochondrial adaptations to exercise training (29).

### *1.2.2. Cardiorespiratory fitness*

Cardiorespiratory fitness, as objectively measured through a peak oxygen uptake ( $\text{VO}_{2\text{peak}}$ ) test, is considered to be a stronger predictor of risk for adverse health outcomes than traditional risk factors such as hypertension, smoking, obesity and hyperlipidemia (58). Low-volume SIT (4, 44, 118) or HIIT (31, 56), involving as few as six sessions over 2 weeks, has been shown to rapidly increase  $\text{VO}_{2\text{peak}}$ , although this is not a universal finding (15) and seems to depend in part on the initial fitness or activity level of the subjects. A recent systematic review and meta-analysis of 16 studies concluded that the aggregate improvement in  $\text{VO}_{2\text{max}}$  after SIT in young healthy individuals was 3.6 ml/kg/min (8 %), which

was no different than that elicited by MICT (38). These findings are consistent with a review by Sloth et al. (104) who analyzed 19 studies and reported  $\text{VO}_{2\text{max}}$  increases in the range of 4-13 % after 2-8 weeks of SIT in healthy sedentary or recreationally active individuals. This is important from a clinical perspective, as improvements of this magnitude ( $\sim 3.5$  ml/kg/min or 1 MET) have been shown to translate into a 15 and 19 % reduced risk of all-cause and cardiovascular disease mortality, respectively (63).

It has been suggested that SIT-induced improvements in  $\text{VO}_{2\text{peak}}$  may be mediated by peripheral factors (i.e., enhanced oxygen extraction), at least over the short-term (104). Although improvements in  $\text{VO}_{2\text{peak}}$  following MICT are traditionally believed to be centrally-mediated (7), several studies have failed to observe an increase in cardiac output following short-term SIT or HIIT (56, 70). In contrast, Esfandiari et al. (31) recently reported that Doppler-derived measures of end-diastolic volume, stroke volume, and cardiac output, as well as blood volume and  $\text{VO}_{2\text{max}}$ , were increased to a similar extent after a 2 weeks of HIIT or MICT. Continued investigation is needed to decipher the mechanisms mediating the rapid improvement in  $\text{VO}_{2\text{peak}}$  following low-volume interval training.

### *1.2.3. Glycemic control*

Several studies have reported improvements in glycemic control following short-term, low-volume interval training, in both healthy and diseased populations.

As little as 2-6 weeks of low-volume SIT has been shown to increase insulin sensitivity based on oral-glucose tolerance tests (OGTTs) in young healthy individuals (5, 22, 91, 95) and overweight/obese men (21, 118). Perhaps the most convincing evidence comes from Richards et al. (87) who reported increased insulin sensitivity 72 hours following a 2-week SIT intervention using the hyperinsulinemic-euglycemic clamp in recreationally active adults. Low-volume HIIT interventions lasting 2 weeks have also been shown to improve glycemic control in sedentary adults based on fasting-derived indices (51), and in patients with type 2 diabetes using continuous glucose monitoring (65). All of these studies have been relatively short-term interventions, with few direct comparisons to high-volume MICT protocols (21, 22, 91, 95).

While a coordinated response from multiple tissues is likely involved, exercise-induced adaptations within skeletal muscle are likely central to the improvement in glycemic control following low-volume SIT and HIIT. Skeletal muscle is the primary disposal site for ingested glucose (24) and is recognized to be a key contributor to the improvement in insulin sensitivity following MICT (49). The high degree of muscle fiber-recruitment (28) and glycogen utilization (19, 97) during acute SIT and HIIT may contribute to the training-induced improvements in insulin sensitivity (49). As little as 2 weeks of training increases whole-muscle GLUT4 protein content (12, 51, 65, 67), which is believed to play a role in exercise training-induced improvements in glycemic control (42, 49). In rodents, the training-induced increase in total GLUT4 protein content is



proportional to the increase in sarcolemmal GLUT4 translocation in response to a given insulin concentration (86), supporting the notion that elevated GLUT4 after training may help to improve glycemic control.

It is also possible that increased skeletal muscle mitochondrial content plays a role in mediating the improvement in insulin sensitivity following low-volume interval training. Individuals with obesity, insulin resistance and type 2 diabetes have reduced markers of skeletal muscle mitochondrial content compared to lean, healthy controls (46, 88, 89, 99, 100). There is also evidence of impaired *in vivo* mitochondrial function in individuals with insulin resistance (75, 76) and type 2 diabetes (93), supporting the theory that reduced mitochondrial content or oxidative capacity of skeletal muscle contributes to the development of insulin resistance and type 2 diabetes (68, 72, 77). Reduced mitochondrial content or function can result in defects in lipid oxidation (50, 55) and accumulation of deleterious lipid intermediates (e.g., fatty acyl CoA, diacylglycerol and ceramides) that can impair insulin signaling and GLUT4 translocation to the cell surface (68, 72, 114). If this theory holds true, increased mitochondrial content following low-volume interval training could improve insulin sensitivity by enhancing the capacity to oxidize lipid substrates. However, the hypothesis that reduced skeletal muscle oxidative capacity causes insulin resistance has been questioned (48). It is also possible that the increased skeletal muscle capillary density observed following low-volume SIT (21, 22) contributes to improvements in glucose transport and insulin sensitivity (1, 21).

#### *1.2.4. Body composition*

Limited evidence highlights the potential for low-volume interval training to induce favorable changes in body composition. Short-term SIT and HIIT interventions lasting 2 weeks are not associated with changes in body composition, however a 6-week running SIT intervention was shown to reduce fat mass in men (70). Trapp et al. (111) also reported that a cycling-based SIT protocol involving 60 repetitions of 8-second sprints, interspersed with 12 seconds of recovery, performed three times per week for 15 weeks, was more effective than a MICT protocol involving 40 minutes of cycling at 60 % of  $\text{VO}_{2\text{peak}}$  for decreasing whole body and abdominal fat mass in women. It is currently unknown if changes in body composition occur earlier than 15 weeks in women however, or if they can be elicited by less demanding HIIT protocols. Various factors have been suggested to mediate the observed changes in body composition after low-volume interval training (11), with likely candidates being increased post-exercise oxygen consumption (45, 94, 101) or changes in appetite (98, 119).

### **1.3. Potential for nutrition to augment the adaptive response to interval training**

Nutrient availability is a potent modulator of the adaptive response to endurance exercise training (43). Arguably one of the most effective nutritional interventions purported to augment select markers of the training response is

carbohydrate restriction. Originally proposed by Hansen et al. (40), it was suggested that repeated exercise sessions commenced in a low glycogen state would enhance physiological remodeling due to a greater relative stress placed upon the muscle. Indeed, using a unique design – involving once daily versus twice every other day training – several authors have reported superior training adaptations when half of the weekly training sessions are commenced with low endogenous carbohydrate (40, 54, 73, 127). Specifically, greater increases in resting muscle glycogen content (40, 127), maximal activities of mitochondrial enzymes including CS and  $\beta$ -hydroxy acyl CoA dehydrogenase ( $\beta$ -HAD) (40, 54, 127), and fat oxidation during submaximal exercise (54, 127) have been conferred using this “train low” strategy.

The superior training response is believed to be mediated, at least in part, by the enhanced activation of upstream signaling pathways believed to regulate mitochondrial biogenesis in response to exercise training. Indeed, a number of studies have reported an inverse relationship between skeletal muscle carbohydrate availability and the activation and/or nuclear translocation of AMPK $\alpha$ 2 (79, 106, 122, 126) and p38 MAPK (16, 19), which coincide with greater PGC-1 $\alpha$  mRNA expression (83) and nuclear translocation (79) 3 hours post exercise. Other pathways recently implicated in mitochondrial biogenesis, including p53 (6) and peroxisome proliferator activated receptor- $\delta$  (PPAR- $\delta$ ) (79), have been shown to be up-regulated to a greater extent when exercise is performed with low muscle glycogen. Indeed muscle glycogen is implicated as an

important signaling molecule (78) that can regulate skeletal muscle adaptation to exercise training.

An alternative strategy to alter carbohydrate availability before exercise is manipulation of exogenous carbohydrate sources by commencing exercise in the overnight fasted state. Fasted exercise is well recognized to alter substrate use during exercise, namely by increasing energy provision from fat oxidation compared to fed-state exercise (23, 26, 52, 60). The elevated circulating plasma epinephrine and low insulin concentrations (8) associated with fasting conditions stimulate lipolysis of both intramuscular (9) and peripheral adipose tissue depots (52). In contrast, carbohydrate ingestion prior to or during exercise reduces fatty acid lipolysis (8, 52) and oxidation (23), and increases the contribution of plasma glucose to energy provision (3, 23, 60). These differences in skeletal muscle fuel availability, and subsequent oxidation, have been suggested to alter the adaptive response to training (81, 82). Van Proeyen et al. (82) reported that 6 weeks of high-volume MICT in the fasted state, involving 60-90 minutes of cycling at 70 %  $\text{VO}_{2\text{peak}}$  four times per week, increased the maximal activities of CS and  $\beta$ -HAD to a greater extent than fed-state training in young healthy males. This is not a universal finding, however, as others have failed to detect differences in the extent of improvement in oxidative capacity following fasted versus fed-state training (2, 81, 105). In addition to potential differences in skeletal muscle remodeling, fasted but not fed-state MICT has been shown to increase insulin sensitivity and prevent weight gain in the face of a hyper-caloric fat-rich diet in young healthy males (81).

The improvement in insulin sensitivity in the fasted group was associated with a larger increase in GLUT4 protein content compared to fed-state training (81). Based on these findings, it is possible that performing HIIT in the fasted state could augment improvements in skeletal muscle oxidative capacity and insulin sensitivity. It is also possible that fasted HIIT could be a highly effective means to induce fat loss in overweight/obese individuals.

#### **1.4. How low can you go? Reducing the time commitment of low-volume interval training**

The interval training stimulus is infinitely variable and can be manipulated in various ways. Traditional Wingate-based SIT involving ‘all-out’ efforts is the most common, and perhaps most potent, low-volume interval training protocol employed to date. Despite being a highly effective training stimulus, SIT is extremely demanding and may not be well tolerated or appealing for most individuals (41). The relative “time efficiency” of SIT has also been questioned (37, 41), as once a warm-up, cool-down and rest between intervals are included, each session requires ~25 minutes. When performed three times per week, the total time commitment is in line with the 75 minutes of vigorous-intensity physical activity that some public health agencies suggest as an alternative to 150 minutes of moderate-intensity exercise (32, 123). A “lack of time” remains a commonly cited barrier to regular exercise participation (113), and therefore it is important to identify more time-efficient exercise alternatives.

A variety of approaches have been employed in an attempt to reduce the time commitment of low-volume interval training further, including reducing the duration (44, 69, 71, 102) or number (71) of high-intensity intervals, or the recovery period duration between sprints (20, 44, 69). A few of these protocols require  $\leq 10$  minutes per session, yet maintain the capacity to induce physiological remodeling and improve markers of cardiometabolic health. Reminiscent of the early work of Tabata and colleagues (108), Ma et al. (69) reported a 19 % increase in  $\text{VO}_{2\text{peak}}$  in young men following a 4-week SIT intervention, involving eight, 20-second cycling efforts at 170 % peak power, interspersed with 10 seconds recovery, when performed three times per week. These findings and those of others (20, 44, 71, 110) confirm that brief bursts of intense exercise, requiring  $\leq 10$  minutes a few times per week, improve cardiorespiratory fitness.

With respect to glycemic control, Metcalfe et al. (71) reported that a SIT protocol involving two, 20 second ‘all-out’ sprints within a 10-minute period of otherwise low-intensity cycling, improved insulin sensitivity in men, but not women, when performed three times per week for 6 weeks. While the low sample size ( $n=8$  for women) may have hindered the researchers ability to detect an improvement in the women, it is also possible that there are sex-based differences in the adaptive response to this type of training. It has been suggested that high rates of glycogen breakdown and subsequent resynthesis following acute intense exercise may explain the rapid improvement in insulin sensitivity following short-

term SIT (5). However, women are reported to break down ~50 % less muscle glycogen in type I fibers during a single Wingate sprint compared to their male counterparts (30). Further work is needed to clarify if women might in fact “respond less” to low-volume SIT.

Improvements in mitochondrial capacity are well established in response to traditional Wingate-SIT, however there is limited and equivocal data regarding the effect of very low-volume interval training on skeletal muscle remodeling. A 2-week SIT intervention, involving six sessions of 8-12, 10-second ‘all-out’ cycling sprints against 5.0 % body weight interspersed with 80 seconds of rest, did not improve oxidative capacity in overweight men as evidenced by no change in the protein content of COXII and IV (102). Ma and colleagues (69) however, reported increased protein abundance of COXI and IV after 4 weeks of “Tabata-style” SIT in young men. The maximal activity of CS was unchanged following training however, which is reportedly one of the best markers of mitochondrial content in human skeletal muscle (62). Further research is needed to clarify whether these brief SIT protocols induce similar physiological remodeling as those elicited by traditional SIT or MICT.

## **1.5. SIT compared to MICT for improving cardiometabolic health: The relative importance of exercise intensity versus volume**

### *1.5.1. Intensity versus volume for improving cardiometabolic health*

Exercise volume is a product of the duration, frequency and intensity of the activity performed (80), and is the major focus of public health recommendations (32, 112, 123). The positive relationship between exercise volume and cardiometabolic health is well established (80), with numerous dose response studies indicating that a greater exercise volume confers larger improvements in cardiometabolic health. For example, the DREW study (Dose Response to Exercise) compared three different volumes of exercise training on improvements in cardiorespiratory fitness in > 400 overweight or obese women (18). Women were assigned to one of three groups, required to expend either 4, 8 or 12 kcal/kg per week at a fixed intensity of 50 %  $\text{VO}_{2\text{peak}}$ . Following the 24-week intervention, there was a clear dose response between exercise volume and the improvement in cardiorespiratory fitness (4 vs. 6 vs. 8 % increase) (18). Similar results were obtained from STRRIDE (Studies of a Targeted Risk Reduction Intervention through Defined Exercise), as authors concluded that exercise volume was the greatest determinant of the improvement in cardiorespiratory fitness (27), insulin sensitivity (53) and body composition (103) following a 32-week exercise intervention in overweight adults. In addition to these functional outcomes, there is strong epidemiological evidence supporting an inverse and curvilinear relationship between exercise dose and risk of all-cause mortality (80, 116).

It has been more difficult however, to tease out the importance of exercise intensity, independent of its contribution to total volume of energy expended.



While some studies have reported added benefit of vigorous-intensity exercise, the greater volume of exercise associated with higher-intensity activity confounds these results. Thus, it is seemingly impossible to determine the importance of exercise intensity irrespective of exercise volume, unless exercise duration and frequency are manipulated.

Several authors have attempted to decipher the importance of exercise intensity on health indices when exercise is matched for total volume. In these scenarios, exercise duration is commonly reduced in the vigorous-intensity group in an attempt to maintain a given dose of energy expenditure. Results from studies of this nature suggest that when exercise is matched for total volume, higher-intensity exercise training confers larger improvements in  $\text{VO}_2\text{peak}$  (10, 39, 47, 59, 90, 107, 109, 121) and glycemic control (10, 25, 59, 90, 109). For example, Gormley and colleagues (39) assessed changes in cardiorespiratory fitness in response to three training programs that differed in exercise intensity, but were matched for total volume. Young men performed either moderate- (50 %  $\text{VO}_2$  reserve), vigorous- (75 %  $\text{VO}_2$  reserve) or near maximal- (95 %  $\text{VO}_2$  reserve) intensity exercise training for 6 weeks. Following training, there was a clear effect of exercise intensity on cardiorespiratory fitness, with the near maximal-intensity group conferring the largest improvement in  $\text{VO}_2\text{peak}$  (21 vs. 14 vs. 10 %). This is in agreement with work from Ulrik Wisloff's lab, suggesting that 12-16 weeks of high-volume HIIT is more effective than an equal volume of MICT for increasing  $\text{VO}_2\text{peak}$  in patients with heart failure (121) and metabolic syndrome

(109). Recent work from Ross et al. (90) also indicates that for a fixed amount of exercise, increases in cardiorespiratory fitness and glycemic control are intensity dependent. In this randomized control trial, 300 obese adults trained for 24 weeks in one of three exercise groups: 1. low-amount, low-intensity (300 kcal at 50 %  $\text{VO}_{2\text{peak}}$ ); 2. high-amount, low-intensity (600 kcal at 50 %  $\text{VO}_{2\text{peak}}$ ); or 3. high-amount, high-intensity (600 kcal at 75 %  $\text{VO}_{2\text{peak}}$ ). At the end of the intervention, improvements in  $\text{VO}_{2\text{peak}}$  and glycemic control, assessed by the 2-hour blood glucose concentration following an OGTT, were greater in those performing a given volume of exercise at a high- compared to low-intensity. Exercise-induced reductions in waist circumference however, were independent of exercise intensity (90). The intensity-dependent increase in glycemic control is consistent with other reports suggesting that volume-matched exercise performed at a higher intensity confers larger improvements (10, 25, 59, 109). Recent epidemiological evidence also suggests that there is an inverse relationship between relative exercise intensity and risk of coronary heart disease (64, 92), metabolic syndrome (57) and all-cause mortality (92, 120).

In general, findings highlighting the importance of exercise intensity oppose current physical activity guidelines which imply that, in comparison with moderate-intensity exercise, the benefits of engaging in vigorous-intensity exercise are attributed to the greater energy expenditure dose per unit of time and do not relate to intensity *per se*. However, given the evidence discussed above, it has been suggested that population-based physical activity interventions with a

focus on high-intensity exercise should be implemented at the national and international level (33, 85).

#### *1.5.2. Low-volume SIT versus high-volume MICT*

A number of studies have revealed positive improvements in cardiometabolic health following short-term SIT, yet relatively few provide direct comparison to adaptations elicited by MICT as often reflected in public health guidelines. Comparison between these diverse training strategies pose a unique question however, as exercise is *not matched* for total volume. Instead, studies of this nature raise the question: *Can a small dose of high-intensity exercise improve cardiometabolic health similar to a large dose of moderate-intensity exercise?*

There are currently no studies comparing adaptations to very brief SIT protocols requiring  $\leq 10$  minutes per session to those elicited by traditional MICT. However, comparisons between Wingate-based SIT and MICT reveal similar increases in skeletal muscle oxidative capacity (14, 34), cardiorespiratory fitness (14) and insulin sensitivity derived from OGTTs (22, 91, 95), at least over the short-term (i.e. up to 6 weeks). It has been suggested that the relatively short-term studies may favour SIT with respect to the early time course of physiological adaptations, and potential divergent adaptations from MICT may be more apparent if the duration of training is extended (104). However, to date no studies have directly compared adaptations to SIT and MICT protocols lasting longer than 6 weeks.

## **1.6. Scope and nature of this work**

The overall purpose of the present thesis is to advance our understanding of the physiological and health-related adaptations to low-volume interval training in sedentary adults. While a number of studies suggest that low-volume interval training induces physiological remodeling traditionally associated with endurance training, a number of fundamental avenues regarding the chronic response to SIT and HIIT remain to be explored.

First, nutrition is recognized to be a potent modulator of adaptive response to endurance exercise training, but little is known regarding the potential for prolonged nutritional manipulation to augment HIIT-induced remodeling. The purpose of Study 1 was to evaluate the effect of HIIT in the fed versus fasted state on skeletal muscle oxidative capacity, body composition and insulin sensitivity based on OGTTs. In light of recent findings following high-volume endurance training (81, 82), we hypothesized that 6 weeks of HIIT performed in the fasted state would improve muscle mitochondrial capacity, body composition and insulin sensitivity to a greater extent than training in the fed state in sedentary women.

Low-volume SIT is a highly effective training stimulus, but it is extremely demanding and may not be well tolerated or appealing for most individuals (41). The relative “time efficiency” of SIT has also been questioned (37, 41). Several recent reports suggest that it may be possible to confer similar benefits using brief SIT protocols requiring  $\leq 10$  minutes per session. The purpose of Study 2 was to

clarify and advance our understanding of the impact of very low-volume SIT on physiological and health related adaptations in sedentary men and women.

Specifically, we examined the impact of a training protocol that involved only 1 minute of intense intermittent exercise within a 10-minute time commitment, including warm-up and cool-down. Our primary hypothesis was that 6 weeks of training would increase skeletal muscle oxidative capacity, improve  $\text{VO}_{2\text{peak}}$ , and reduce mean 24-hour blood glucose concentration measured using continuous glucose monitoring (CGM).

Based on findings from Study 2, the purpose of Study 3 was to directly compare the effects of 12 weeks of SIT and MICT on indices of cardiometabolic health in sedentary men. No other studies have directly compared this type of very low-volume SIT to high-volume MICT reflected in public health guidelines, nor examined changes occurring beyond 6 weeks. We hypothesized that, compared to a non-training control group, SIT and MICT would similarly increase  $\text{VO}_{2\text{peak}}$ , skeletal muscle mitochondrial capacity, and insulin sensitivity based on the intravenous glucose tolerance test (IVGTT) method.

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## CHAPTER 2

### **Interval training in the fed or fasted state improves body composition and muscle oxidative capacity in overweight women**

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## Original Article

CLINICAL TRIALS: BEHAVIOR, PHARMACOTHERAPY, DEVICES, SURGERY



## Interval Training in the Fed or Fasted State Improves Body Composition and Muscle Oxidative Capacity in Overweight Women

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**Objective:** To investigate the effects of low-volume high-intensity interval training (HIT) performed in the fasted (FAST) versus fed (FED) state on body composition, muscle oxidative capacity, and glycemic control in overweight/obese women.

**Design and Methods:** Sixteen women ( $27 \pm 8$  years, BMI:  $29 \pm 6$  kg/m<sup>2</sup>,  $\dot{V}O_{2\text{peak}}$ :  $28 \pm 3$  ml/kg/min) were assigned to either FAST or FED ( $n = 8$  each) and performed 18 sessions of HIT ( $10 \times 60$ -s cycling efforts at  $\sim 90\%$  maximal heart rate, 60-s recovery) over 6 weeks.

**Results:** There was no significant difference between FAST and FED for any measured variable. Body mass was unchanged following training; however, dual energy X-ray absorptiometry revealed lower percent fat in abdominal and leg regions as well as the whole body level (main effects for time,  $P \leq 0.05$ ). Fat-free mass increased in leg and gynoid regions ( $P \leq 0.05$ ). Resting muscle biopsies revealed a training-induced increase in mitochondrial capacity as evidenced by increased maximal activities of citrate synthase and  $\beta$ -hydroxyacyl-CoA dehydrogenase ( $P \leq 0.05$ ). There was no change in insulin sensitivity, although change in insulin area under the curve was correlated with change in abdominal percent fat ( $r = 0.54$ ,  $P \leq 0.05$ ).

**Conclusion:** Short-term low-volume HIT is a time-efficient strategy to improve body composition and muscle oxidative capacity in overweight/obese women, but fed- versus fasted-state training does not alter this response.

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### Introduction

Most adults fail to meet minimum physical activity guidelines, often citing a “lack of time” as a key barrier to regular exercise participation (1). High-intensity interval training (HIT), characterized by brief bursts of intense exercise separated by short periods of recovery, is a time-efficient stimulus to induce physiological adaptations normally associated with continuous moderate-intensity training (2). For example, as little as six sessions of HIT over 2 weeks increased muscle oxidative capacity to the same extent as a continuous moderate-intensity training protocol that required an approximately three-fold greater time commitment and approximately ninefold higher training volume (3). Short-term low-volume HIT has also been reported to improve various health-related indices in healthy adults including insulin sensitivity (4,5) and cardiovascular function (6). We recently showed that a practical, low-volume HIT model consisting of  $10 \times 60$ -s intervals at 90% of maximal heart rate ( $HR_{\text{max}}$ ) reduced 24-h blood glucose concentration immediately following an acute session of HIT (7) and 72 h following a 2-week HIT intervention (8) in patients with type 2 diabetes.

Despite the abundance of evidence linking low-volume HIT to improved metabolic and cardiovascular fitness, the effect on body composition is less known. Short-term HIT interventions lasting 2 weeks yield no change in body composition; however, Trapp and colleagues reported that a 15-week maximal effort HIT intervention was more effective than moderate-intensity continuous exercise at reducing whole body fat mass and in particular, intra-abdominal adiposity in young healthy women (9). Six-week “all-out” sprint interval running has also been shown to reduce fat mass in men (10). It has yet to be determined in a large cohort of females if changes in body composition occur earlier than 15 weeks or if they can be induced by less intense HIT protocols that do not demand all-out efforts.

Nutrient availability is a potent modulator of many acute physiological responses to exercise. Recently, it was reported that 6-week of endurance training in the fasted state potentiated adaptations in muscle oxidative enzymes (11), glucose, and fatty acid transport capacities (11,12) and prevented weight gain in the face of a high calorie

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**TABLE 1** Subject characteristics

Variable	Fed	Fasted	All
Age, years	27 ± 7	27 ± 9	27 ± 8
Height, cm	163 ± 8	163 ± 5	1.63 ± 6
Weight, kg	77 ± 13	78 ± 13	78 ± 12
BMI, kg/m <sup>2</sup>	29 ± 3	29 ± 4	29 ± 3
VO <sub>2peak</sub> , l/min	2.2 ± 0.4	2.1 ± 0.4	2.2 ± 0.4
VO <sub>2peak</sub> , ml/kg/min	29 ± 6	27 ± 6	28 ± 6
Maximal workload, W/kg	2.6 ± 0.03	2.7 ± 0.04	2.7 ± 0.04

Values are means ± SD.  $n = 8$  for the fed- and fasted-state training group. VO<sub>2peak</sub>, peak oxygen uptake. There was no difference between groups in any variable.

fat-rich diet in young healthy men (12). It was speculated that the greater distortion of energy homeostasis associated with fasted-state exercise triggered the augmented training response (11).

The purpose of the present study was to evaluate the effect of HIT in the fed versus fasted state on body composition, skeletal muscle metabolic capacity, and glycemic control. We hypothesized that HIT performed in the fasted state would improve body composition, muscle mitochondrial capacity, and insulin sensitivity to a greater extent than training in the fed state in overweight/obese women.

## Methods and Procedures

### Subjects

Sixteen overweight/obese women took part in the study (Table 1). Subjects were deemed sedentary based on their self-reported habitual physical activity which consisted of  $\leq 2$  sessions/week of structured exercise lasting  $\leq 30$  min. The study protocol was approved by the Hamilton Health Sciences/McMaster University Faculty of Health Sciences Research Ethics Board. Following routine medical screening to rule out any conditions that may have precluded participation, subjects provided written informed consent.

### Experimental protocol

The experimental protocol consisted of three phases: (1) baseline testing; (2) a 6-week HIT protocol under one of the two dietary interventions; and (3) post-training measurements.

**Baseline testing.** Subjects performed an incremental maximal oxygen uptake (VO<sub>2peak</sub>) test on an electronically braked cycle ergometer (Lode Excalibur Sport V 2.0, Groningen, The Netherlands) using procedures similarly to what we have previously described (13). Briefly, following a 5-min warm-up at 50 W, resistance was increased by 1 W every 2 s until volitional exhaustion or the point at which pedal cadence fell below 50 rpm. A metabolic cart with an online gas collection system (Moxus modular oxygen uptake system, AEI Technologies, Pittsburgh, PA) acquired oxygen consumption (VO<sub>2</sub>) and carbon dioxide production (VCO<sub>2</sub>) data. VO<sub>2peak</sub> was defined as the highest average oxygen consumption over a 15-s period. Peak power output ( $W_{max}$ ) and maximal heart rate (HR<sub>max</sub>) were also recorded. Pairs of subjects were matched for age, BMI,

VO<sub>2peak</sub>, and oral contraceptive use and then randomly assigned to either the fed (FED) or fasted (FAST) state training condition.

Baseline testing included an oral glucose tolerance test (OGTT) following a  $\geq 10$  h overnight fast. An indwelling catheter was inserted into a forearm vein, and a single fasting blood sample was obtained before ingestion of a 75 g glucose solution. Thereafter, blood samples were collected into appropriately treated tubes at 10, 20, 30, 60, 90, and 120 min and immediately placed on ice. Plasma and serum were separated by centrifugation (10 min at 4000 rpm) and stored at  $-20^{\circ}\text{C}$  for subsequent analysis. Following the OGTT, participants recorded their dietary intake over a 3-day period before returning to the lab to undergo a dual energy X-ray absorptiometry (DEXA) scan (Lunar Prodigy Advance, Madison, WI) and resting skeletal muscle biopsy procedure as we have previously described (3). Muscle samples were obtained from the vastus lateralis under local anesthesia (1% Lidocaine) using a Bergstrom needle adapted with suction. Samples were blotted to remove excess blood, sectioned into several pieces, immediately snap frozen in liquid nitrogen, and stored at  $-80^{\circ}\text{C}$  for later analysis.

**Training protocol.** The HIT protocol involved 18 supervised sessions over 6 weeks (Monday, Wednesday, Friday each week). Each session consisted of 10  $\times$  60-s cycling bouts interspersed with 60-s recovery as we have previously described (8,13). Training was performed on an ergometer (LifeCycle C1, Life Fitness, Schiller Park, IL) set in constant watt mode at a pedal cadence of 80-100 rpm. Individual workloads were selected to elicit a heart rate of  $\sim 90\%$  HR<sub>max</sub> during the intervals. Heart rate and rating of perceived exertion (RPE; using a 0-10 scale) were recorded at the end of each interval. During the 60-s recovery, participants rested or pedaled slowly at a resistance of 50 W. Training sessions included a 3-min warm-up and a 2-min cool-down at 50 W, for a total time commitment of 25 min. The weekly training protocol therefore involved a 30-min high-intensity exercise within a 75-min time commitment including warm-up and cool-down. All training sessions were performed in the morning from 0700 to 1000 h.

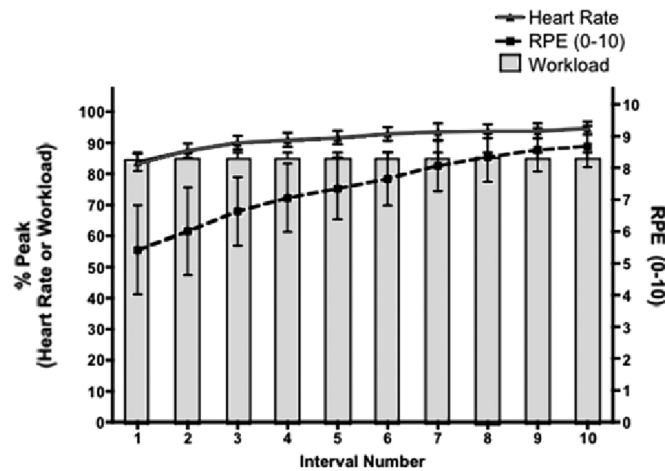
**Nutritional intervention.** Subjects ingested a standardized and identical breakfast on training days. The FED group ingested the meal  $\sim 60$  min prior to training, while the FAST group remained in the overnight fasted state and ingested their meal  $\sim 60$  min following exercise. The meal consisted of an energy bar, yogurt, and orange juice, which provided 439 kcal derived from 74% carbohydrate, 14% fat, and 12% protein. Aside from the standardized breakfast provided on training days, no other dietary controls were applied in order to simulate normal free-living conditions. Subjects were instructed to maintain their pre-training dietary habits throughout this study, which was confirmed by 3-day diet records at weeks 3 and 6 of training ( $P > 0.05$ ; Food Processor SQL).

**Post-testing.** An OGTT was performed 72 h after the last training session, followed by a DEXA scan and resting muscle biopsy procedure 24 h later. A VO<sub>2peak</sub> test was completed 6 days following training cessation. All procedures and controls were identical to those employed during baseline testing.

### Blood analysis

Plasma glucose was analyzed using a kit assay (Pointe Scientific, Canton, MI), and serum insulin was measured by ELISA (ALPCO





**FIGURE 1** Characterization of the HIT training protocol. Training intensity expressed as percent of maximal workload (bars), maximal heart rate (solid line), and RPE (dashed line) averaged across all 18 sessions for all subjects. Values are means  $\pm$  SD ( $n = 16$ ). RPE, ratings of perceived exertion.

Immunoassays, Salem, NH). Glucose and insulin area under the curve (AUC) values were calculated using the trapezoidal rule. Insulin sensitivity was calculated using ISI(HOMA) (14), ISI(composite) (14), and ISI(Cederholm) (15).

#### DEXA analysis

A whole body DEXA scan on a Lunar Prodigy Advance (Madison, WI) was used to measure body composition by one trained technician. The abdominal region included the area distal to T12 and superior to the iliac crest.

#### Muscle analysis

**Enzyme activity.** One piece of muscle (~25 mg) was homogenized using a glass tissue grinder (Kimble/Kontes 885300-0002) in 20 volumes of buffer containing 70 mM sucrose, 220 mM mannitol, 10 mM 4-(2-hydroxyethyl)-1 piperazineethanesulfonic acid (HEPES) supplemented with protease inhibitors (Complete Mini®, Roche Applied Science, Laval, PQ, Canada) and used to determine the maximal activity of citrate synthase (CS) and  $\beta$ -hydroxyacyl-CoA dehydrogenase ( $\beta$ -HAD) as we have previously described (3,13,16). Protein concentration of homogenates was determined using a commercial assay (BCA Protein Assay, Pierce, Rockford, IL), and enzyme activity is expressed as mmol/kg protein/h wet weight.

**Western blotting.** A second piece of muscle (~30 mg) was homogenized in radioimmunoprecipitation assay (RIPA) buffer for Western blot analyses using techniques described previously (3,13). Briefly, protein concentration of homogenates was determined (BCA Protein Assay), and equal amounts of protein (20  $\mu$ g) were prepared in 4 $\times$  Laemmli's buffer, heated to 95°C before being separated by 12.5% sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-

PAGE), and electrotransferred to nitrocellulose membranes. Ponceau S staining was performed following transfer to visualize equal loading and transfer. Following 1-h blocking in 5% fat-free milk Tris-buffered saline 0.1% Tween® 20 (TBS-T), membranes were incubated in the primary antibody (glucose transporter 4 [GLUT4]; Millipore, AB1345) overnight at 4°C or in 3% bovine serum albumin TBS-T based on previously optimized conditions. After 3 $\times$  5-min washes in TBS-T, membranes were incubated in the species-specific secondary antibody diluted (1:10,000) in 3% fat-free milk TBS-T for 1 h at room temperature (RT), washed in TBS-T for 3 $\times$  15 min, and visualized by chemiluminescence (SuperSignal West Dura, Pierce) using a FluorChem® SP Imaging System (Alpha Innotech Corporation, San Leandro, CA). ImageJ software (NIH) was used to quantify the optical density of protein bands.

#### Statistical analysis

Muscle, blood, and DEXA scans were analyzed by way of a two-factor ANOVA, with one between-factor (group; FAST versus FED) and one within-factor (time; pre-training versus post-training). Pearson's product-moment correlation coefficient was used to determine the relationship between variables. The level of significance for all analyses was set at  $P \leq 0.05$ , and all data are presented as means  $\pm$  SD. All data are based on  $n = 8$  for both groups; however owing to difficulties during data collection, we only report an  $n = 7$  for FED blood analyses and  $n = 6$  for FAST muscle data.

## Results

### Exercise responses and training data

All subjects completed the 18 exercise training sessions, except for one who missed two sessions during week 3 owing to an illness

TABLE 2 Health adaptations

Variable	Fed		Fasted	
	Pre	Post	Pre	Post
Body mass, kg	77 ± 12	77 ± 13	79 ± 15	79 ± 15
Total fat mass, kg*	30.3 ± 7.9	29.7 ± 7.9	32.3 ± 10.3	31.7 ± 10.2
Total lean mass, kg	43.5 ± 8.2	44.1 ± 7.8	42.8 ± 5.5	43.3 ± 5.5
Percent body fat*	40.9 ± 5.8	40.1 ± 5.4	42.3 ± 8.1	41.6 ± 7.8
Abdominal fat mass, kg*	2.65 ± 0.5	2.63 ± 0.5	2.76 ± 0.9	2.66 ± 0.9
Abdominal percent fat*	47.7 ± 6.3	47.1 ± 6.4	48.2 ± 7.8	46.7 ± 8.1
Leg lean mass, kg*	15.3 ± 3.1	15.7 ± 3.1	14.7 ± 1.4	15.1 ± 1.9
Gynoid lean mass, kg*	6.4 ± 1.1	6.6 ± 1.0	6.4 ± 0.8	6.5 ± 0.7
Fasting plasma glucose, mmol/l	4.5 ± 0.6	4.4 ± 0.5	5.0 ± 0.8	4.7 ± 0.8
Fasting plasma insulin, pmol	41 ± 15	59 ± 20	54 ± 50	68 ± 66
Glucose AUC, mmol/l 3 h*	752 ± 143	720 ± 120	748 ± 63	691 ± 136
Insulin AUC, $\mu$ U/ml 3 h	4325 ± 2301	5020 ± 1945	6292 ± 4670	6224 ± 4001
ISI(HOMA)	13.9 ± 5.2	10.4 ± 4.0	12.9 ± 7.8	11.2 ± 6.3
ISI(composite)	7.4 ± 1.1	5.7 ± 1.9	6.3 ± 3.1	5.9 ± 3.6
ISI(Cederholm)	70 ± 18	69 ± 18	71 ± 20	78 ± 30

Values are means  $\pm$  SD. \*Significant difference of pre- versus post-training (main effect for condition such that pre-training  $\neq$  post-training,  $P \leq 0.05$ ).

unrelated to this study. Interval intensity averaged  $85 \pm 5\%$  of  $W_{max}$ , elicited  $91 \pm 2\%$   $HR_{max}$ , and was associated with an RPE of  $7.4 \pm 0.9$ , with no differences between groups (Figure 1). Training increased  $VO_{2peak}$  with no difference between groups (FED:  $34.3 \pm 5.2$  ml/kg/min vs.  $28.2 \pm 6.1$  ml/kg/min; FAST:  $31.3 \pm 5.7$  ml/kg/min vs.  $27.4 \pm 6.4$  ml/kg/min; main effect for time,  $P < 0.001$ ). This was associated with a 12% and 10% increase in peak power output for FED ( $228 \pm 23$  W vs.  $200 \pm 27$  W) and FAST ( $240 \pm 37$  W vs.  $215 \pm 34$  W), respectively (main effect for time,  $P < 0.001$ ).

### Body composition

Body mass was unchanged following training (FED:  $77 \pm 13$  kg vs.  $77 \pm 12$  kg; FAST:  $79 \pm 15$  kg vs.  $79 \pm 15$  kg,  $P > 0.05$ ). DEXA revealed lower percent fat after training in abdominal and leg regions as well as at the whole body level (Table 2,  $P \leq 0.05$ ). HIT also reduced abdominal (Table 2,  $P \leq 0.05$ ) and total body fat mass (Figure 2,  $P \leq 0.05$ ). Lean mass increased in leg and gynoid regions (Table 2,  $P \leq 0.05$ ) and tended to increase at the whole body level (Figure 2,  $P = 0.07$ ). There was no effect of the dietary intervention on changes in body composition (Table 2).

### Skeletal muscle and blood metabolites

The maximal activity of CS increased by 23% and 22% following HIT in FED and FAST, respectively (Figure 3, main effect for time,  $P \leq 0.01$ ).  $\beta$ -HAD tended to increase more after FAST ( $\sim 19\%$ ) compared to FED ( $\sim 10\%$ ), but the difference was not significant (main effect for time,  $P \leq 0.05$ ). GLUT4 protein content increased by 42% in FED and 61% in FAST following training (Figure 3, main effect for time,  $P \leq 0.05$ ). Glucose AUC during the OGTT was lower after training (Table 2, main effect for time,  $P \leq 0.05$ ). However, there was no change in any marker of insulin sensitivity owing to a slight but non-significant change in insulin concentration

(Table 2,  $P > 0.05$ ). Interestingly, changes in insulin AUC were positively correlated with changes in the abdominal percent fat (Figure 4,  $r = 0.54$ ,  $P = 0.04$ ).

## Discussion

The major novel finding from the present study was that, regardless of nutritional intake around the training sessions, 6-week low-volume HIT favorably altered body composition in overweight and obese women despite no change in body mass. Following training, percent fat was reduced in both the abdominal and leg regions as

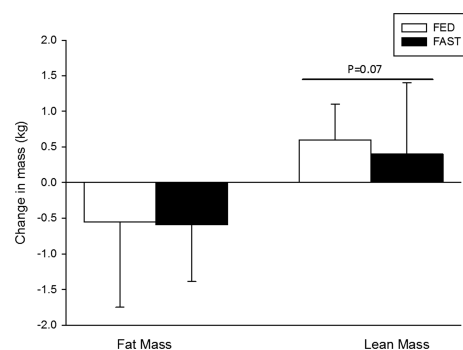
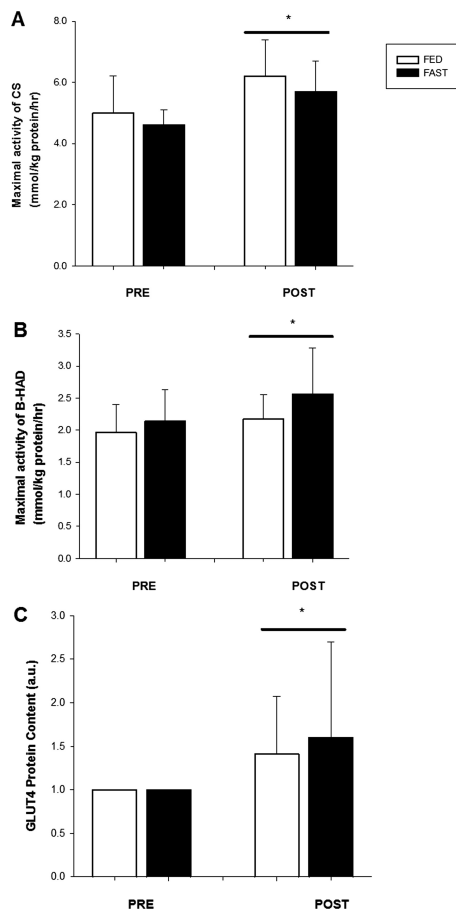


FIGURE 2 Change in total body fat and lean mass analyzed from DEXA scans taken before and 96 h following 6-week HIT in either the fed (FED; white bar) or fasted (FAST; black bar) state.  $P < 0.05$  unless otherwise stated.

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## Obesity



**FIGURE 3** Skeletal muscle mitochondrial and glucose transport capacity measured in biopsy samples obtained from the vastus lateralis before (PRE) and 96 h after (POST) 6-week HIT performed in the fed (FED) or fasted state (FAST). Maximal activity of CS (**A**) and  $\beta$ -HAD (**B**) and protein content of GLUT4 (**C**). Values are means  $\pm$  SD ( $n = 6$ , FAST;  $n = 8$ , FED). \* $P < 0.05$ , main effect for time.

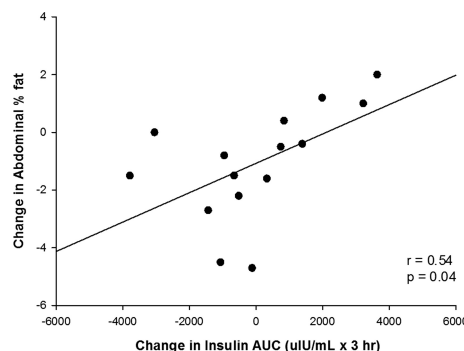
well as at the whole body level. To our knowledge, we are also the first to report a gain in fat-free mass following low-volume HIT in women. Considering the exercise protocol involved only 30-min intense exercise per week, our data suggest that HIT represents a time-efficient exercise alternative for improving body composition. We cannot comment on how this compares to endurance training as the major goal of the present investigation was to assess the potential for nutrition to potentiate the effects of HIT as opposed to making a direct comparison versus endurance training *per se*. That said, we did not detect any measurable differences between fed- and fasted-state HIT on training outcomes. Power analyses performed on

variables which were the closest to being significantly different between groups ( $VO_{2peak}$ ,  $\beta$ -HAD maximal activity, and abdominal fat mass) revealed that a sample size of 21-63 per group would have been necessary to detect a significant difference with 80% power. The practical message from our findings would seem to be that the beneficial effects of HIT can be realized regardless of when food is ingested around the acute training sessions.

## Effect of low-volume HIT on body composition

Public health guidelines recommend adults accumulate  $\sim 150$  min of “moderate-to-vigorous-intensity” aerobic physical activity each week (17); however, most adults do not meet these guidelines. While we recognize reasons for inactivity including numerous psychological, social, and environmental factors, one commonly cited barrier to regular exercise participation is lack of time (1). In the present study, we show that 6-week low-volume HIT induced small but significant improvements in body composition, including reduced adiposity at the abdominal and whole body level and increased leg lean mass. Few studies have investigated the effect of HIT on fat mass; however, early reports suggest that 20-week sprint training reduced subcutaneous fat, as measured by skin folds, to a greater extent than moderate-intensity continuous exercise training (18). More recently, Trapp and colleagues reported superior fat loss in young women following 15-week HIT compared to moderate-intensity continuous endurance training (9). The present data are consistent with the findings from Trapp et al. and demonstrate that low-volume HIT can induce fat loss in as early as 6 weeks and does not need to be performed using “supramaximal” efforts in order to be effective. Interestingly, Macpherson and colleagues did not detect a change in fat mass following 6-week sprint interval running in young healthy women (10). While these findings appear to conflict with the present study, the lack of improvement could be attributed to low sample size ( $n = 4$ ) and different HIT protocol and/or subject characteristics.

The mechanisms mediating the reductions in fat mass following HIT remain elusive. The large hormonal response associated with HIT may be important, as catecholamines are known to drive lipolysis of



**FIGURE 4** Relation between the change in abdominal fat percentage and the change in insulin AUC after 6-week HIT.  $P < 0.05$ .

both subcutaneous and intramuscular fat stores. Post-exercise oxygen consumption (EPOC) could also play a role (19), as Hazell and colleagues recently reported equivalent 24 h EPOC following an acute bout of HIT (4× 30-s sprints with 4-min recovery) and a 30-min bout of continuous moderate-intensity exercise (20). Lastly, appetite suppression following high-intensity exercise could be involved, as suggested by Boutcher (21) in a recent review on HIT-induced fat loss.

To our knowledge, we are the first to report a gain in fat-free mass following low-volume HIT in women. Heydari et al. found increased fat-free mass in the leg (0.4 kg) and trunk (0.7 kg) regions following 12-week HIT in young men (22). In the present study, we found similar gains in leg fat-free mass (0.3 kg) as Heydari et al. (0.4 kg) despite our training program lasting only 6 weeks. A direct comparison between HIT and endurance training at this time point would be interesting, as it is likely that the increase in fat-free mass is unique to HIT. Future studies could investigate whether low-volume HIT acutely stimulates muscle protein synthesis, which could be related to the observed increase in leg fat-free mass.

#### Skeletal muscle adaptations after fed- versus fasted-state HIT

Acute endurance exercise performed in the fasted state enhances the energy contribution from fat oxidation compared to fed-state exercise (23,24). This difference in skeletal muscle fuel utilization has been proposed to alter the training response (11,12). Van Proeyen et al. (11) recently reported that following 6-week high-volume endurance training in young healthy males, increases in the maximal activity of CS and  $\beta$ -HAD as well as glucose and fatty acid transport capacity were significantly greater in subjects who trained in the fasted compared to the carbohydrate fed state. This, however, is not a universal finding, as another work has shown no difference between fed- and fasted-state endurance training on improvements in muscle oxidative capacity (25). In the present study, we did not detect a difference in training-induced gains in the maximal activity of CS and  $\beta$ -HAD or GLUT4 protein content between FAST and FED after 6-week HIT.

Although HIT induces similar metabolic adaptations as continuous endurance training, there are fundamental differences in the duration, intensity, and thus the pattern of substrate utilization during each exercise bout compared to END. In the work by Van Proeyen et al. (11), subjects cycled for 60-90 min/session, a stimulus previously shown to deplete muscle glycogen by ~70% (26). In this scenario, exogenous glucose availability likely becomes an important substrate, and the differential metabolic states of FAST and FED could alter muscle remodeling. The short nature and higher intensity of training employed in the present study however likely rely more on intramuscular substrates (particularly glycogen), with relatively little difference in glucose utilization. Mixed venous blood glucose concentration measured before and immediately following the first exercise bout in a subset of participants ( $n = 9$ ) revealed no change in glucose availability during exercise in either FAST or FED (data not shown). We did not measure acute muscle glycogen utilization; however, previous work has reported that five 3-min bouts (27) or five 4-min bouts (28) of interval exercise decrease muscle glycogen content by only 30-35%. It is also possible that the potency of the HIT stimulus may overshadow any effect of the nutritional intervention, especially in untrained individuals like those in the present

investigation. We also recognize that our relatively small sample size may have limited our ability to detect small differences that potentially exist between FAST and FED for some outcome variables. Finally, it is possible that sex-based differences exist as Stannard and colleagues found that fasted END stimulates greater increases in CS activity in men than women (25).

#### Low-volume HIT and markers of insulin sensitivity

We found no training-induced change in insulin sensitivity when measured 3 days following the last training bout in either FED or FAST. Previous work has reported improved insulin sensitivity in young healthy males at this time point following HIT (4,5); however, this is not universal (22,29,30). It has been proposed that the rapid improvement in insulin sensitivity following HIT is attributed to muscle glycogen breakdown and its subsequent resynthesis following each exercise bout (4); however, women have been shown to utilize up to 50% less glycogen during a single Wingate sprint compared to men (31). Indeed, a recent report suggested that improvements in insulin sensitivity following a very low-volume HIT model were sex-specific, with improvements observed in men but not the women when measured 3 days after training (29). Individual analyses revealed that some of our subjects appeared to be “nonresponders,” and it has been demonstrated that up to 25% of the population does not improve insulin sensitivity following a period of exercise training with 15% actually showing a decline (32). Despite the lack of change in insulin sensitivity, an interesting observation was that the change in insulin AUC was positively correlated with the change in abdominal percent fat. While this does not provide any insight into a cause and effect, abdominal adiposity has been implicated as a major determinant of insulin resistance in women, and it is in keeping with the findings of a significant correlation between loss of abdominal fat and improvements in insulin sensitivity following energy restriction (33).

#### Conclusion

In summary, we report that 6-week low-volume HIT, consisting of only 30-min exercise within a 1-h time commitment per week, improved body composition and skeletal muscle oxidative capacity in overweight and obese women. These adaptations were realized regardless of when food was ingested around the acute training sessions and provide evidence to suggest that HIT is a time-efficient and effective exercise strategy to improve fitness in overweight women. The HIT protocol employed in our study is more practical than previous models such as Wingate-based HIT that demands maximal efforts and may be especially attractive for those with limited time available. It is currently unknown how such a small volume of total exercise can induce these favorable adaptations in body composition. The lack of control group is a limitation of the present investigation, and future studies are needed to identify how these findings compare to 6-week traditional endurance training. It is also possible that sex-based differences influence the adaptive response to HIT and future studies should make comprehensive comparisons in this regard. **O**

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JBG and MJG designed the study; JBG, MEP, MAT, and MJG were involved in data collection; data analysis was completed by JBG,

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MEP, and AL; JBG, MEP, AL, MAT, and MJG interpreted results of experiments; JBG prepared figures; JBG drafted the manuscript; JBG, MEP, AL, MAT, and MJG edited and revised this manuscript; and all authors approved the final version. This project was supported by a Natural Science and Engineering Research Council (NSERC) operating grant and McMaster University internally sponsored research grant to MJG. JBG was supported by a NSERC Canada Graduate Scholarship (Masters). MEP held an undergraduate student research award from the Canadian Institutes of Health Research (CIHR).

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### CHAPTER 3

**Three minutes of all-out intermittent exercise per week increases skeletal muscle oxidative capacity and improves cardiometabolic health**

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# Three Minutes of All-Out Intermittent Exercise per Week Increases Skeletal Muscle Oxidative Capacity and Improves Cardiometabolic Health

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## Abstract

We investigated whether a training protocol that involved 3 min of intense intermittent exercise per week — within a total training time commitment of 30 min including warm up and cool down — could increase skeletal muscle oxidative capacity and markers of health status. Overweight/obese but otherwise healthy men and women ( $n = 7$  each; age =  $29 \pm 9$  y; BMI =  $29.8 \pm 2.7$  kg/m<sup>2</sup>) performed 18 training sessions over 6 wk on a cycle ergometer. Each session began with a 2 min warm-up at 50 W, followed by  $3 \times 20$  s “all-out” sprints against 5.0% body mass (mean power output:  $\sim 450$ – $500$  W) interspersed with 2 min of recovery at 50 W, followed by a 3 min cool-down at 50 W. Peak oxygen uptake increased by 12% after training ( $32.6 \pm 4.5$  vs.  $29.1 \pm 4.2$  ml/kg/min) and resting mean arterial pressure decreased by 7% ( $78 \pm 10$  vs.  $83 \pm 10$  mmHg), with no difference between groups (both  $p < 0.01$ , main effects for time). Skeletal muscle biopsy samples obtained before and 72 h after training revealed increased maximal activity of citrate synthase and protein content of cytochrome oxidase 4 ( $p < 0.01$ , main effect), while the maximal activity of  $\beta$ -hydroxy acyl CoA dehydrogenase increased in men only ( $p < 0.05$ ). Continuous glucose monitoring measured under standard dietary conditions before and 48–72 h following training revealed lower 24 h average blood glucose concentration in men following training ( $5.4 \pm 0.6$  vs.  $5.9 \pm 0.5$  mmol/L,  $p < 0.05$ ), but not women ( $5.5 \pm 0.4$  vs.  $5.5 \pm 0.6$  mmol/L). This was associated with a greater increase in GLUT4 protein content in men compared to women (138% vs. 23%,  $p < 0.05$ ). Short-term interval training using a 10 min protocol that involved only 1 min of hard exercise, 3x/wk, stimulated physiological changes linked to improved health in overweight adults. Despite the small sample size, potential sex-specific adaptations were apparent that warrant further investigation.

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## Introduction

Interval exercise is characterized by repeated bursts of relatively intense effort, interspersed by periods of rest or lower-intensity exercise for recovery. Short-term interval training protocols can induce physiological remodeling similar to continuous moderate-intensity training, despite reduced time commitment and a relatively small total exercise volume [1]. Recent studies have also shown improvements in various health indices including markers of glycemic control in both healthy individuals [2–4] and people with cardiometabolic disorders including type 2 diabetes [5] after low-volume interval training. These studies have been conducted on relatively small numbers of subjects and involved relatively short training interventions. Nonetheless, the findings have garnered significant interest from a public health perspective, given one of the most commonly cited barriers to regular exercise participation is “lack of time” [6].

A common interval training model is the Wingate Test, which involves a 30 s “all out” burst of cycling on a specialized ergometer. Typically, 4–6 such intervals are performed, separated by  $\sim 4$ – $5$  min of recovery, with three training sessions performed each week [1]. Despite the very small total amount of exercise, a training session typically lasts  $\sim 25$  min, given the brief warm-up and cool down that are usually included in addition to the recovery periods. The relative “time efficiency” of Wingate-based training has therefore been questioned [7], considering the  $\sim 75$  min time commitment per week, which falls within the physical activity guidelines advocated by some public health agencies. While 150 min of moderate-intensity exercise per week is the general recommendation [8,9] some guidelines include 75 min of vigorous physical activity as an alternative [9].

Several recent studies investigated physiological and health-related adaptations to very low-volume interval training protocols that involved a time commitment of  $\leq 15$  min per session [10–12]. For example, Metcalfe and colleagues [10] reported that a 10 min



training protocol, involving low-intensity cycling except for 2, 20 sec all out sprints, improved cardiorespiratory fitness ( $\text{VO}_2$  peak) in previously sedentary adults when performed 3x/wk for 6 wk. The potential for very low-volume interval training protocols to improve  $\text{VO}_2$  peak has also been described by Ma et al. [11] and Hazell et al. [12]. Metcalfe et al. [10] also reported that insulin sensitivity based on oral glucose tolerance tests was improved after training in men but not women, highlighting the potential for sex-based differences in the adaptive response. Only one study has examined muscle adaptations to this type of training, with Ma et al. [11] reporting increased protein content of some mitochondrial enzymes after training, although the maximal activity of citrate synthase was unchanged.

The purpose of the present study was to clarify and advance our understanding of the impact of very low-volume interval training on physiological and health related adaptations to very low-volume SIT. Specifically, we examined the impact of a training protocol that involved only 1 minute of intense intermittent exercise within a 10 min time commitment, including warm-up and cool-down. Sedentary but otherwise healthy subjects trained 3x/wk for 6 wk, and needle biopsies were obtained before and after training to examine skeletal muscle remodeling. We also assessed changes in several markers reflective of cardiometabolic health. In light of the findings by Metcalfe et al. [11], a secondary aim was to explore potential sex-based differences in the adaptive response to this type of training. We hypothesized that the training intervention would increase skeletal muscle oxidative capacity, as reflected by the maximal activity and protein content of mitochondrial enzymes, increase  $\text{VO}_2$  peak, and reduce resting blood pressure and 24 h mean blood glucose concentration measured using continuous glucose monitoring (CGM) under conditions of controlled activity and feeding. We further hypothesized that reductions in 24 h glucose would be superior in men.

## Materials and Methods

The protocol for this study and supporting TREND checklist are available as supporting information; see Checklist S1 and Protocol S1.

## Subjects

Fourteen overweight or obese men and women were recruited by poster advertisement from the McMaster University community and took part in the study (Table 1). Subjects were deemed sedentary based on their self-reported habitual physical activity, which consisted of  $\leq 2$  sessions/wk of structured exercise lasting  $\leq 30$  min. Participants were allocated into the male or female intervention group and matched for age, body mass index and  $\text{VO}_2$  peak. The experimental protocol, which consisted of familiarization and baseline testing, a 6 wk training intervention, and post-training measurements, was approved by the Hamilton Integrated Research Ethics Board and all visits took place at McMaster University. All subjects provided written informed consent prior to their participation.

## Experimental Protocol

**Familiarization and baseline testing.** Participants reported to the laboratory on four separate occasions over 14 d for familiarization and baseline testing during May–July 2013. On the first visit, subjects initially sat quietly for 10 min prior to 3 separate measurements of blood pressure using an automatic blood pressure cuff (Contec 08A, Qinhuaangdao, China), with the lowest of these values used for analysis as previously reported [13]. Subjects subsequently performed an incremental maximal oxygen

uptake ( $\text{VO}_2$  peak) test on an electronically braked cycle ergometer (Lode Excalibur Sport V 2.0, Groningen, The Netherlands) as previously described [14,15]. Briefly, following a 2 min warm-up at 50 W, the resistance was increased by 1 W every 2 s until volitional exhaustion or the point at which pedal cadence fell below 50 rpm. A metabolic cart with an on-line gas collection system (Moxus modular oxygen uptake system, AEI Technologies, Pittsburgh, PA) acquired oxygen consumption ( $\text{VO}_2$ ) and carbon dioxide production ( $\text{VCO}_2$ ) data.  $\text{VO}_2$  peak was defined as the highest average oxygen consumption over a 30 s period. Approximately 15 min following the  $\text{VO}_2$  peak test, participants performed 1–2  $\times$  20 s all-out sprints on an electronically braked cycle ergometer (Veletron, RacerMate, Seattle, WA, USA) to become acquainted with the interval protocol.

Approximately 5 d after the familiarization session, participants returned to the laboratory and were fitted with a continuous glucose monitor (CGM; CGMS; iPro, Medtronic, Northridge, CA) and chest-worn accelerometer (Actiheart; Camtech, Cambridge, United Kingdom). Subjects were also given a glucose meter (OneTouch UltraMini, Lifescan, Milpitas, CA) with instructions on how to perform capillary blood sampling. Participants received a standardized food parcel, which they were instructed to consume at prescribed meal times over the subsequent 24 h. The diet was individualized for each participant and energy intake was estimated using the Mifflin-St Jeor equation [16]. Mean total energy was  $2623 \pm 123$  and  $1886 \pm 146$  kcal for men and women, respectively, derived from  $56 \pm 1\%$  carbohydrate,  $30 \pm 1\%$  fat and  $14 \pm 1\%$  protein.

Starting at 600 h the day following CGM insertion, participants began consuming the control diet under free-living conditions and CGM data was collected for a 24 h period. Participants obtained capillary blood glucose samples at four points over the 24 h period when blood glucose was expected to be stable (i.e. upon awakening, before lunch, before dinner and before bed) and were automatically stored in the glucose meter provided. Average blood glucose concentration, glucose area under the curve (AUC) and the daily peak glucose concentration ( $G_{\text{max}}$ ) were calculated from CGM data for a 24 h period from 600 to 559 h before and after training. Physical activity was monitored continuously over this 24 h period using a chest-worn device (Actiheart) that simultaneously measured heart rate and activity with an internal accelerometer that senses the frequency and intensity of torso movements to calculate energy expenditure. Following CGM removal at  $\sim 1200$  h, glucose data were uploaded as previously described [5].

Approximately 2 d later, participants reported to the lab following a 10 h overnight fast. A single resting blood sample was obtained by venipuncture from an antecubital vein. Plasma and serum were separated by centrifugation (10 min at 4000 rpm) and stored at  $-20^\circ\text{C}$  for subsequent analysis. A resting skeletal muscle biopsy was obtained using procedures we have previously described [17]. Briefly, muscle samples were obtained from the vastus lateralis under local anesthesia (1% lidocaine) using a Bergstrom needle adapted with suction. Samples were sectioned into several pieces, immediately snap frozen in liquid nitrogen and stored at  $-80^\circ\text{C}$  for later analysis.

**Training protocol.** At least 5 d following the muscle biopsy, subjects initiated the interval training program, which consisted of 18 supervised sessions over 6 wk during June–August 2013. Training was performed on Monday, Wednesday and Friday each week. Each session consisted of 3  $\times$  20 s all-out cycling efforts against a load corresponding to 0.05 kg/kg body mass, separated by 2 min of low intensity cycling (50 W), on an electronically braked ergometer (Veletron, RacerMate, Seattle, WA, USA). All



**Table 1.** Subject Characteristics.

VARIABLE	MEN	WOMEN
Age (y)	29±9	30±10
Height (cm)	176±5	162±8
Weight (kg)	97±8	75±12*
Body Mass Index (kg/m <sup>2</sup> )	31±2	29±2
VO <sub>2</sub> peak (L/min)	3.0±0.5	2.0±0.2*
VO <sub>2</sub> peak (ml/kg/min)	31±4	28±4
Maximal Workload (W)	262±30	202±23*

Values are means ± S.D. N=7 for men and women. VO<sub>2</sub>peak, maximal oxygen uptake.

\*Significantly different from men (p=0.05).

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training sessions included a 2 min warm-up and 3 min cool-down at 50 W, for a total time commitment of 10 min. The weekly training protocol therefore involved a total of 3 min of very intense intermittent exercise within a time commitment of 30 min including warm-up, cool-down and the recovery between efforts. Peak and mean power output was recorded for each sprint and an average determined for each session. Heart rate (HR) was measured continuously on the first training session.

**Post-testing.** Resting blood pressure was measured 24 h after the final training session, before subjects were fitted with the CGM and Actiheart. CGM data was collected for a 24 h period starting ~48 h after the final exercise session and diet was controlled to be the same as baseline. There was no difference in activity counts between the baseline and post-testing CGM period ( $P>0.05$ ). Fasting blood and a resting muscle biopsy sample were obtained 72 h following the last training bout. Approximately 4 d following the biopsy and 1 wk after the final training session, a maximal exercise test was performed using the same procedures as at baseline. All procedures and controls were identical to those employed during baseline testing and took place during July–September 2013. A flow chart of all participants involved in the trial is depicted in Figure 1.

#### Blood Analysis

Plasma glucose was analyzed using a kit assay (Pointe Scientific, Canton MI, USA) and serum insulin was measured by ELISA according to manufacturer instructions (ALPCO Immunoassays, Salem NH, USA). Insulin resistance was calculated using HOMA-IR [18].

#### Muscle Analysis

**Enzyme activity.** One piece of muscle (~25 mg) was homogenized in Lysing Matrix D tubes (MP Biomedicals, Solon, OH, USA) using the FastPrep-24 Tissue and Cell Homogenizer (MP Biomedicals, Solon, OH, USA) for 10×5 s cycles at a speed of 4 m/s with samples placed on ice for 5 min in between cycles. Samples were homogenized in 20 volumes of buffer containing 70 mM sucrose, 220 mM mannitol, 10 mM HEPES supplemented with protease inhibitors (Complete Mini, Roche Applied Science, Laval, PQ, Canada) and used to determine the maximal activity of citrate synthase and 3-β-hydroxyacyl CoA dehydrogenase (β-HAD) as we have previously described [14,19,20]. Protein concentration of homogenates was determined using a commercial assay (BCA Protein Assay, Pierce, Rockford, IL, USA) and enzyme activity is expressed as mmol/kg protein/hr.

**Western blotting.** A second piece of muscle (~30 mg) was homogenized in RIPA buffer using Lysing Matrix D tubes (MP Biomedicals, Solon, OH, USA) with the FastPrep-24 Tissue and Cell Homogenizer (MP Biomedicals, Solon, OH, USA). Samples were processed for 4×20 s cycles at 4.0 m/s, with samples placed on ice for 5 min in between cycles, followed by 2×20 s cycles at 4.0 m/s, with samples placed on ice for 2 min in between cycles. Western blot analysis was conducted using techniques described previously [14,19]. Briefly, protein concentration of homogenates was determined (BCA Protein Assay) and equal amounts of protein were prepared in 4× Laemmli's buffer and heated to 95°C before being separated by 10% SDS-PAGE and electrotransferred to nitrocellulose membranes. Ponceau S staining was performed following transfer to visualize equal loading and transfer. Following 1 h blocking in 5% fat-free milk Tris-buffered saline 0.1% Tween 20 (TBS-T), membranes were incubated in the primary antibody (glucose transporter 4; Millipore, AB1345 or COXIV; Mitosciences, MS408) overnight at 4°C in 3% fat-free milk TBS-T based on previously optimized conditions. After 3×5 min washes in TBS-T, membranes were incubated in the species-specific secondary antibody diluted (1:10,000) in 3% fat-free milk TBS-T for 1 h at room temperature, washed in TBS-T for 6×5 min, and visualized by chemiluminescence (SuperSignal West Dura, Pierce) using a FluorChem SP Imaging System (Alpha Innotech Corporation, San Leandro, CA, USA). ImageJ software (NIH) was used to quantify the optical density of protein bands. Protein content was expressed as a fold change relative to pre-training for all subjects. α-tubulin (Cell Signaling Technology, #2125), which did not change following training ( $p=0.91$ ), was used as a loading control.

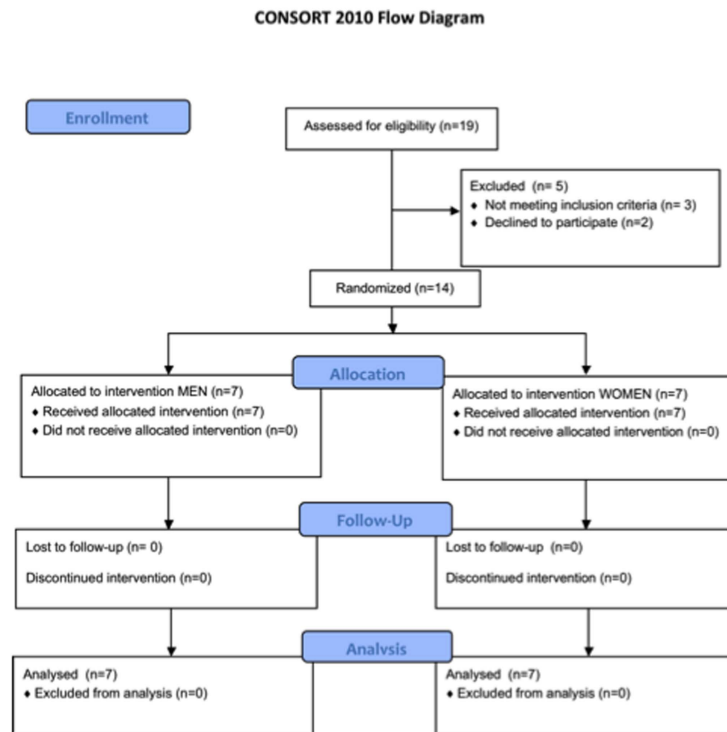
#### Statistical Analysis

All data were analyzed using a two-factor analysis of variance (ANOVA), with the between factor group (men, women) and the within factor time (pre-, post-training) using SPSS Statistics software. Significant interactions and main effects were subsequently analyzed using a Tukey's honestly significant difference post hoc test. The level of significance for all analyses was set at  $P<0.05$  and all data are presented as means ± S.D for  $n=7$  in each group, except for the CGM data which represents  $n=6$  per group.

#### Results

##### Descriptive characteristics of training

Adherence to the training sessions was 100%. Mean HR, measured continuously during the first training session and



**Figure 1. Flow diagram of participants through all phases of the trial.**  
doi:10.1371/journal.pone.0111489.g001

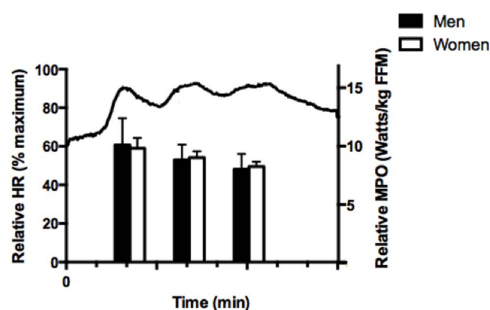
averaged over the entire 10 min protocol including warm-up and cool-down, was  $83 \pm 2\%$  of  $HR_{max}$ . Relative PPO and MPO measured on the first and last training session did not differ between men and women and increased with training (Table 2,

main effect for time,  $p < 0.01$ ). The average HR response for all subjects and average MPO for men and women during session 1 is depicted in Figure 2.

**Table 2. Markers of Health and Fitness.**

VARIABLE	MEN		WOMEN	
	PRE	POST	PRE	POST
Body Mass (kg)	$77 \pm 12$	$77 \pm 13$	$79 \pm 15$	$79 \pm 15$
FPG (mmol/L)	$5.1 \pm 0.3$	$5.2 \pm 0.3$	$5.0 \pm 0.3$	$5.0 \pm 0.3$
FPI (uIU/ml)	$13.5 \pm 7.9$	$10.7 \pm 7.0^*$	$9.6 \pm 4.0$	$7.1 \pm 3.0^*$
HOMA-IR	$3.1 \pm 1.9$	$2.5 \pm 1.5^*$	$2.1 \pm 0.9$	$1.5 \pm 0.6^*$
Gmax (mmol/L)	$8.0 \pm 1.3$	$6.8 \pm 1.1^*$	$7.3 \pm 0.6$	$7.6 \pm 0.9$
Resting SBP (mmHg)	$124 \pm 8$	$116 \pm 8^*$	$109 \pm 11$	$100 \pm 11^*$
Resting DBP (mmHg)	$71 \pm 11$	$67 \pm 5$	$66 \pm 9$	$60 \pm 9$
Resting MAP (mmHg)	$88 \pm 8$	$83 \pm 4^*$	$80 \pm 10$	$74 \pm 9^*$
Relative PPO (W/kg FFM)	$11.3 \pm 4.1$	$12.2 \pm 3.6^*$	$10.0 \pm 0.6$	$11.8 \pm 1.1^*$
Relative MPO (W/kg FFM)	$9.0 \pm 1.6$	$10.6 \pm 1.5^*$	$9.0 \pm 0.5$	$12.0 \pm 0.1^*$

Values are means  $\pm$  S.D. N = 7 for men and women. \*Significantly different than pre-training ( $p \leq 0.05$ ).  
FPG, fasting plasma glucose; FPI, fasting plasma insulin; Gmax: daily peak glucose concentration.  
doi:10.1371/journal.pone.0111489.t002



**Figure 2. Characterization of the low-volume SIT protocol.** Solid line represents average heart rate (HR) response, expressed as a % of maximum, for all subjects during the first training session (left side y-axis). Bar graph represents relative mean power output (MPO) per kilogram fat-free mass (FFM) for men (dark bar) and women (white bar) during the first training session (right side y-axis). doi:10.1371/journal.pone.0111489.g002

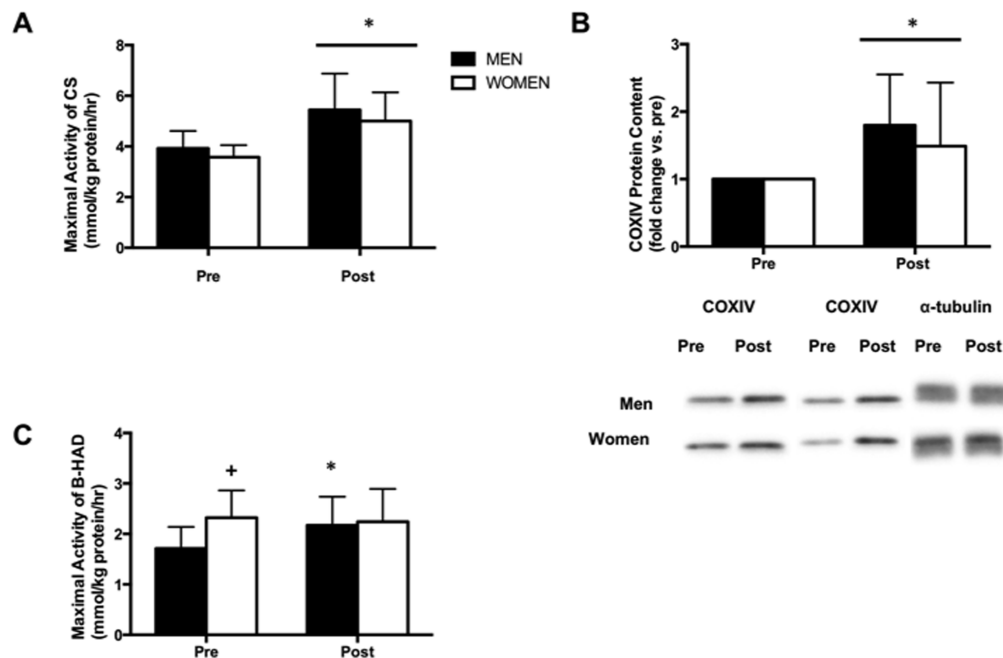
#### Skeletal muscle adaptations to very low-volume SIT

The maximal activity of citrate synthase increased by ~40% after training (Fig. 3A, main effect for time,  $p<0.001$ ). COXIV

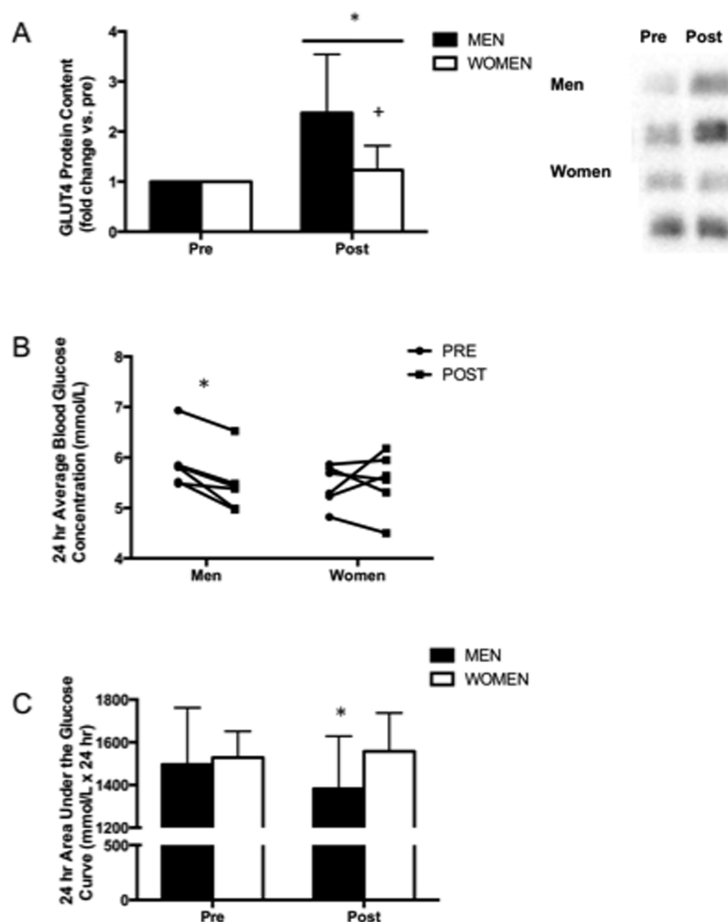
protein content also increased after training with no differences between groups (Fig. 3B, main effect for time,  $p<0.01$ ), however  $\beta$ -HAD maximal activity only increased after training in the men (Fig. 3C; interaction between training and sex,  $p<0.05$ ). GLUT4 protein content increased in both men and women following training (Fig. 4A, main effect for time,  $p<0.01$ ), however men increased to a greater extent compared to women (138% vs. 23%, interaction between training and sex,  $p<0.05$ ).

#### Indices of cardiometabolic health

Very low-volume interval training increased  $\text{VO}_2$  peak by 12% in both men and women (Fig. 5, main effect for time,  $p<0.001$ ), which was associated with a 14% increase in maximal workload (Table 1, main effect for time,  $p<0.001$ ). Mean arterial pressure (MAP) was reduced by 6% and 8% in men and women respectively following training (Table 2, main effect for time,  $p<0.01$ ). Systolic blood pressure (SBP) was also reduced following training (Table 2, main effect for time,  $P<0.01$ ), while diastolic blood pressure (DBP) trended towards being lower (Table 2,  $p=0.07$ ). Insulin sensitivity measured by HOMA-IR was improved after training (Table 2, main effect for time,  $p<0.05$ ), owing mainly to a decrease in fasting serum insulin (Table 2, main effect for time,  $p\leq 0.05$ ). There was no change in fasting plasma glucose in either group (Table 2,  $p>0.05$ ). CGM revealed a lower 24 h average blood glucose concentration after training in men ( $5.4\pm 0.6$  vs.  $5.9\pm 0.5$  mmol/L,  $p<0.05$ ) but not women (Fig. 4B,



**Figure 3. Very low-volume SIT improves skeletal muscle mitochondrial capacity.** Measured in muscle biopsy samples obtained from the vastus lateralis before (PRE) and 72 h after (POST) 6-week SIT in men and women. Maximal activity of citrate synthase (A), protein content of COXIV (B) and maximal activity of  $\beta$ -HAD (C). Values are means  $\pm$  SD ( $n=7$  per group). Representative Western blots for 2 men and 2 women are shown for COXIV.  $\alpha$ -tubulin was used as a loading control and representative Western blots are shown. \* $P<0.05$ , pre- vs. post-training; + $p<0.05$ , men vs. women at same time point; line denotes a main effect. doi:10.1371/journal.pone.0111489.g003



**Figure 4. Improved indices of blood glucose control in men following very low-volume SIT.** GLUT4 protein content measured in muscle biopsy samples obtained from the vastus lateralis before (PRE) and 72 h after (POST) 6 week SIT in men and women (A). Individual 24 h average blood glucose concentration (B) and 24 h blood glucose area under the curve (AUC) measured before (PRE) and 48–72 h after (POST) 6 week SIT in men and women using continuous glucose monitoring (CGM). Values are means  $\pm$  SD ( $n=7$  per group for muscle data,  $n=6$  per group for CGM data). Representative Western blots for 2 men and 2 women are shown for GLUT4. \* $P<0.05$ , pre- vs. post-training; + $p<0.05$ , men vs. women at same time point.

doi:10.1371/journal.pone.0111489.g004

$5.5 \pm 0.6$  vs.  $5.5 \pm 0.4$  mmol/L,  $p>0.05$ ). Similarly, 24 h glucose AUC was reduced in men only (Fig. 4C, interaction between training and sex,  $p<0.05$ ).  $G_{max}$  was lower in men following training, but not in women (Table 2, interaction between training and sex,  $p<0.01$ ).

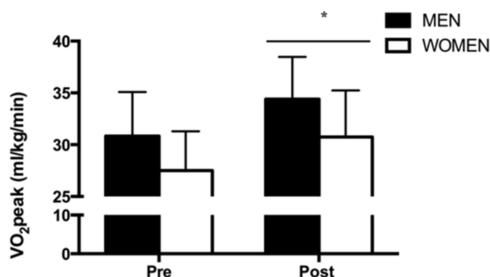
## Discussion

The main finding from the present study was that short-term interval training, using a protocol that involved only 1 min of very intense exercise within a total time commitment of 10 min, was a potent stimulus to induce physiological adaptations that are linked to improved health in overweight and obese adults. Our general

design, which involved 3 sessions per week for 6 wk, was similar to recent studies by Metcalfe [10] and Ma [11], but clarified outstanding questions regarding the potential for very low-volume interval training to increase muscle oxidative capacity, resting blood pressure and aspects of glycemic control. Despite the small sample size, we also found evidence of potential sex-specific adaptations to this type of training that warrant further investigation.

## Very low-volume interval training increases muscle oxidative capacity

A recent systematic review and meta-analysis [21] proposed a classification scheme for interval training in an effort to



**Figure 5. Very low-volume SIT increases  $\text{VO}_2$  peak.** Measured before (PRE) and 1 week following (POST) 6 wk SIT in men and women. Values are means  $\pm$  SD ( $n=7$  per group). \* $P<0.05$ , pre- vs. post-training; line denotes a main effect. doi:10.1371/journal.pone.0111489.g005

standardize terminology employed in future studies. Using the descriptors proposed by Weston and colleagues, we have opted to classify the present protocol as “sprint interval training” (SIT) given the “all-out” efforts, as opposed to “high-intensity interval training” (HIIT), which the authors define as bouts performed at relatively intense but nonetheless submaximal workloads corresponding to 80–100% of maximal heart rate [21]. We report here for the first time that very low-volume SIT can increase the maximal activity of citrate synthase, which is reportedly one of the best indicators of mitochondrial content in human skeletal muscle, as it is highly correlated with gold-standard measures made by electron microscopy [22]. While skeletal muscle adaptations to SIT are well established, there are limited and equivocal data regarding the effect of very low-volume SIT on mitochondrial content. Skleryk et al. [23] reported that a protocol involving 8–12 $\times$ 10 s all-out cycle sprints against 5% body weight interspersed with 80 s rest, performed six times over 2 wk, did not improve mitochondrial capacity in overweight men as reflected by a lack of change in the protein content of COXII and COXIV. In contrast, Ma et al. [11] showed that a protocol consisting of 8, 20 s cycling efforts at an intensity of 170% of  $\text{VO}_2$  peak and interspersed with 10 s of recovery, performed four times per week for 4 wk, increased the protein content of COXI and COXIV, however, the maximal activity of citrate synthase was unchanged. The results from the present study confirm that 6 wk of very low-volume SIT, involving a total of only 3 min of all out intermittent exercise within a 30 min time commitment per week, was a sufficient stimulus to elicit a robust increase in citrate synthase similar to what has been reported after protocols involving a larger volume of SIT or traditional moderate-intensity continuous training that involve a much greater total volume of exercise and training time commitment [24]. Clearly, there is some minimal total volume of SIT necessary to acutely stimulate mitochondrial biogenesis, which when performed repeatedly leads to measureable increases in enzyme protein content or maximal activity. The various short-term, very low-volume SIT protocols that have been employed to date are likely on the lower end of this threshold, which may in part explain the equivocal results to date. Additional studies, like the elegant work by Perry et al. [25], which characterized the early time course of adaptation to HIIT, will help to resolve this matter.

#### Effect of very low-volume interval training on markers of cardiometabolic health

Seminal work by Tabata and colleagues over two decades ago showed that 7–8 bouts of 20 s all out sprints, with 10 s rest in

between, improved  $\text{VO}_2$  peak in young men by 15%, when performed four times per week for 6 wk [26]. The beneficial effect of “Tabata style” training on  $\text{VO}_2$  peak, which is a popular exercise strategy among many personal trainers, was recently confirmed by Ma et al. [11] who reported a 19% increase in young men after 4 wk. The present work, and recent studies by others [10–12,27], confirm that very brief bouts of all-out exercise, performed a few times per week, is a very time efficient strategy to improve  $\text{VO}_2$  peak, which is a strong predictor of all-cause morbidity and mortality [28]. A novel, important finding from the present work was the significant reduction in MAP when measured 24 h after the final training bout, which is of similar magnitude to findings following traditional Wingate-based SIT in overweight/obese men and women [13], as well as 16 wk of high volume aerobic interval or continuous moderate intensity training in individuals with metabolic syndrome [29]. Based on findings from a recent systematic review and meta-analysis, the blood pressure reduction in the present study is of similar magnitude to those following intermittent isometric resistance training [30], which is emerging as a very effective exercise strategy for lowering resting blood pressure [30,31]. It is unclear if our findings represent an acute effect of the last training bout, however if one performs SIT every other day as in the present study, the beneficial effect on blood pressure would be maintained.

#### Potential sex-specific adaptations to low-volume interval training

SIT has been shown to improve insulin sensitivity, based on hyperinsulinemic euglycemic clamps performed on sedentary and recreationally active individuals [2] as well as oral glucose tolerance tests (OGTTs) performed on young healthy [4] and overweight/obese [13] men. Metcalfe et al. [10] recently reported that a 10 min low-intensity cycling protocol that included 2 $\times$ 20 s all-out sprints, performed 18 times over 6 wk, improved insulin sensitivity measured by OGTTs in men but not women [10]. Consistent with the observations of Metcalfe et al. [10], we found using CGM that 24 h average blood glucose concentration, glucose AUC and  $G_{\text{max}}$  measured under standard dietary conditions from 48–72 h after the final training session, were improved in men but not women. Interestingly, the training-induced increase in total GLUT4 protein content was approximately 6-fold higher in men compared to women.

The lack of change in peripheral glucose control in women in the present study is consistent with recent reports by others [10,15], although this is not a universal finding [2]. It is possible that our low sample size ( $n=6$  for CGM data), or the fact that the women had higher 24 h blood glucose control at baseline, influenced our findings and resulted in a type 2 statistical error. Nonetheless, by way of a possible related mechanism, it has also been speculated that the rapid improvement in insulin sensitivity following SIT is attributed to high rates of glycogen breakdown and subsequent re-synthesis following each exercise bout [10], and women have been shown to break down 42% less muscle glycogen in type I fibers during a single Wingate sprint [32]. Future studies are needed to investigate if GLUT4 translocation following acute SIT is blunted in women, and definitively determine in larger cohorts of subjects if improvements in glucose control following SIT are sex-specific. HOMA-IR was improved in both men and women after training, owing to significant reductions in fasting plasma insulin, and consistent with previous studies [3,33].

We also found sex-specific differences in a marker of lipid oxidation capacity, based on changes in the maximal activity of  $\beta$ -HAD which were detected in men but not women. A similar period of Wingate-based SIT was reported to improve the

maximal activity of  $\beta$ -HAD in both men and women, but that study did not involve a specific comparison between sexes [24]. Similar to the pre-training CGM data, it is possible that the higher baseline value for  $\beta$ -HAD in women in the present study reduced their potential to increase the capacity for lipid oxidation compared to men. Other recent studies however, have also highlighted sex-based differences in the skeletal muscle adaptive response to SIT in active young men and women [34]. Scalzo et al. [34] reported higher rates of muscle protein synthesis in men compared to women following a 3 wk SIT intervention, based on oral administration of deuterium oxide. Future research is needed, using designs which control for menstrual cycle phase and initial fitness level [35], to evaluate if sex-based differences exist in the skeletal muscle adaptive response low-volume SIT.

## Conclusions

In summary, we report that 3 min of all-out exercise performed within a 30 min time commitment per week including warm-up and cool-down, improved skeletal muscle oxidative capacity and indices of cardiometabolic health including  $\text{VO}_2$  peak and blood pressure, in overweight/obese adults. The protocol employed in the present study involved a training time commitment that was considerably lower than in previous Wingate-based SIT studies (i.e., 10 versus ~25 min per session) and provides further evidence

of the potential for very brief, intense bursts of exercise to elicit physiological adaptations that are associated with improved health status in a time-efficient manner. Despite the small sample size, potential sex-specific adaptations were apparent that warrant further investigation.

## Supporting Information

**Checklist S1 TREND checklist.**  
(PDF)

**Protocol S1 Trial study protocol.**  
(DOCX)

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## Author Contributions

Conceived and designed the experiments: JBG MJG. Performed the experiments: JBG MEP LES BJM RBT MAT MJG. Analyzed the data: JBG MEP LES BJM RBT. Contributed reagents/materials/analysis tools: MAT MJG. Wrote the paper: JBG MJG.

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**CHAPTER 4:**

**Twelve weeks of sprint training improves cardiometabolic health similar to traditional endurance training despite a five-fold lower exercise volume**

*In preparation*



Twelve weeks of sprint training improves cardiometabolic health similar to traditional endurance training despite a five-fold lower exercise volume

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## KEY POINTS SUMMARY

- Sprint interval training (SIT) protocols involving  $\leq 10$  minutes per session improve indices of cardiometabolic health similar to moderate-intensity continuous training (MICT) protocols that involve larger exercise volumes and time commitments.
- No studies have directly compared this type of very low-volume SIT to traditional high-volume MICT, nor examined changes occurring beyond 6 weeks.
- We investigated changes in cardiometabolic health indices after 12 weeks of thrice weekly SIT (involving three, 20-second ‘all-out’ cycling intervals within a 10 minute exercise session) or MICT (50 minutes of continuous cycling per session).
- SIT and MICT elicited similar improvements in cardiorespiratory fitness, skeletal muscle oxidative capacity and insulin sensitivity based on intravenous glucose tolerance tests, whereas a control group showed no changes.
- Twelve weeks of brief intense interval exercise improves indices of cardiometabolic health to the same extent as traditional endurance training, despite a five-fold difference in total exercise volume and time commitment.

## ABSTRACT

We investigated whether sprint interval training (SIT), involving 1 minute of intense exercise within a 10-minute time commitment per session, could improve indices of cardiometabolic health to the same extent as traditional moderate-intensity continuous training (MICT) involving 50 minutes of continuous exercise. Sedentary men ( $27 \pm 8$  y; BMI =  $26 \pm 6$  kg/m<sup>2</sup>) performed three weekly sessions of SIT (n=9) or MICT (n=10) for 12 weeks or served as non-training controls (n=6). SIT involved 3x20-second ‘all-out’ cycle sprints (~500 W) interspersed with 2 minutes of cycling at 50 W, whereas MICT involved 45 minutes of continuous cycling at ~70% maximal heart rate (~110 W). Both protocols involved a 2-minute warm-up and 3-minute cool-down at 50 W. Peak oxygen uptake increased after training by 19% in both groups (SIT:  $32 \pm 7$  to  $38 \pm 8$ ; MICT:  $34 \pm 6$  to  $40 \pm 8$  ml/kg/min;  $p < 0.05$ ). Skeletal muscle mitochondrial content also increased similarly after SIT and MICT, as reflected by the maximal activity of citrate synthase and protein content of electron transport chain subunits ( $p < 0.05$ ). Insulin sensitivity, determined by intravenous glucose tolerance tests performed before and 72 hours after training, also increased similarly after SIT ( $4.9 \pm 2.5$  to  $7.5 \pm 4.7$ ) and MICT ( $5.0 \pm 3.3$  to  $6.7 \pm 5.0 \times 10^{-4}$  min<sup>-1</sup> [ $\mu$ U/mL]<sup>-1</sup>) and this was accompanied by similar increases in muscle GLUT4 protein content ( $p < 0.05$ ). There was no change in the control group for any variable ( $p > 0.05$ ). Twelve weeks of brief intense interval exercise improved indices of

cardiometabolic health to the same extent as traditional endurance training, despite a five-fold lower total exercise volume and time commitment.

Abbreviations: AUC, area under the curve;  $\Delta\text{AUC}_{\text{INS}}$ , insulin area under the curve above the fasting concentration;  $\beta$ -HAD, 3- $\beta$ -hydroxyacyl CoA dehydrogenase; CGM, continuous glucose monitoring; COX, cytochrome c oxidase; CS, citrate synthase;  $\text{CS}_I$ , insulin sensitivity index; CTL, non-training control; HR, heart rate;  $\text{HR}_{\text{max}}$ , maximal heart rate; IVGTT, intravenous glucose tolerance test;  $K_G$ , glucose disappearance rate; MICT, moderate-intensity continuous training;  $M_{\text{ISI}}$ , Matsuda composite index; OGTT, oral glucose tolerance test; RPE, rating of perceived exertion;  $S_I$ , minimal model insulin sensitivity index; SIT, sprint interval training;  $\text{VO}_{2\text{peak}}$ , peak oxygen uptake;  $W_{\text{max}}$ , maximal workload.

## INTRODUCTION

Regular endurance exercise training enhances cardiorespiratory fitness (4) induces skeletal muscle remodeling towards a more oxidative phenotype (22) and promotes favorable changes in cardiometabolic health indices including insulin sensitivity (21). These well established training responses provide support for current physical activity guidelines that generally recommend 150 minutes of moderate-intensity or 75 minutes of vigorous-intensity aerobic physical activity per week to achieve health benefits (15, 54, 62). Despite the association between low amounts of physical activity and increased risk of many chronic diseases, including cardiovascular disease and type 2 diabetes (58), most adults do not meet the minimum physical activity guidelines, as confirmed by studies that incorporated accelerometer data (11, 55). The reasons for not engaging in regular physical activity are numerous and complex, but “lack of time” remains one of the most commonly cited barriers (56).

In contrast to traditional endurance training, sprint interval training (SIT) is characterized by brief intermittent bursts of very intense exercise, i.e., at an ‘all-out’ pace or an intensity above that which elicits peak oxygen uptake ( $\text{VO}_{2\text{peak}}$ ), separated by short periods of rest or low-intensity exercise for recovery (59). At least over the short-term (i.e., up to 6 weeks), SIT has been shown to induce numerous physiological adaptations similar to endurance training, despite a lower total exercise volume and time commitment (17, 18). The most commonly studied SIT protocol involves repeated Wingate Tests; typically four to six, ‘all-out’ 30-

second cycling efforts are performed each session, interspersed by 4-5 minutes of recovery. The few studies that have directly compared Wingate-based SIT versus moderate-intensity continuous training (MICT) have reported similar improvements in cardiorespiratory fitness (6), skeletal muscle oxidative capacity as reflected by the maximal activity or protein content of mitochondrial enzymes (6, 16) and indices of cardiovascular structure and function (10, 41). Other studies have reported improved insulin sensitivity after short-term SIT (2, 42), including studies that have incorporated brief running efforts at maximal or near-maximal effort (45).

Given that Wingate-based SIT or similar run-based protocols involve ~20-30 minutes per session, not including warm-up or cool-down, the purported “time efficiency” of this type of training has been questioned. Indeed, the weekly time commitment is comparable to the lower end of physical activity guidelines that call for at least 75 minutes of vigorous-intensity aerobic physical activity per week (15, 62). Intriguingly, several recent studies have shown that very brief SIT protocols elicit adaptations similar to longer SIT protocols and MICT. We found that a modified Wingate-based protocol involving three, 20-second ‘all-out’ sprints, within a training session that lasted 10 minutes including warm-up and cool-down, improved  $\text{VO}_2\text{peak}$  in previously sedentary adults when performed three times per week for 6 weeks (19). Other adaptations included a reduction in resting blood pressure and lower 24-hour blood glucose concentration, as well as higher skeletal muscle mitochondrial and glucose transport capacities (19). These

data, which are supported by recent findings by others (32, 36, 53), highlight the potential for very brief SIT protocols involving  $\leq 10$  min of exercise per session to improve cardiometabolic health similar to traditional endurance training. However, no study has directly compared these diverse training strategies in a comprehensive manner, nor studied potential adaptations occurring beyond six weeks.

The purpose of the present study was to compare the effects of 12 weeks of SIT or MICT on indices of cardiometabolic health, including cardiorespiratory fitness, skeletal muscle oxidative capacity and glycemic control. The two protocols differed markedly with respect to total exercise volume and time commitment: SIT involved 1 minute of intense intermittent exercise within a session that lasted 10 minutes, whereas MICT consisted of 50 minutes of moderate-intensity continuous exercise. Both groups performed three sessions per week, such that total exercise volume and time commitment was five-fold lower in SIT compared to MICT. We hypothesized that, compared to a non-training control group (CTL), SIT and MICT would similarly increase  $\text{VO}_{2\text{peak}}$ , the maximal activity and protein content of mitochondrial enzymes, and insulin sensitivity based on the intravenous glucose tolerance test method.

## **METHODS**

### **Subjects and Ethics Approval**



A total of 27 sedentary but otherwise healthy men were recruited by poster advertisement to take part in the study. Participants were generally deemed inactive based on an International Physical Activity Questionnaire score of less than 600 MET-minutes per week. Participants were matched for age, body mass index and  $\text{VO}_{2\text{peak}}$ , and assigned to SIT, MICT or CTL. One subject in each of the two training groups dropped out for reasons unrelated to the study, resulting in a total of  $n=9$ , 10 and 6 in the SIT, MICT and CTL groups, respectively (Table 1). The experimental protocol, which included baseline testing, a 12-week intervention, and post-testing, was approved by the Hamilton Integrated Research Ethics Board at McMaster University and conformed to the Declaration of Helsinki. All participants provided written informed consent prior to their participation.

## **Experimental Protocol**

### *Baseline testing and exercise familiarization*

Following entry into the study, participants reported to the laboratory on four occasions over 2 weeks. On visit 1, participants performed an incremental  $\text{VO}_{2\text{peak}}$  test on an electronically-braked cycle ergometer (Lode Excalibur Sport V 2.0, Groningen, The Netherlands), as described previously (19). Briefly, following a 1-minute warm-up at 50 W, the resistance was increased by 1 W every 2 seconds until exhaustion or when pedal cadence fell below 50 rpm. Oxygen consumption and carbon dioxide production data were acquired through a metabolic cart with an on-line gas collection system (Moxus modular oxygen

uptake system, AEI Technologies, Pittsburgh, PA), and  $\text{VO}_2\text{peak}$  was defined as the highest average oxygen consumption over a 30-second period. Before departing from the laboratory, participants were provided with a standardized meal that they were instructed to consume the evening before visit two. Approximately 5 days later, participants reported to the laboratory following a 10-hour overnight fast to undergo a body composition test and a 50-minute intravenous glucose tolerance test (IVGTT) recently validated by Tura et al. (57). Food intake was recorded for 24 hours prior to the IVGTT, and participants consumed a standardized meal the evening before the visit consisting of  $561 \pm 99$  kcal derived from  $47 \pm 2\%$  carbohydrate,  $31 \pm 3\%$  fat and  $22 \pm 4\%$  protein. Upon arrival to the laboratory, fat and fat-free masses were determined through air-displacement plethysmography (BodPod®, COSMED Inc., Concord, CA, USA). Subsequently, two indwelling catheters were inserted into forearm veins (one in each arm) by a trained nurse. A fasting blood sample (12 ml) was obtained from the “sampling arm”, and a bolus dose of glucose ( $0.5 \text{ g/kg}$  up to a maximum of 35 g) was manually delivered to the contralateral “infusion arm” at an even pace over 3 minutes using two 60 ml syringes. A  $38 \pm 2\%$  glucose solution (Hospira LifeCare) was used in a total volume of 90 ml. Following infusion, the catheter was removed from the “infusion arm”, and blood samples (8 ml) were obtained from the “sampling arm” at 10, 20, 30, 40 and 50 minutes post-infusion. The catheter was immediately flushed with 5 ml of 0.9 % saline after each blood draw.

Plasma and serum were separated by centrifugation (10 minutes at 1300g) and stored at -80°C for subsequent analyses.

Approximately 2 days later, a resting skeletal muscle biopsy was obtained using procedures described previously (51). Briefly, a single muscle sample (~100 mg) was obtained from the *vastus lateralis* under local anesthesia (1% lidocaine) using a Bergström needle adapted with suction. Samples were sectioned into several pieces, snap frozen in liquid nitrogen and stored at -80°C for later analysis.

At least 5 days following the muscle biopsy, participants assigned to SIT or MICT returned to the laboratory for exercise familiarization. Participants in the SIT group performed one or two 20-second ‘all-out’ sprints on an electronically-braked cycle ergometer (Veletron, RacerMate, Seattle, WA, USA) to become acquainted with the interval protocol. Participants in the MICT group were fitted with a heart rate (HR) monitor (Polar A3, Lake Success, NY, USA) and cycled on an ergometer (Kettler, Ergo Race I, Germany) for ~20 minutes to determine the workload that elicited 64-76 % of maximal heart rate ( $HR_{max}$ ). The wattage was initially set to 30% of maximal workload ( $W_{max}$ ), and was subsequently adjusted by investigators in an effort to elicit the desired stimulus. The target HR for MICT was based on the classification for “moderate-intensity” put forth by the American College of Sports Medicine (15).

#### 12-week intervention

Participants in SIT and MICT completed 12 weeks of supervised exercise training. A “lead-in” phase to training was provided, in which one session was completed

in week 1, and two sessions in week 2. Exercise was performed three times per week thereafter, with the exception of week 7 where two sessions were replaced with a “mid-training assessment” for  $\text{VO}_{2\text{peak}}$  and arterial ultrasound imaging (a collaborative measure not reported in the present manuscript). Training was typically performed on Monday, Wednesday and Friday each week, and all participants completed  $\geq 30$  training sessions over 12 weeks. During training, a chest-worn monitor (Polar Team System) recorded HR every 5 seconds, from which the average HR during each exercise session was determined. The SIT protocol consisted of 3 x 20-second ‘all-out’ cycling efforts against 0.05 kg/kg body mass, separated by 2 minutes of low intensity cycling (50 W), on an electronically-braked ergometer (Veletron, RacerMate, Seattle, WA, USA). The MICT protocol consisted of 45 minutes of continuous cycling at  $\sim 70\%$   $\text{HR}_{\text{max}}$  on an ergometer set in constant-watt mode (Kettler, Ergo Race I, Germany). Both protocols involved a 2-minute warm-up and 3-minute cool-down at 50 W, resulting in 10- and 50-minute sessions, respectively, for SIT and MICT. To accommodate progression, training loads were adjusted throughout the 12 weeks to maintain the desired relative exercise intensity. Specifically, if the average HR elicited by SIT was  $< 75\%$   $\text{HR}_{\text{max}}$  for three consecutive sessions, the resistance was increased by 0.005 kg/kg body mass to a maximal resistance of 0.07 kg/kg. Likewise, if the average HR elicited by MICT was  $< 70\%$   $\text{HR}_{\text{max}}$  for three consecutive sessions, the resistance was increased by 5-10 W. Ratings of perceived exertion (RPE; Borg 6-20 scale) were also recorded at the end of each

sprint (SIT) or at 15, 30 and 45 minutes of exercise (MICT), on the 1<sup>st</sup>, 15<sup>th</sup> and 30<sup>th</sup> sessions to ensure the appropriate stimulus was maintained throughout the intervention. Overall, the SIT and MICT training programs involved 30 and 150 minutes per week, respectively, resulting in a 5-fold difference in weekly time commitments. Participants in CTL did not report to the laboratory during the 12 weeks intervention, with the exception of week 7 for the mid-assessment. CTL participants were asked to maintain their current activity levels throughout the 12 weeks.

#### Post-testing

Participants were asked to refrain from all physical activity during the post-testing period. 72 hours after the intervention, participants repeated the body composition test and IVGTT adhering to the baseline pre-visit nutritional controls. A resting muscle biopsy was obtained 96 hours following the last training bout.

Approximately 4 days following the biopsy and 1 week after the final training session, a VO<sub>2</sub>peak test was performed. All procedures and controls were identical to those employed during baseline testing.

#### **Glucose and Insulin Analysis**

Plasma glucose was analyzed using a kit assay (Pointe Scientific, Canton MI, USA), and serum insulin was measured with ELISA, according to manufacturer instructions (ALPCO Immunoassays, Salem NH, USA). The insulin sensitivity index (CS<sub>I</sub>) was calculated as proposed by Tura et al. (57) using glucose and insulin data from the 50-minute IVGTT. CS<sub>I</sub> has been shown to be highly

correlated with the Minimal Model insulin sensitivity index ( $S_I$ ) obtained from a 3-hour IVGTT, as well as the gold standard glucose infusion rate measured during a hyperinsulinemic-euglycemic clamp in individuals with both normal and impaired glucose tolerances (57). This method has also been used to assess insulin sensitivity in response to acute exercise (38, 39), and was also shown to have greater day-to-day reproducibility than the Matsuda composite index ( $M_{ISI}$ ) derived from a 120-minute oral glucose tolerance test (OGTT) (39). Briefly,  $CS_I$  was calculated as follows:

$$CS_I = \alpha [K_G / (\Delta AUC_{INS} / T)]$$

where  $\alpha$  is a scaling factor (0.604),  $K_G$  is the glucose disappearance rate (mmol/L; calculated as the slope of log [glucose]),  $\Delta AUC_{INS}$  is the insulin area under the curve above the basal/fasting sample (uIU/ml) and T is the time interval between 10 and 50 minutes (40 minutes) from which  $K_G$  and  $\Delta AUC_{INS}$  were calculated (57).

Delta glucose and insulin area under the curve (AUC) from 0-50 minutes were also calculated, and fasting insulin resistance was determined using HOMA-IR (34).

### **Muscle Analysis**

Enzyme activity. One piece of muscle (~25 mg) was homogenized in Lysing Matrix D tubes (MP Biomedicals, Solon, OH, USA) using the FastPrep-24 Tissue and Cell Homogenizer (MP Biomedicals, Solon, OH, USA) for 5 x 5-second cycles at a speed of 4.0 m/s with samples placed on ice for 5 minutes between

cycles. Samples were homogenized in 20 volumes of buffer containing 70 mM sucrose, 220 mM mannitol, 10mM HEPES, 1mM EGTA, supplemented with protease inhibitors (Complete Mini<sup>®</sup>, Roche Applied Science, Laval, PQ, Canada). The maximal activities of citrate synthase (CS) and 3- $\beta$ -hydroxacyl CoA dehydrogenase ( $\beta$ -HAD) were determined with modification to that previously described (8). For determination of CS maximal activity, 15  $\mu$ l of muscle homogenate was added to cuvette containing: 825 $\mu$ l 0.1M Tris Buffer (pH 8.0), 100 $\mu$ l 5,5'-dithiobis(2-nitrobenzoic acid) (DTNB, 0.5mg/mL Tris Buffer) and 10  $\mu$ l acetyl CoA (6mg/mL Tris Buffer). The cuvette was warmed to 37°C, the spectrophotometer (Cary Bio-300, Varion, Inc., Palo Alto, CA, USA) was zeroed and 50  $\mu$ L of oxaloacetate (6.1mg/mL Tris buffer) was added to initiate the reaction. Absorbance was recorded at 412 nm for 120 seconds and the slope between 30 and 90 seconds was recorded. For determination of  $\beta$ -HAD, 25  $\mu$ l of muscle homogenate was added to a quartz cuvette containing: 800  $\mu$ l Tris buffer (50mM Tris-Cl, 1mM EDTA, pH 7.0) with 0.2% Triton X-100 and 10  $\mu$ L NADH (3.54mg/mL Tris buffer). The cuvette was incubated at 30°C for 5 minutes, at which point 10  $\mu$ l acetoacetyl CoA (4.8mg/mL) was added to initiate the reaction. Absorbance was recorded at 340 nm for 120 seconds and the slope between 30 and 90 seconds was recorded. Samples were run in duplicate and the intra-assay coefficient of variation (CV) for CS and  $\beta$ -HAD were 3.0 and 6.5% respectively. Protein concentration of the homogenates was determined using a commercial

assay (BCA Protein Assay, Pierce, Rockford, IL, USA) and enzyme activity is expressed as mmol/kg protein/h.

Western blotting. A second piece of muscle (~30 mg) was homogenized in RIPA buffer supplemented with protease (Complete Mini<sup>®</sup>, Roche Applied Science) and phosphatase inhibitors (PhosSTOP, Roche, Applied Science) using Lysing Matrix D tubes (MP Biomedicals, Solon, OH, USA) with the FastPrep-24 Tissue and Cell Homogenizer (MP Biomedicals, Solon, OH, USA). Samples were processed for 6 x 20-second cycles at 4.0 m/s, with samples placed on ice for 5 minutes in between cycles. Samples were rotated end-over-end for 1 hour at 4°C, underwent a 10 minute spin at 14,000 g, and the supernatant was aliquoted for use. Western blot analysis was conducted using techniques described previously (31). Briefly, the protein concentrations of homogenates were determined (BCA Protein Assay), and equal amounts of protein were prepared in 4X Laemmli's buffer and heated to 95°C before being separated by 10% SDS-PAGE. Protein was electrotransferred to nitrocellulose membranes and Ponceau S staining was performed to visualize equal loading and transfer. Following 1 hour blocking in 3-5% fat-free milk Tris-buffered saline 0.1% Tween<sup>®</sup> 20 (TBS-T), membranes were incubated in the primary antibody overnight at 4°C in 3-5% fat-free milk TBS-T based on previously optimized conditions. After 3 x 5-minute washes in TBS-T, membranes were incubated in the species-specific secondary antibody diluted (1:10,000) in 3% fat-free milk TBS-T for 1 hour at room temperature. Membranes were washed in TBS-T for 3 x 15-minutes and visualized by chemiluminescence



(SuperSignal West Dura, Pierce) using a FluorChem® SP Imaging System (Alpha Innotech Corporation, San Leandro, CA, USA). ImageJ software was used to quantify the optical density of protein bands.  $\alpha$ -tubulin (Cell Signaling Technology, #2125), which did not change following training ( $p=0.85$ ), was used as a loading control. Primary antibodies for the following proteins of interest were used: NDUFA9 (Mitosciences, MS111), Complex II 70 kDa subunit (Mitosciences, MS204), Complex III Core 2 protein (Mitosciences, MS304), cytochrome c oxidase (COX) subunit II (MitoSciences, MS405), COX subunit IV (Mitosciences, MS408), ATP synthase  $\alpha$ -subunit (MitoSciences, MS507) and GLUT4 (Millipore, AB1345).

### **Statistics**

Baseline subject characteristics (Table 1) were analyzed using a one-way (group) analysis of variance (ANOVA). Muscle, blood and body composition data were analyzed using a two-way ANOVA with the between factor group and the within factor time (pre- and post-training for all variables except for  $\text{VO}_2\text{peak}$  which included a mid-point test as well). Significant group x time interactions ( $p<0.05$ ) were subsequently analyzed using a Tukey's honestly significant difference post hoc test. All analyses were conducted using SPSS software, and the level of significance was set at  $p<0.05$ . All data are presented as means  $\pm$  standard deviation for  $n=10$  (MICT),  $n=9$  (SIT) and  $n=6$  (CTL), unless otherwise stated. Due to difficulties during data collection, we report  $n=9$  (MICT) and  $n=5$  (CTL) for body composition data and  $n=8$  (SIT) for blood analyses.

## RESULTS

### *Descriptive characteristics of training*

A total of  $31 \pm 1$  and  $32 \pm 2$  sessions were completed for SIT and MICT, respectively. Mean HR, measured continuously over the 10- and 50-minute protocols and averaged over all training sessions, was  $79 \pm 4\%$  and  $71 \pm 5\%$  of  $HR_{\max}$  for SIT and MICT, respectively. Mean RPE, measured during the 1<sup>st</sup>, 15<sup>th</sup> and 30<sup>th</sup> exercise sessions, was  $16 \pm 1$  for SIT and  $13 \pm 1$  for MICT. Mean total work per session, calculated over the course of training based on average power outputs, was  $\sim 60$  and  $\sim 310$  kJ per session for SIT and MICT, respectively. While there was no change in total body mass (Table 2), there was a slight reduction in whole-body percent fat following SIT and MICT ( $p < 0.05$  vs. pre-training for both, Table 2).

### *Cardiorespiratory fitness*

$VO_{2\text{peak}}$  increased by  $\sim 12\%$  after 6 weeks of training in both SIT and MICT ( $p < 0.05$  vs. pre training for both; Fig. 1).  $VO_{2\text{peak}}$  increased further at 12 weeks compared to 6 weeks in both exercise groups ( $p < 0.05$  for SIT and MICT; Fig. 1), resulting in a  $\sim 19\%$  overall improvement in both groups vs. pre-training.

Accordingly,  $W_{\max}$  increased by  $\sim 12\%$  following 12 weeks of training ( $p < 0.05$  vs. pre-training, Table 2).  $VO_{2\text{peak}}$  and  $W_{\max}$  were unchanged at all time points in CTL ( $p > 0.05$ ).

### *Skeletal muscle adaptations*

The maximal activity of CS increased after SIT and MICT ( $p < 0.05$  vs. pre-training for both), respectively, such that both were greater than CTL post-training ( $p < 0.05$  vs. CTL; Fig. 2A). Individual changes in CS activity are depicted for all participants in Figure 2B. SIT and MICT also increased mitochondrial protein content as evidenced by changes in Complex II 70 kDa, Complex III Core 2 protein, COX subunit IV and ATP Synthase  $\alpha$ -subunit (all  $p < 0.05$  vs. pre-training), all of which were also greater than CTL post-training ( $p < 0.05$ ; Fig. 2C). The increase in the protein content of NDUFA9 after SIT and MICT did not reach statistical significance ( $p = 0.26$ ; Fig 2C). There was no training by time interaction for  $\beta$ -HAD maximal activity, although the absolute changes in SIT (28%) and MICT (17%) were larger compared to CTL (-2%; data not shown). GLUT4 protein content increased by ~50% following SIT and MICT ( $p < 0.05$  vs. pre-training for both), and SIT was greater than CTL post-training ( $p < 0.05$ ; Fig. 3).

#### *Indices of glycemic control*

$CS_I$  from the IVGTT, and associated variables ( $K_G$  and  $\Delta AUC_{INS}$ ), are reported in Table 2.  $CS_I$  increased 53 and 35% following 12 weeks of SIT and MICT, respectively ( $p < 0.05$  vs. pre-training for both; Fig. 4). Glucose AUC during the 50-minute IVGTT was lower following SIT and MICT ( $p < 0.05$  vs. pre-training for both; Table 2); however, no significant changes were observed in insulin AUC ( $p > 0.05$  vs. pre-training; Table 2). Fasting indices of glycemic control, including plasma glucose, serum insulin and HOMA-IR, were unchanged following training

( $p > 0.05$ , Table 2). There were no changes in CTL for any index of glycemic control ( $p > 0.05$ , Fig. 4, Table 2).

## DISCUSSION

The major novel finding from the present study was that 12 weeks of SIT in previously inactive men improved cardiorespiratory fitness, skeletal muscle oxidative capacity and insulin sensitivity to the same extent as MICT that involved a five-fold greater exercise volume. SIT involved 1 minute of intense intermittent exercise, within a time commitment of 10 minutes per session, whereas MICT consisted of 50 minutes of continuous exercise at a moderate pace. A few previous studies have reported similar improvements in skeletal muscle remodeling and markers of health status after SIT and MICT protocols lasting up to 6 weeks (6, 10, 16). The present work was more ambitious in scope, involving a SIT protocol that required less than half the time of previous interventions, a training program that was twice the duration of previous studies, and the inclusion of a non-training control group. Our data demonstrate that 12 weeks of brief intense exercise improves indices of cardiometabolic health to the same extent as traditional endurance training involving a five-fold greater time commitment and exercise volume.

### *Cardiorespiratory fitness*

Tabata and colleagues showed over two decades ago that a low-volume SIT protocol involving eight 20-second cycling sprints, interspersed with 10 seconds of rest, increased  $\text{VO}_{2\text{peak}}$  by  $\sim 13\%$  when performed 4 days per week for 6 weeks (50). The finding that brief, intense exercise training can improve cardiorespiratory fitness has been demonstrated by others using various protocols that involve a total time commitment of  $\leq 10$  minutes per session (19, 32, 36). One such study included a comparison to MICT (35) and showed that 16 sessions of body-weight type SIT over 4 weeks improved  $\text{VO}_{2\text{peak}}$  to the same extent ( $\sim 7\text{--}8\%$ ) as 30 minutes of continuous cycling at 85% of  $\text{VO}_{2\text{peak}}$  per session. In the present study, we observed a strikingly similar rate of improvement between groups over 12 weeks of training, which amounted to 19% overall after both SIT and MICT, despite a five-fold difference in the weekly training time commitment. This mean increase compares favorably with the typical change in  $\text{VO}_{2\text{peak}}$  reported after several months of traditional endurance training (37, 44), although individual responses can be highly variable (48) as also observed in the present study. Exercise intensity is generally regarded to be the more critical factor in the trainability of  $\text{VO}_{2\text{peak}}$ , with higher intensity exercise conferring larger improvements in cardiorespiratory fitness when exercise is matched for total volume (5, 20, 27, 52). The present data show it is possible for previously sedentary individuals to markedly improve  $\text{VO}_{2\text{peak}}$  using interval training, to a similar extent as traditional endurance despite requiring a five-fold lower time commitment. These findings are noteworthy given the well-established link

between low cardiorespiratory fitness and increased risk of cardiovascular disease and all-cause mortality (28, 29).

Improvements in  $\text{VO}_2\text{peak}$  following MICT are traditionally believed to be centrally mediated (4), however SIT studies of this nature are limited and controversial (14, 24, 33). Esfandiari et al. (14) reported similar increases in stroke volume during exercise following 2 weeks of HIIT or MICT, as a result of increased plasma volume and left ventricular filling. This is not a universal finding however (24, 33), and it has also been suggested that SIT-induced improvements in  $\text{VO}_2\text{peak}$  may be mediated by peripheral factors, at least over the short-term (49). It remains to be determined if the similar rate of improvement in  $\text{VO}_2\text{peak}$  at both 6 and 12 weeks of training in SIT and MICT is a result of similar or distinct mechanisms and this is a fruitful area for continued investigation.

#### *Mitochondrial content*

It is well established that short-term SIT can markedly enhance skeletal muscle oxidative capacity as reflected by increases in the maximal activity and total protein content of mitochondrial enzymes (6, 19). With respect to potential underlying mechanisms, SIT appears to activate many of the same molecular signaling pathways that are believed to regulate skeletal muscle remodeling in response to MICT (31). A few previous studies have shown that very low-volume SIT, similar to that employed in the present study, also increases mitochondrial

content. For example, Ma et al. (32) showed that Tabata-style training involving eight, 20-second cycling efforts at an intensity equivalent to 170%  $\text{VO}_{2\text{peak}}$ , interspersed with 10 seconds of rest, increased the protein content of COXI and COXIV when performed three times per week for 4 weeks. Similarly, we have shown that 6 weeks of training, using the protocol employed in the present work, increased the maximal activity of citrate synthase (19). The present study extends these observations and demonstrates that 12 weeks of SIT increases multiple markers of mitochondrial content to the same extent as MICT despite a five-fold difference in weekly time commitment.

We did not specifically examine the time course of skeletal muscle remodeling in the present study, but the mean increase in citrate synthase maximal activity in the two groups after 12 weeks of training was similar to the 30-40% increase that we previously observed after 2 (7) and 6 weeks (6, 19) of SIT and MICT. Consistent with the recent observations by Egan et al. (13), who examined the time course for the increase in mitochondrial content in response to 14 sessions of endurance training, these data would seem to collectively imply that much of the increase in mitochondrial content occurs relatively early in response to training. The robust remodeling induced by SIT would also seemingly suggest that training intensity, rather than volume, may be the more critical determinant of the improvement in mitochondrial capacity, or at least that brief bouts of very intense exercise can stimulate pathways leading to mitochondrial biogenesis to a similar extent as longer periods of less intense contractile activity. As recently

reviewed by Bishop et al. (3), we know surprisingly little regarding the optimal exercise stimulus for inducing mitochondrial adaptations in human skeletal muscle, and well-controlled studies directly manipulating training intensity and volume are required.

### *Insulin sensitivity*

Previous studies lasting up to 6 weeks have employed a variety of techniques to assess changes in glycemic control after SIT. Babraj et al. (2) were the first to show that short-term SIT could improve insulin sensitivity, as reflected by OGTTs performed before and after six sessions of SIT performed over 2 weeks. Others have replicated this finding using the same method (60), and Richards et al. (42) verified the observation using the gold standard hyperinsulinemic-euglycemic clamp method. We have also observed lower 24-hour blood glucose concentration in men after a 6-week SIT protocol that was otherwise similar to the one employed in the present experiment (19). Previous studies directly comparing short-term SIT and MICT have reported similar improvements based on OGTT-derived measures (9, 45, 47). In the present study, we measured insulin sensitivity using data obtained from a 50-minute IVGTT ( $CS_I$ ) recently established by Tura and colleagues (57). The technique was shown to be highly correlated with the gold standard glucose infusion rate obtained during a hyperinsulinemic-euglycemic clamp (57), and  $CS_I$  has been shown to have greater reproducibility than  $M_{ISI}$  derived from OGTTs (39).



Houmard et al. (23) previously reported that a continuous training protocol involving 170 minutes of exercise per week improved insulin sensitivity based on IVGTT-derived measures more substantially than 115 minutes per week, regardless of exercise intensity and volume. However, several recent reports suggest that higher-intensity exercise training confers larger improvements in insulin sensitivity, when exercise is matched for total volume (5, 12, 27, 43, 52). In the present study, SIT improved insulin sensitivity to the same extent as MICT despite a five-fold lower exercise volume.

The potential mechanisms that mediate exercise-training induced increases in whole-body insulin sensitivity are obviously complex (21). With respect to potential changes in skeletal muscle that might in part explain the improved insulin sensitivity, we found similar increases in GLUT4 protein content after the two different training protocols despite large differences in total exercise volume. SIT and MICT have also been shown to similarly increase skeletal muscle microvascular density (9), which is also associated with improved glucose transport and insulin sensitivity (1, 9), despite large differences in training volume. It is also possible that the improvement in mitochondrial content (21) or an increased capacity for intramuscular triglyceride utilization (47) could be involved.

*Is exercise intensity the key for improving cardiometabolic health?*

There is currently no consensus on the importance of exercise intensity for improving cardiometabolic health (40). Physical activity guidelines imply that, in

comparison with moderate-intensity exercise, the benefits of engaging in vigorous-intensity exercise are attributed to the greater volume or energy expenditure dose per unit of time, and do not relate to intensity *per se*. Epidemiological evidence however, suggests there is an inverse relationship between relative exercise intensity and risk of coronary heart disease (30, 46), metabolic syndrome (25) and all-cause mortality (46, 61). Accumulating evidence also indicates that when exercise is matched for total volume, higher intensity exercise confers larger improvements in cardiorespiratory fitness (5, 20, 27, 52) and insulin sensitivity (5, 12, 27, 43, 52). Our findings compliment this general concept and suggest that when exercise dose is *not matched*, a low volume of high-intensity exercise is as effective as a high volume of moderate-intensity exercise for improving cardiometabolic health. These findings may be of interest to individuals citing a “lack of time” as a barrier to regular exercise participation. Large-scale randomized control trials are needed to confirm these findings in individuals at risk for, or afflicted with, cardiometabolic disease.

## CONCLUSION

In summary, we report that a SIT protocol involving 3 minutes of intense intermittent exercise per week, within a total time commitment of 30 minutes, is as effective as 150 minutes per week of moderate-intensity continuous training for improving cardiorespiratory fitness, skeletal muscle oxidative capacity and insulin sensitivity in previously inactive men. While largely a proof-of-concept study, the

investigation represents the longest comparison of SIT and MICT to date and demonstrates the profound effectiveness of short, intense bursts of exercise for improving cardiometabolic health. Future studies should examine the potential for other protocols that are relatively intense, but not necessarily ‘all-out’ efforts, to induce similar effects in an equally time-efficient manner. Considering a large number of individuals do not meet the minimum physical activity recommendations (11, 55), and preliminary evidence suggesting greater adherence to interval compared to traditional endurance training (26), it is worth exploring the potential for this exercise strategy on a larger scale to improve public health.

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## **ADDITIONAL INFORMATION**

### **Competing Interests**

Authors have no competing interests to declare.

### **Author contributions**

Conception and design of the experiments: JBG, MJG. Collection, assembly, analysis and interpretation of data: JBG, BJM, MJM, LES, MAT, MJG. Drafting the article: JBG, MJG. Revising the article critically for important intellectual content: JBG, BJM, MJM, LES, MAT, MJG. All authors approved the final manuscript prior to submission.

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## TABLES

TABLE 1: Subject Characteristics

<b>VARIABLE</b>	<b>MICT (10)</b>	<b>SIT (9)</b>	<b>CON (6)</b>
<b>Age (y)</b>	28 ± 9	27 ± 7	26 ± 8
<b>Height (cm)</b>	176 ± 10	177 ± 11	176 ± 5
<b>Weight (kg)</b>	84 ± 20	84 ± 23	78 ± 25
<b>Body Mass Index (kg/m<sup>2</sup>)</b>	26 ± 6	27 ± 5	25 ± 7
<b>VO<sub>2</sub>peak (ml/kg/min)</b>	33 ± 6	32 ± 7	32 ± 7
<b>VO<sub>2</sub>peak (L/min)</b>	2.7 ± 0.5	2.6 ± 0.8	2.5 ± 0.7
<b>Maximal Workload (W)</b>	248 ± 30	243 ± 68	219 ± 60

Values are means ± S.D. VO<sub>2</sub>peak, maximal oxygen uptake

TABLE 2: Markers of Health and Fitness

VARIABLE	MICT (10)		SIT (9)		CON (6)	
	PRE	POST	PRE	POST	PRE	POST
Weight (kg)	84 ± 20	82 ± 20	84 ± 23	83 ± 22	78 ± 25	78 ± 23
Body Mass Index (kg/m <sup>2</sup> )	26 ± 6	26 ± 6	27 ± 5	26 ± 5	25 ± 7	25 ± 7
Percent Fat (%)	27 ± 10	25 ± 10*	30 ± 7	28 ± 8*	24 ± 6	25 ± 6
VO <sub>2</sub> peak (L/min)	2.7 ± 0.5	3.2 ± 0.5*	2.6 ± 0.8	3.0 ± 0.7*	2.5 ± 0.7	2.5 ± 0.7
Maximal Workload (W)	248 ± 30	271 ± 33*	243 ± 68	275 ± 50*	219 ± 60	213 ± 52
CS <sub>I</sub>	5.0 ± 3.3	6.7 ± 5.0*	4.9 ± 2.5	7.5 ± 4.7*	7.4 ± 5.8	7.0 ± 4.9
K <sub>G</sub> (%/min)	2.0 ± 0.9	2.1 ± 0.7	2.1 ± 0.9	2.4 ± 0.8	2.1 ± 0.6	2.1 ± 0.7
ΔAUC <sub>INS (10-50 min)</sub> (uIU/ml)	1171 ± 591	1007 ± 545	1231 ± 705	1149 ± 844	1095 ± 843	1158 ± 908
ΔInsulin AUC (uIU/ml)	1423 ± 712	1223 ± 647	1515 ± 917	1454 ± 1065	1317 ± 946	1425 ± 1035
ΔGlucose AUC (mmol/L)	321 ± 114	257 ± 103*	303 ± 92	225 ± 75*	201 ± 52	222 ± 54
FPG (mmol/L)	5.3 ± 0.8	5.7 ± 0.9	5.0 ± 1.2	5.4 ± 0.8	5.5 ± 1.6	5.4 ± 0.8
FPI (uIU/mL)	10.1 ± 6.0	8.4 ± 6.9	9.5 ± 5.3	7.8 ± 4.1	7.5 ± 6.4	10.8 ± 13.2
HOMA-IR	2.4 ± 1.6	2.3 ± 2.1	2.1 ± 1.3	1.9 ± 1.0	2.0 ± 2.3	2.7 ± 3.7

Values are means ± S.D. **VO<sub>2</sub>peak**, maximal oxygen uptake; **CS<sub>I</sub>**, insulin sensitivity index from IVGTT; **K<sub>G</sub>**, glucose rate of disappearance during 10-50 min of IVGTT; **ΔAUC<sub>INS</sub>**, insulin area under the curve from 10-50 min of IVGTT; **ΔInsulin AUC**, insulin area under the curve from 0-50 min of IVGTT; **ΔGlucose AUC**, glucose area under the curve from 0-50 min of IVGTT; **FPG**, fasting plasma glucose; **FPI**, fasting plasma insulin. \*Significantly different vs. pre-training (p<0.05)

## FIGURE LEGENDS

**Figure 1. Similar improvements in  $\text{VO}_2\text{peak}$  after 6 and 12 weeks of SIT or MICT.** Measured at baseline (PRE), 6 weeks (MID), and 12 weeks (POST) in MICT, SIT and CTL. Values are means  $\pm$  S.D. \*  $p < 0.05$ , vs. same group at PRE; #  $p < 0.05$ , vs. same group at MID.

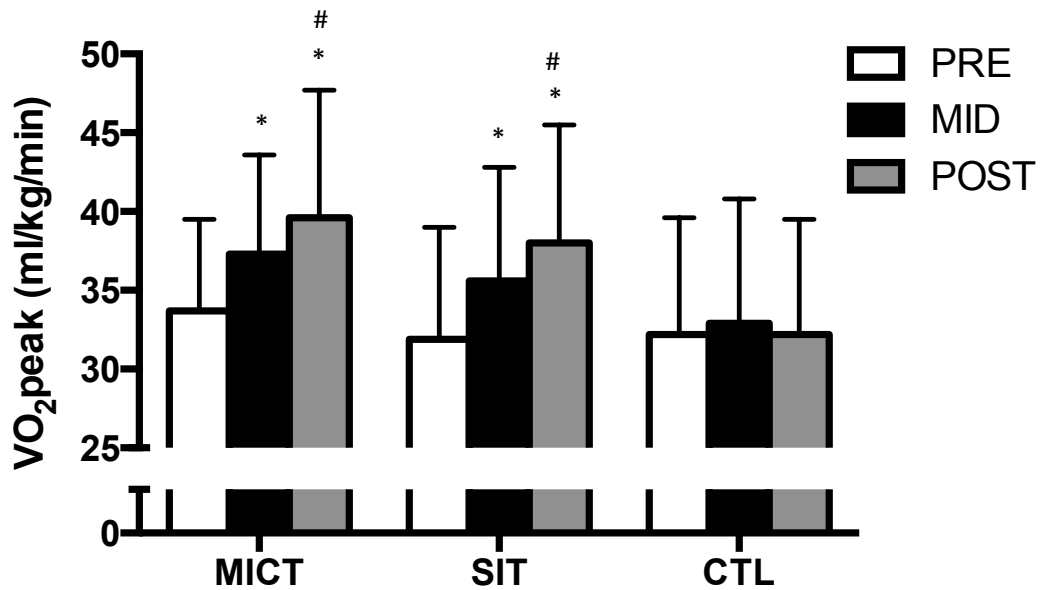
**Figure 2. 12 weeks of SIT or MICT increases skeletal muscle mitochondrial capacity.** Measured in muscle biopsy samples obtained from the vastus lateralis before (PRE) and 96 h after (POST) the 12-week intervention in MICT, SIT and CTL. Maximal activity of citrate synthase (A), individual changes in maximal activity of citrate synthase (B) and protein content of various subunits from complexes in the electron transport chain (C). Representative western blots are shown. Values are means  $\pm$  S.D. \*  $p < 0.05$ , vs. same group at PRE; †  $p < 0.05$ , vs. CTL at POST.

**Figure 3. 12 weeks of SIT or MICT improves GLUT4 protein content.** GLUT4 protein content measured in muscle biopsy samples obtained from the vastus lateralis before (PRE) and 96 h after (POST) the 12-week intervention in MICT, SIT and CTL. Values are means  $\pm$  S.D. \*  $p < 0.05$ , vs. same group at PRE; †  $p < 0.05$ , vs. CTL at POST.

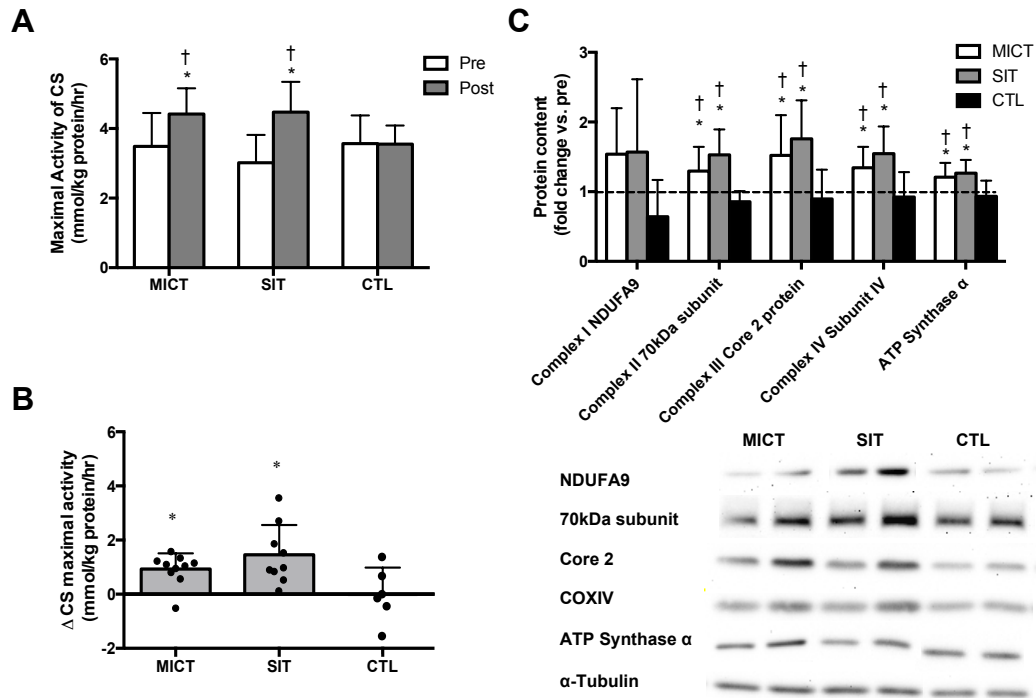
**Figure 4. Similar increases in insulin sensitivity following 12 weeks of SIT or MICT.**

The change in insulin sensitivity ( $CS_I$ ) over the 12-week intervention, measured from a 50-minute IVGTT in MICT, SIT and CTL. Closed circles denote individual responses. Values are means  $\pm$  S.D. \*  $p < 0.05$ , PRE vs. POST.

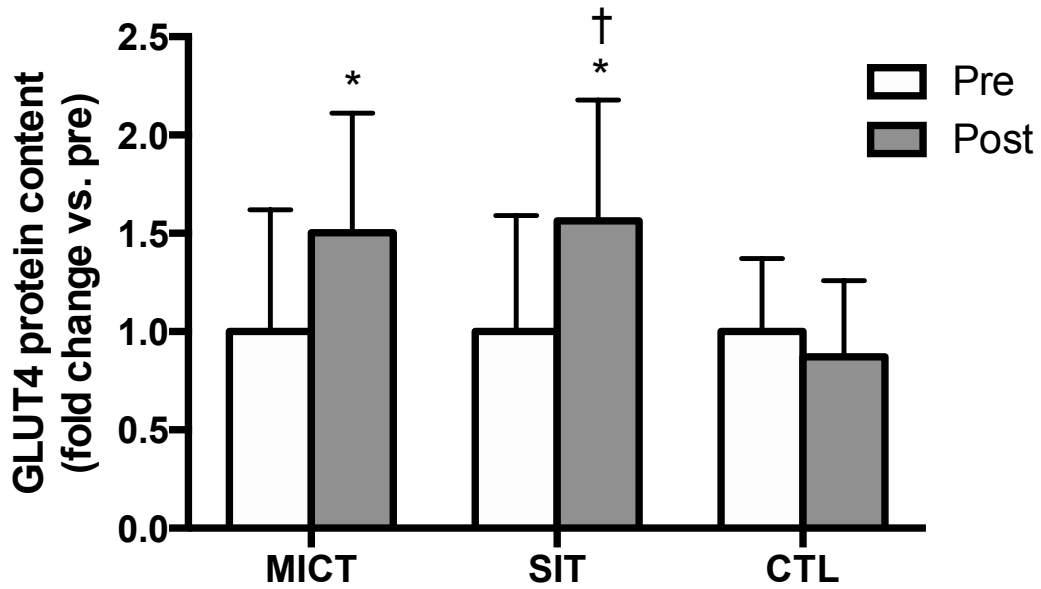




**Figure 1. Similar improvements in  $VO_2$  peak after 6 and 12 weeks of SIT or MICT.** Measured at baseline (PRE), 6 weeks (MID), and 12 weeks (POST) in MICT, SIT and CTL. Values are means  $\pm$  S.D. \*  $p < 0.05$ , vs. same group at PRE; #  $p < 0.05$ , vs. same group at MID.

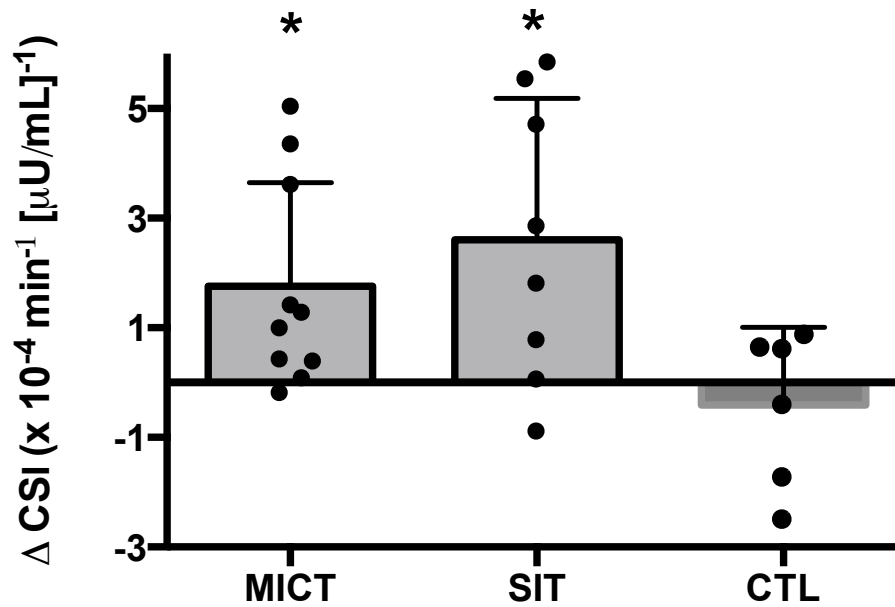


**Figure 2. 12 weeks of SIT or MICT increases skeletal muscle mitochondrial capacity.** Measured in muscle biopsy samples obtained from the vastus lateralis before (PRE) and 96 h after (POST) the 12-week intervention in MICT, SIT and CTL. Maximal activity of citrate synthase (A), individual changes in maximal activity of citrate synthase (B) and protein content of various subunits from complexes in the electron transport chain (C). Representative western blots are shown. Values are means  $\pm$  S.D. \*  $p < 0.05$ , vs. same group at PRE; †  $p < 0.05$ , vs. CTL at POST.



**Figure 3. 12 weeks of SIT or MICT improves GLUT4 protein content.**

GLUT4 protein content measured in muscle biopsy samples obtained from the vastus lateralis before (PRE) and 96 h after (POST) the 12-week intervention in MICT, SIT and CTL. Values are means  $\pm$  S.D. \*  $p < 0.05$ , vs. same group at PRE; †  $p < 0.05$ , vs. CTL at POST.



**Figure 4. Similar increases in insulin sensitivity following 12 weeks of SIT or MICT.**

The change in insulin sensitivity ( $\text{CSI}$ ) over the 12-week intervention, measured from a 50-minute IVGTT in MICT, SIT and CTL. Closed circles denote individual responses. Values are means  $\pm$  S.D. \*  $p < 0.05$ , PRE vs. POST.

**CHAPTER 5:**  
**GENERAL DISCUSSION**

## **5.1. Introduction**

The present thesis sought to advance our understanding of the physiological and health-related adaptations to low-volume interval training. The first study (Chapter 2) investigated whether performing 6 weeks of HIIT, in the fasted- versus fed-state, could potentiate adaptations in skeletal muscle oxidative capacity, insulin sensitivity and body composition in previously sedentary women. In contrast to the augmented responses seen after 6 weeks of MICT when physically active men trained in the fasted compared to fed state (80, 81), there was no effect of the nutritional manipulation on HIIT-induced improvements in skeletal muscle oxidative capacity or body composition. A surprising observation however, was the lack of improvement in insulin sensitivity after HIIT, as determined from OGTTs. This finding is in contrast to previous low-volume interval training studies in men, which consistently showed improved markers of glycemic control (4, 83, 107), revealing the potential for sex-based differences in the adaptive response. In Study 2 (Chapter 3), we attempted to advance our understanding of the physiological and health-related adaptations to very low-volume SIT by examining the impact of a protocol involving only 3 minutes of intense intermittent exercise within a 30-minute weekly time commitment. Following a 6-week intervention in previously sedentary men and women, we observed improvements in  $\text{VO}_{2\text{peak}}$  and mitochondrial capacity, as reflected by increased maximal activity of CS and protein content of COXIV. Intriguingly, 24-hour blood glucose concentration, assessed by CGM, was improved in men but

not women, which is consistent with observations from Study 1. In the final study (Chapter 4), we conducted a 12-week training intervention in men directly comparing the low-volume SIT protocol from Study 2 to high-volume MICT reflective of current public health guidelines. Despite a five-fold lower exercise volume and time commitment in SIT, both protocols elicited strikingly similar improvements in  $\text{VO}_{2\text{peak}}$ , skeletal muscle mitochondrial content and insulin sensitivity derived from IVGTTs. Collectively, these findings highlight the potency of low-volume SIT and HIIT for improving cardiometabolic health in a time-efficient manner. The present chapter attempts to integrate findings from all studies and highlight the collective contribution of the thesis to the larger field. Potential limitations, unanswered questions, and future directions are discussed throughout.

## **5.2. Fasted- versus fed-state HIIT: No differences in the adaptive response**

MICT performed in the fasted state is characterized by a greater contribution of fat-derived energy provision as compared to fed-state exercise (20, 23, 49, 56). The low circulating insulin and high plasma epinephrine concentrations associated with fasting are believed to limit carbohydrate flux and oxidation (20, 56), and concomitantly increase oxidation of fatty acids derived from both adipose tissue (49) and intramuscular depots (10). These metabolic differences in substrate use have been suggested to alter the skeletal muscle adaptive response (80, 81). However, we found that 6 weeks of HIIT performed in

the fasted state did not potentiate training-induced gains in mitochondrial capacity, as reflected by similar increases in the maximal activity of CS and  $\beta$ -HAD in fasted and fed groups (37).

The disparity between our findings and those reported following 6 weeks of MICT could be due to a number of factors, including differential effects of the fasted state on moderate- versus high-intensity exercise. Evidence suggesting the fasted state is associated with increased fat oxidation during exercise are mainly derived when exercise is performed at 50 % of  $\text{VO}_{2\text{peak}}$  (7, 20, 49, 56), coinciding with the optimal intensity to promote fat utilization. It is well established however, that as exercise intensity increases, the relative and absolute contribution of fatty acids to energy provision declines (104). Alternatively, carbohydrate, particularly muscle glycogen, is the primary fuel source during exercise above 70 %  $\text{VO}_{2\text{peak}}$  (85, 104) and appears quite resistant to modification. Indeed, no difference in fuel oxidation was reported during 30 minutes of MICT at 75 %  $\text{VO}_{2\text{peak}}$  when performed in the fasted- or fed-state (7). Similarly, Whitley et al. (106) showed that regardless of whether exercise was performed after an overnight fast, or following a high-carbohydrate or high fat meal, substrate selection during 90 minutes of MICT at 70 % of  $\text{VO}_{2\text{peak}}$  was unaltered. It is likely that substrate selection was also similar between fasted- and fed-state HIIT during each 25-minute exercise session, considering the high-intensity nature and likely resilient reliance on muscle glycogen for energy provision. Thus, the metabolic perturbation induced by training was presumably



quite similar between groups, explaining the lack of difference in the adaptive response to training. It is also possible that the potency of the HIIT stimulus in an untrained population may have overshadowed any effect of the nutritional manipulation, which may have been difficult to tease out with our limited sample size. There is also evidence of sex-based differences in the response to fasted-state training. Stannard et al. (95) reported that 4 weeks of MICT performed at 65 %  $\text{VO}_2\text{peak}$  in the fasted- compared to fed-state potentiated training-induced gains in CS maximal activity in men but not women. Although not a universal finding (61), greater activation of AMPK $\alpha$ 2 during exercise in the fasted versus fed-state (1) has been suggested to mediate the superior training response. Women reportedly have lower AMPK $\alpha$ 2 activation than men following acute exercise performed in the fasted state however (84), which could explain the lack of response to fasted-state training in women (37, 95).

### **5.3. Low-volume interval training improves cardiorespiratory fitness**

Cardiorespiratory fitness increases following short-term low-volume interval training programs (3, 11, 13, 43, 70). The improvement in  $\text{VO}_2\text{peak}$  is comparable to that elicited by traditional MICT, at least in short-term studies lasting up to 6 weeks (14, 18, 30, 92). In Study 1, we observed a 15 % increase in  $\text{VO}_2\text{peak}$  following 6 weeks of low-volume HIIT in sedentary women, which is comparable to previous studies of similar duration (14, 18). More recently, brief SIT protocols, requiring  $\leq 10$  minutes per session including warm-up and cool-

down, have been shown to increase  $\text{VO}_{2\text{peak}}$  (43, 64, 72). In Study 2, we found that a SIT protocol involving 1 minute of intense intermittent exercise, within a 10-minute time commitment, improved  $\text{VO}_{2\text{peak}}$  by 12 % in men and women when 18 sessions were performed over 6 weeks (38). Improvements of this magnitude ( $\sim 3.5$  ml/kg/min or 1 metabolic equivalent) have been associated with a 15 and 19 % lower risk of all-cause and cardiovascular disease mortality (60), respectively, highlighting the clinical significance of these findings.

It has been speculated that the rapid improvement in  $\text{VO}_{2\text{peak}}$  observed following short-term interval training programs may begin to plateau over time, with divergent responses to MICT becoming more apparent over long-term interventions (94). Findings from Study 3, however, suggest that the increase in  $\text{VO}_{2\text{peak}}$  during SIT and MICT follow a strikingly similar pattern up to 12 weeks of training, amounting to a 19 % (6 ml/kg/min) improvement overall in both groups. In the longest previous comparison to date, Nybo et al. (76) showed that the increase in  $\text{VO}_{2\text{peak}}$  after 12 weeks of near-maximal interval running was double that elicited by a continuous running protocol (14 vs. 7 %) despite a lower weekly training time commitment ( $\sim 40$  vs.  $\sim 150$  minutes). Our findings and those of Nybo and colleagues (76), highlight that brief bursts of very intense exercise are as effective as a higher volume of MICT for improving cardiorespiratory fitness over the longer-term.

The mechanisms mediating the improvement in  $\text{VO}_{2\text{peak}}$  following low-volume SIT and HIIT cannot be ascertained from the present thesis.

Improvements in  $\text{VO}_2\text{peak}$  following MICT are traditionally believed to be mediated by central factors (i.e., enhanced oxygen delivery) (6, 9), with an increased stroke volume likely being the single most important determinant (9, 88). Studies measuring cardiac responses to short-term low-volume interval training are limited, however, with equivocal evidence suggesting that stroke volume is increased (30, 100) or unchanged (50, 66) after training. Recently, a 2-week low-volume HIIT versus MICT intervention in young healthy men revealed similar increases in Doppler-derived measures of end-diastolic volume, stroke volume and cardiac output, as well as plasma volume and  $\text{VO}_2\text{peak}$  (30). Other studies, however, found no change in cardiac output using the nitrous oxide rebreathing method following a similar 2-week HIIT intervention (50), or after 6 weeks of SIT (66). As a result, it has been proposed that short-term improvements in  $\text{VO}_2\text{peak}$  following low-volume interval training are primarily attributed to peripheral factors (i.e., enhanced oxygen extraction) (50, 66, 94), although few studies have directly examined potential cardiovascular adaptations after very short-term protocols. While additional work is warranted to clarify the precise nature of the mechanisms involved, the present thesis highlights the potency of short, intense bursts of exercise for improving cardiorespiratory fitness.

#### **5.4. Low-volume interval training increases skeletal muscle mitochondrial content**

Results from the present thesis are consistent with other reports highlighting the potency of low-volume interval training for improving skeletal muscle oxidative capacity (35). In all three studies, the maximal activity of CS was used as the primary indicator of mitochondrial adaptation to training, with the protein content of various mitochondrial enzymes employed secondary. CS activity is a commonly used biomarker of mitochondrial content in exercise training studies, as it is highly correlated with gold-standard measures made by electron microscopy (59). The most significant finding from the present thesis with regards to mitochondrial content was the profound improvement that can be elicited by a very small amount of high-intensity exercise. Specifically, we observed a 40 and 50 % increase in the maximal activity of CS following 6 and 12 weeks of low-volume SIT, respectively, with the latter being comparable to a similar period of MICT involving a 5-fold larger exercise volume.

Although increased mitochondrial content is among the most well described adaptations to aerobic exercise training (46), surprisingly little is known regarding the optimal stimulus for promoting this favorable metabolic adaptation (8). Exercise-induced increases in mitochondrial content are largely the result of an increased size of mitochondria (97), which likely reflects the incorporation of new proteins into existing mitochondria. This expanded mitochondrial network primarily serves to alter substrate metabolism during exercise and enhance endurance capacity (14, 39, 47, 79). As little as three sessions of aerobic exercise is sufficient to increase mitochondrial content (27, 78), but the extent to which

exercise duration and intensity influence this adaptation has received little attention (8).

The robust remodeling induced by SIT in Study 3 may suggest that intensity rather than duration is the more critical determinant of the improvement in mitochondrial content following exercise training. Indeed, evidence from acute studies reveal that when exercise volume is matched, higher intensity exercise elicits greater increases in signaling pathways believed to regulate mitochondrial biogenesis in response to training (24, 26, 108). However, caution is warranted when extrapolating these findings, as acute protein signaling events do not necessarily predict long-term phenotypic adaptations (16). We currently lack chronic studies in humans that directly manipulate training duration and intensity in an effort to determine the optimal stimulus for inducing mitochondrial biogenesis (8). The most comprehensive study of this nature was performed in rodents, which included 19 different groups of various exercise intensity and duration combinations (25). Dudley et al. (25) reported that exercise intensity, rather than duration, was more closely associated to the extent of improvement in cytochrome c concentration following 8 weeks of aerobic training. The authors also concluded that as exercise intensity increased, the duration required to achieve a given mitochondrial response decreased, which is consistent with findings from Study 3. The strength of the relationship in the study by Dudley and colleagues was most pronounced in muscle groups containing a greater proportion of “fast white” (type II) fibers (25). This suggests that the greater motor unit

recruitment during high-intensity exercise (87), and subsequent remodeling of type II fibers, could largely mediate the positive relationship between exercise intensity and mitochondrial adaptations.

A limited number of studies have examined muscle fiber type-specific responses to interval training in humans. There is evidence to suggest that interval training induces a modest shift in fiber type, with most studies showing an increase in type IIa fibers (i.e.  $I \rightarrow IIa \leftarrow IIb$ ) (58), consistent with an enhanced oxidative capacity. Specifically with respect to mitochondrial adaptations, Henriksson and Reitman (44) showed almost four decades ago that 8 weeks of maximal-intensity intermittent cycle training induced greater increases in succinate dehydrogenase (SDH) activity in type II compared to type I fibers. In comparison, a similar amount of work performed continuously at a moderate intensity increased SDH activity in type I fibers only (44). Kristensen et al. (57) recently characterized fiber type-specific responses to an acute bout of continuous (30 minutes at 70 %  $VO_{2peak}$ ) and interval (6 x 90 second sprints 95 %  $VO_{2peak}$ , 150 seconds recovery) exercise by dissecting muscle samples into type I and type II fiber pools. In contrast to the continuous protocol, interval exercise elicited fiber type-specific responses such that glycogen utilization and AMPK expression were greater in type II versus type I fibers (57). This in turn could preferentially trigger mitochondrial adaptations in type II fibers over the course of training. Recent investigations into fiber type-specific responses to low-volume interval training do not support this hypothesis however, as similar increases in SDH (90)

and COXIV (92) expression were reported after 6 weeks of SIT in type I and II fibers. This conclusion is based on serial section immunofluorescent staining, however, and investigations using recent methodological advances for fiber type-specific analyses remain ripe for future research (75).

## **5.5. Low-volume interval training and indices of glycemic control**

### *5.5.1. Methods of assessment*

The hyperinsulinemic-euglycemic clamp, originally proposed by DeFronzo et al. (22) is widely accepted as the reference standard for direct assessment of insulin sensitivity in humans (74). However, performing the test is time consuming, labour intensive, technically complex and expensive (74, 105). Thus, a variety of surrogate methods are available for measurement of insulin sensitivity and/or glycemic control, ranging in accuracy, complexity and invasiveness. In this thesis, four methods were employed for assessment of insulin sensitivity and/or glycemic control, all of which are associated with different merits and limitations.

Insulin sensitivity derived from fasting samples is arguably the easiest, quickest and least expensive surrogate index. The homeostasis model assessment (HOMA) (69), which was employed in all three studies, is commonly derived from fasting samples for determination of insulin sensitivity or insulin resistance. However, the fasting condition represents a basal steady state for glucose and insulin, which primarily reflects hepatic insulin sensitivity (74). In contrast,

skeletal muscle is the primary disposal site in response to a glucose load (21), perhaps making glucose tolerance tests a more effective method for assessing exercise-induced changes in glycemic control.

OGTTs are recognized as the accepted diagnostic method for type 2 diabetes (2) and are commonly employed in exercise interventions (method described in Study 1, ref 37). This 2-hour test is designed to provide information on glucose tolerance, however insulin sensitivity can also be obtained from surrogate indexes, such as the Matsuda composite index (68). The OGTT is easy to perform, minimally invasive and mimics glucose and insulin dynamics of physiological conditions more closely than the glucose clamp. However, intra-variability in the OGTT has been reported as high as 17 % (91), and poor reproducibility is documented in multiple studies (12, 73, 77). This could be attributed to variable glucose absorption, splanchnic glucose uptake and incretin effects associated with the oral glucose load (74). Indeed, this may have limited our ability to detect training-induced changes in insulin sensitivity in Study 1 (37).

An alternative method overcoming some of the limitations associated with the OGTT is the IVGTT. The Minimal Model insulin sensitivity index is calculated based on frequent glucose and insulin samples obtained during a 3-hour IVGTT (74, 105). IVGTTs are more labour intensive than OGTTs, but the insulin sensitivity index obtained is reliable (31) and correlates well with the glucose clamp method in healthy individuals (86). However, IVGTTs are time consuming and a specialized computer program is required to calculate insulin sensitivity,



making this a difficult measure to implement in clinical and/or research settings (74, 105). Tura and colleagues (103) recently validated an insulin sensitivity index calculated from glucose and insulin data during a modified 50-minute IVGTT (method described in Study 3). This method is highly correlated with the Minimal Model insulin sensitivity index obtained from the 3-hour IVGTT, as well as the gold standard glucose infusion rate measured during the glucose clamp in individuals with both normal and impaired glucose tolerances (103). This method has also been shown to have greater day-to-day reproducibility than the Matsuda composite index derived from a 2-hour OGTT (77). While the intravenous delivery of glucose generates a more artificial stimulus than OGTTs, the high reliability and validity of the 50-minute IVGTT makes it a superior method for determination of insulin sensitivity. Indeed, we were successful in capturing changes in insulin sensitivity using this method following 12 weeks of SIT or MICT in Study 3.

Lastly, CGM has emerged as an effective tool for assessing glucose control and was implemented in Study 2. While insulin sensitivity cannot be determined from this method, CGM provides detailed information on the direction, magnitude and frequency of blood glucose excursions over several days under free-living conditions (55). CGM proved to be a sensitive measure to detect training-induced reductions in 24-hour blood glucose concentration in Study 2, consistent with other reports (36, 54, 62, 67). In fact, a recent meta-analysis revealed that a number of exercise interventions reduced mean daily glucose

concentration as detected by CGM, despite no changes in fasting glucose or insulin concentrations (65). This suggests that CGM may provide greater insight into the effects of exercise on glucose regulation than simple fasting blood markers.

*5.5.2. Are changes in glycemic control after low-volume interval training sex-specific?*

Low-volume SIT and HIIT have been shown to improve insulin sensitivity, based on hyperinsulinemic euglycemic clamps performed on recreationally active individuals (83) as well OGTTs performed on young healthy (4, 18) and overweight/obese (17, 107) men. These studies have been relatively short-term interventions lasting up to 6 weeks, but nonetheless indicate that low-volume interval training elicits similar improvements in glycemic control as MICT (18, 89, 92). Results from Study 2 advance these findings, as they suggest that a brief SIT protocol – involving only 3 minutes of intense exercise within a 30-minute time commitment per week – lowered mean 24-hour blood glucose concentration in men after 6 weeks of training (38). These findings were confirmed and expanded upon in Study 3 when we directly compared 12 weeks of this low-volume SIT protocol to MICT as reflected in public health guidelines. Despite a five-fold lower time commitment in SIT, insulin sensitivity derived from IVGTTs performed 72 hours following training were similarly improved in SIT and MICT, with no change in the non-training control group. The mechanisms mediating

improvements in insulin sensitivity following low-volume interval training could not be ascertained from the present thesis, however it is tempting to speculate that adaptations within skeletal muscle were involved. As discussed in Chapter 1, these adaptations could include the observed elevation in GLUT4 protein content or mitochondrial capacity, as well as previously reported increases in capillarization following low-volume SIT (17, 18).

Intriguingly, our results also revealed potential sex-based differences in the adaptive response, as we did not detect a change in glycemic control following 6 weeks of training in women. In Study 1, fasting and OGTT-derived measures of insulin sensitivity were unchanged following 6 weeks of HIIT in previously sedentary women (37), which contrasts earlier findings observed in men. Similarly, in Study 2 we observed no change in the mean 24-hour blood glucose concentration in women following 6 weeks of SIT, whereas improvements were detected in men (38). These findings are consistent with those of Metcalfe and colleagues (72) who reported that a 10-minute low-intensity cycling protocol that included 2 x 20 second ‘all-out’ sprints, performed 18 times over 6 weeks, improved insulin sensitivity measured by OGTTs in men but not women.

The training-induced increase in GLUT4 protein content in our study was 6-fold higher in men compared to women (38), which could in part explain the observed differences in glucose control. It has been suggested that high rates of glycogen utilization and subsequent resynthesis following high-intensity exercise may be related to the rapid improvement in insulin sensitivity following low-

volume interval training (72). In comparison to men, however, women are reported to break down 50 % less muscle glycogen during a single Wingate sprint (29), which is supported by lower blood lactate accumulation following single (28, 51) and repeated 30-s sprints (28). In comparison to men, a lower rate of glycogenolysis in women may be due to a reduced activity of glycolytic enzymes (40, 51), a greater proportion of type I fibers (63, 96) or a reported predisposition for aerobic metabolism during a 30-second sprint (45). It is important to note, however, that other studies involving mixed cohorts of men and women have not described sex-based differences in insulin sensitivity following low-volume interval training (48, 83, 89), although these studies were not specifically designed to address this issue. It is also possible that the higher glycemic control in women compared to men at baseline, consistent with other reports (34, 72), influenced our findings. Clearly, well-controlled studies are warranted to determine whether women might in fact “respond less” to low-volume interval training, using best practice designs that control for various factors, such as menstrual cycle phase and relative fitness, that can increase variance and lead to false conclusions regarding potential sex-based differences (98).

#### **5.6. Low-volume interval training: A time efficient strategy to improve public health?**

It is well established that regular endurance training enhances cardiorespiratory fitness (9), increases skeletal muscle oxidative capacity (47) and

improves indices of cardiometabolic health including insulin sensitivity (42). These findings provide support for current public health guidelines, which recommend 150 minutes of moderate- or 75 minutes of vigorous-intensity physical activity per week to achieve health benefits (32, 99, 109). Unfortunately, objectively measured accelerometer data from population-based studies in Canada (19) and the United States (101) indicate that only 15-20 % of adults meet these minimum guidelines. Reasons for not engaging in regular exercise are numerous and complex, but a “lack of time” remains a commonly cited barrier (15, 93, 102).

The present thesis suggests that there may be an intensity-duration trade-off with respect to stimulating the health benefits of exercise. Indeed, in the final study we report that a 12-week intervention involving 3 minutes of ‘all-out’ intermittent exercise per week improved cardiometabolic health to the same extent as 150 minutes per week of moderate-intensity physical activity. From an applied perspective, our findings may appeal to individuals citing a “lack of time” as a barrier to regular exercise participation. Nonetheless, concerns have been raised regarding the feasibility of implementing this type of exercise at the public level (41). SIT requires a specialized cycle ergometer and a high degree of participant motivation, perhaps making it impractical for many individuals. All studies in the present thesis employed a cycling model, however other types of traditional whole-body exercise may also be effective, e.g., climbing stairs, brisk uphill walking or running. A recent study found that subjects who performed 8 x 20 second efforts of a single exercise (burpees, jumping jacks, mountain climbers,

or squat thrusts) interspersed by 10 seconds of rest, four times per week for 4 weeks increased  $\text{VO}_{2\text{peak}}$  to the same extent as a group who performed 30 minutes of traditional endurance training per session (71). Nonetheless, future studies should examine the potential for HIIT protocols that are relatively intense, but not necessarily ‘all-out’ efforts, to induce similar effects in an equally time-efficient manner. Emerging evidence suggests that HIIT is perceived to be more enjoyable than MICT in recreationally active individuals (5, 53) and also results in greater exercise adherence in individuals with pre-diabetes (52). Given the improved health outcomes observed in the present thesis, it is worth exploring the potential for low-volume interval training to improve public health on a larger scale.

## **5.7. Conclusions**

The studies in the present thesis advance our understanding of the physiological and health-related adaptations to low-volume interval training in humans. Our findings suggest that low-volume HIIT and SIT are time-efficient exercise strategies to increase cardiorespiratory fitness, glycemic control and skeletal muscle oxidative capacity in previously sedentary adults. Importantly, these beneficial adaptations are realized despite a very low exercise volume and time commitment, highlighting the potency of short, intense bursts of exercise for improving cardiometabolic health. While previous studies have been relatively short-term interventions, including studies 1 and 2 in the present thesis, the

strikingly similar response to 12 weeks of SIT and MICT in Study 3 reveals the efficacy of SIT to improve health over the longer-term. Large-scale randomized control trials are needed to confirm these findings in individuals at risk for, or afflicted with, cardiometabolic disease. Nonetheless, the present thesis raises fundamental questions regarding the minimum exercise stimulus necessary to induce physiological remodeling that is linked to improved health. As proposed by others (33, 82), there is value in studying the potential for vigorous-intensity exercise to maximize the health benefits of physical activity, which could have implications for clinical and public health guidelines.

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**(Figure 1; Chapters 1)**



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