

SKELETAL MUSCLE METABOLIC AND PERFORMANCE ADAPTATIONS TO
HIGH-INTENSITY SPRINT INTERVAL TRAINING.

SKELETAL MUSCLE METABOLIC AND PERFORMANCE ADAPTATIONS TO
HIGH-INTENSITY SPRINT INTERVAL TRAINING.

By

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ABSTRACT

This thesis examined the effect of high-intensity “sprint” interval training (SIT) on aerobic-based exercise performance and metabolic adaptations in human skeletal muscle. It has long been recognized that several weeks of interval-based training increased skeletal muscle oxidative capacity; however, little was known regarding the minimum “dose” of SIT necessary to elicit this adaptive response or the time-course and magnitude of adaptation in other markers of skeletal muscle metabolic control. Our general hypothesis was that low-volume SIT would induce rapid improvements in a wide array of metabolic variables that were comparable to traditional high-volume endurance training (ET). Healthy young men and women were recruited to perform four to six 30-second “all out” Wingate Tests, three times per week with one to two days of recovery, for up to six weeks. The weekly dose of SIT corresponded to ~10 minutes of maximal cycling exercise (~225-300 kJ) over a total training time commitment of 60-90 minutes, including recovery. The SIT response was compared against control subjects who performed no training or an ET group who performed up to one hour per day of moderate-intensity cycling exercise, five days per week for six weeks (~2250 kJ per week). Our major findings were that one to two weeks of SIT increased performance during aerobic-based exercise (time-to-fatigue tests and time-trials of varying duration) and the maximal activity or total protein content of mitochondrial enzymes and transport

proteins associated with carbohydrate metabolism (e.g., citrate synthase, cytochrome oxidase, glucose transporter 4). Six weeks of SIT or ET induced similar increases in markers of skeletal muscle carbohydrate (pyruvate dehydrogenase E1 α protein content) and lipid oxidation (3-hydroxyacyl CoA dehydrogenase maximal activity) and peroxisome-proliferator-activated receptor-gamma coactivator-1 α protein content, and similar reductions in phosphocreatine and glycogen utilization during matched-work exercise. These data suggest that SIT is a time-efficient strategy to increase skeletal muscle oxidative capacity and to induce specific metabolic adaptations during exercise that are comparable to ET.

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FORMAT AND ORGANIZATION OF THESIS

This thesis includes material which sets the context of the candidate's work, published journal articles, and overall implications of this work, and has been prepared in the "sandwich format" as outlined in the School of Graduate Studies' Guide for the Preparation of Theses. This thesis is comprised of 4 original research papers (Chapters 2-5), preceded by a general introduction and followed by a general discussion. All research papers have been peer-reviewed and Chapters 2-4 are published with the candidate as first author, and Chapter 5 is pending final acceptance.

CONTRIBUTION TO PAPERS WITH MULTIPLE AUTHORSHIP

Chapter 2

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Burgomaster, K.A., Hughes, S.C., Heigenhauser, G.J.F., Bradwell, S.N. & Gibala, M.J. (2005). Six sessions of sprint interval training increases muscle oxidative potential and cycle endurance capacity in humans. *J.Appl.Physiol.*, 98, 1985-1990.

Contribution

The experiments were coordinated and conducted by S.C Hughes and K.A. Burgomaster, with assistance from the co-authors. This research was conducted as partial fulfillment for the Master's degree of S.C. Hughes, and fulfillment of a Natural Sciences and Engineering Research Council of Canada (NSERC) Undergraduate Student Research Award (USRA) for K.A. Burgomaster. The supervisor for this study was M.J. Gibala. Muscle biopsies were obtained by G.J.F. Heigenhauser. S.N. Bradwell assisted with reproducibility measurements. K.A. Burgomaster collected breath samples and processed blood samples. S.C. Hughes and K.A. Burgomaster conducted the training program and analyzed all muscle samples. Manuscript preparation was completed by K.A. Burgomaster with input from M.J. Gibala and the co-authors.

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Burgomaster, with the exception of the western blot analyses which were performed by M.J. Gibala and S.L. McGee. The training program was conducted by K.A. Burgomaster, K.R. Howarth and M. Rakobowchuk. Statistical analyses were completed by K.A. Burgomaster. Manuscript preparation was completed by K.A. Burgomaster with input from M.J. Gibala and the co-authors.

CHAPTER 1

GENERAL INTRODUCTION

1.1 INTRODUCTION

Repeated sessions of prolonged moderate-intensity exercise (e.g., ≥ 1 hour at $\sim 65\%$ of VO_{2peak}) performed over several weeks elicit physiological adaptations that result in improved endurance capacity and exercise performance. Increases in skeletal muscle oxidative capacity and shifts in substrate utilization contribute to the training response and facilitate improvements in exercise capacity (Gollnick *et al.* 1973). Rapid increases in muscle oxidative potential, as indicated by changes in the maximal activities of “marker” enzymes such as citrate synthase have been reported after only 6-7 days of traditional endurance training (ET) (i.e., 2 hours per day at $\sim 65\%$ of VO_{2peak}) (Chesley *et al.* 1996; Spina *et al.* 1996). Several weeks of high-intensity sprint interval training (SIT), which is characterized by brief repeated sessions of very intense exercise, has been shown to induce marked changes in skeletal muscle metabolism including increases in the maximal activities of enzymes involved in glycolytic and oxidative energy provision (Saltin *et al.* 1976; Jacobs *et al.* 1987; MacDougall *et al.* 1998). More recently it has been suggested that increases in skeletal muscle oxidative capacity occur quite quickly as demonstrated by large increases in citrate synthase maximal activity and peak oxygen uptake after only two weeks of daily SIT (Rodas *et al.* 2000; Parra *et al.*

2000). These findings illustrate the potency of SIT to elicit rapid adaptations in skeletal muscle metabolism and suggest that repeated sessions of very intense exercise may induce changes in skeletal muscle normally associated with ET.

The purpose of this introductory chapter is to review the regulation of skeletal muscle metabolism during acute high-intensity sprint exercise and following several weeks of SIT. The chapter begins with an overview of skeletal muscle energy provision during an acute bout of high-intensity exercise, and is followed by a review of metabolic and performance adaptations to SIT, including a brief outline of the time-course for such adaptations. Finally, the physiological adaptations induced by SIT and traditional ET are compared. Although the major focus of this review is skeletal muscle metabolic and performance adaptations, other physiological changes (e.g., alterations in cardiovascular regulation) during acute high-intensity exercise and following several weeks of SIT will be briefly discussed.

1.2 ENERGY PROVISION DURING HIGH-INTENSITY EXERCISE

1.2.1 Definition of sprint or high-intensity exercise

Although there is no universal agreement regarding the definition of sprint or high-intensity exercise, a sprint effort is often referred to as a single bout of brief, intense exercise performed at a relatively high exercise intensity (e.g., $\geq 90\%$ of VO_{2peak}). A single sprint is normally associated with activities such as cycling or running and may last 5 seconds up to several minutes in duration,

depending on the intensity at which the exercise is performed. With maximal cycling efforts, extremely high power outputs (i.e., power outputs three-fold greater than that which elicits peak oxygen uptake) are reached within a few seconds of contraction, however, as the duration of the bout increases, power output declines accordingly (Spriet 1995) (Figure 1.1). A common method of assessing sprint performance is the Wingate Test which consists of 30 seconds of “all out” cycling on an ergometer against a resistance equivalent to 7.5% of body mass.

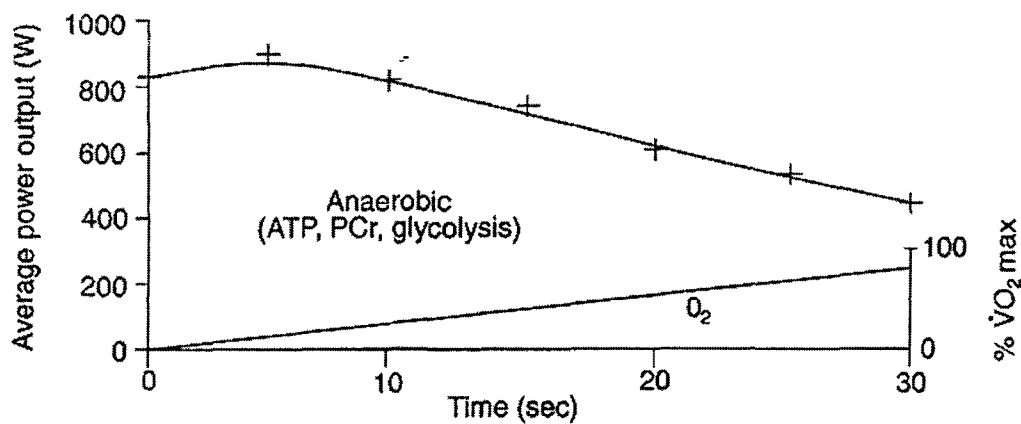


Figure 1.1 Average power output during 30 seconds of maximal isokinetic cycling. Approximate oxygen uptake during 30 seconds of cycling is indicated on the right axis (taken from Spriet 1995).

Sprint interval training is often referred to as repeated sessions of relatively brief, intermittent high-intensity exercise, separated by several minutes of recovery. A common training intervention in exercise studies is the incorporation of repeated Wingate Tests performed with an “all-out” effort, interspersed by 3-5 minutes of recovery (Barnett *et al.* 2004; MacDougall *et al.* 1998; Stathis *et al.* 1994). Alternatively, other studies have incorporated brief sprint running efforts performed at an intensity close to that which elicits maximal oxygen uptake, separated by relatively short recovery periods (Dawson *et al.* 1998; Nevill *et al.* 1989).

1.2.2 Skeletal muscle metabolism during an acute bout of high-intensity exercise

In order for muscle contractile activity to be maintained for longer than a few seconds, ATP must be supplied at a rate equivalent to demand. During exercise, phosphagen hydrolysis, non-oxidative glycolysis and oxidative phosphorylation contribute to ATP provision in order to meet the energy demands of the working muscle. It has been estimated that during a single 30-second maximal sprint the anaerobic and aerobic systems contribute ~80 and ~20% of total ATP provision, respectively (Parolin *et al.* 1999). The muscle metabolic response to a single bout of high-intensity exercise (e.g., 30-second sprint) is characterized by significant reductions in phosphocreatine (PCr) and glycogen, and increases in intramuscular lactate (Spriet *et al.* 1989). At the onset of high-intensity exercise, non-oxidative metabolism provides the majority of ATP

production when the energy demand is greater than can be provided through oxidative phosphorylation (Spriet 1995). For example, Bogdanis and colleagues (1998) demonstrated that during the initial 10 seconds of an intense bout of exercise, rapid rates of phosphagen hydrolysis and glycogenolysis resulted in marked reductions in muscle PCr and glycogen concentration.

Oxidative metabolism contributes to ATP provision particularly during the latter phase of short-term sprint exercise when phosphagen hydrolysis and glycogenolysis are reduced, compared to the initial seconds of exercise (Bogdanis *et al.* 1998). Parolin *et al.* (1999) demonstrated that within 15 seconds of a 30-second maximal effort sprint, glycogen phosphorylase approached full activation, allowing for maximal rates of glycogenolysis and pyruvate production. However, as the duration of the bout increased, the relative contribution from substrate-level phosphorylation decreased, while reliance on oxidative ATP production progressively increased (Parolin *et al.* 1999). Bogdanis *et al.* (1998) observed a similar reduction in substrate-level phosphorylation during 20 seconds of maximal sprint exercise and proposed that an increased reliance on oxidative metabolism during the latter part of the sprint partially compensated for the reduction in non-oxidative metabolism, and attenuated the loss of power output.

Several metabolic signals serve to activate enzymes of energy metabolism during exercise including changes in intracellular calcium concentration, the cytoplasmic phosphorylation potential of the muscle cell, and the mitochondrial

reduction/oxidation (redox) state (Spriet & Howlett 1999). At the onset of high-intensity exercise, increases in intracellular calcium activate glycogen phosphorylase within the first few seconds of contraction, allowing for rapid rates of glycogenolysis. In addition, during intense exercise when ATP is rapidly degraded, the increase in free AMP, ADP and inorganic phosphate “fine-tune” the activity of glycogen phosphorylase, while simultaneously increasing PFK activity (Spriet 1995). The rate of oxidative phosphorylation is primarily regulated by the mitochondrial pyruvate dehydrogenase (PDH) complex which is a multi-enzyme unit that exists in both an active and inactive form. PDH kinase catalyzes the phosphorylation of PDH resulting in inactivation, whereas PDH phosphatase dephosphorylates PDH resulting in activation of the enzyme (Spriet & Howlett 1999). During exercise, increases in calcium stimulate PDH phosphatase, whereas elevated levels of pyruvate and the ratios of CoASH/acetyl-CoA and NAD/NADH inhibit PDH kinase, thereby promoting PDH activation. During high-intensity sprint exercise, rapid increases in calcium, inorganic phosphate and AMP stimulate high rates of glycogenolysis resulting in enhanced pyruvate production. The increase in calcium and pyruvate concentration exert a positive allosteric effect on the activation of PDH, thereby enhancing oxidative phosphorylation during sprinting (Parolin *et al.* 1999).

1.2.3 Changes in skeletal muscle metabolism during repeated bouts of high-intensity exercise

Several studies have demonstrated that during repeated bouts of intense

exercise there is a progressive increase in the contribution of oxidative metabolism to ATP provision (Bogdanis *et al.* 1996; Gaitanos *et al.* 1993; Spriet *et al.* 1989; Parolin *et al.* 1999). Earlier work by Spriet *et al.* (1989) examined alterations in energy provision during three 30-second bouts of maximal cycling, and demonstrated a marked reduction in glycogenolysis and a concomitant increase in muscle hydrogen ion concentration ($[H^+]$) during the second and third bout of exercise. Despite reductions in muscle glycogen utilization during the final bout of exercise, the total work performed in bout 3 represented 82% of that in bout 2, suggesting that a greater reliance on oxidative metabolism permitted intense activity to be continued. Similarly, Gaitanos and colleagues (1993) observed a marked reduction in glycogenolysis after ten consecutive 6-second maximal sprints compared to the first bout of exercise. Despite a reduction in glycogenolysis, the mean power output elicited during the tenth sprint was 73% of that generated in the first sprint. The authors suggested that during the final sprint, power output was supported by energy that was mainly derived from oxidative phosphorylation (Gaitanos *et al.* 1993).

More recently, Parolin *et al.* (1999) examined the regulation of glycogen phosphorylase and PDH during three 30-second bouts of maximal sprint exercise. During the first bout of exercise, glycogen phosphorylase was maximally activated at 15 seconds resulting in high rates of glycogenolysis (Figure 1.2), however, at 30 seconds of contraction glycogen phosphorylase reverted back to resting values and remained unchanged with each successive

bout. In contrast, PDH was fully activated at 15 seconds of exercise in bout 1, and was more rapidly activated with each successive sprint, resulting in greater ATP turnover rates from oxidative phosphorylation (Figure 1.2). The metabolic signals responsible for the reductions in glycogenolysis and shift towards greater reliance on oxidative phosphorylation during repeated sprinting are complex and modulated by many factors (Parolin *et al.* 1999). Several reports have suggested that an accumulation of muscle H^+ during intense exercise may inhibit the conformational change of glycogen phosphorylase to its active form, while simultaneously promoting activation of PDH (Parolin *et al.* 1999; Spriet *et al.* 1989).

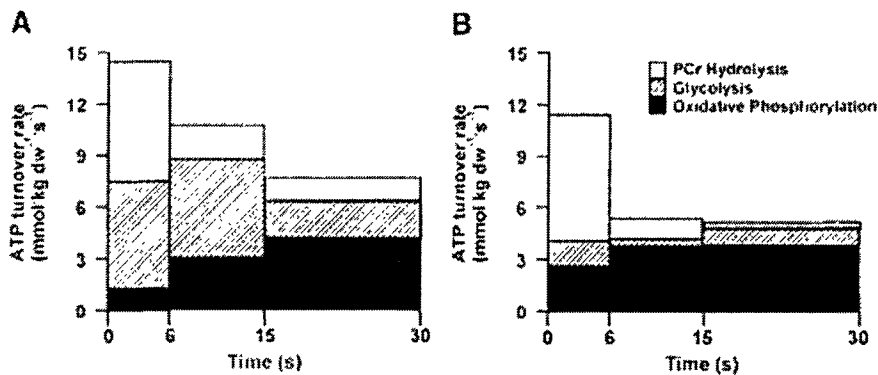


Figure 1.2 ATP turnover rates from phosphocreatine (PCr) hydrolysis, glycolysis, and oxidative phosphorylation in the first (A) and third (B) bouts of maximal isokinetic cycling (taken from Parolin *et al.* 1999).

1.2.4 Other physiological changes during acute high-intensity exercise

In addition to changes in skeletal muscle metabolism, sprint exercise places a major stress on the respiratory and cardiovascular systems. During the first few seconds of intense contraction, pulmonary ventilation rises more than ten-fold which allows for rapid increases in aerobic metabolism while removing CO₂ from the muscle, thereby maintaining acid-base balance (McKenna *et al.* 1997). In addition to increases in ventilation, marked elevations in heart rate occur at the onset of high-intensity exercise, and vascular tone is altered to allow for a rapid increase in muscle perfusion to the working limb (Saltin *et al.* 1998). The increase in blood velocity, and thus blood flow at the start of cycle exercise is related to power output such that blood flow to the femoral artery increases linearly as a function of workload (Saltin *et al.* 1998). For example, during dynamic knee extensor exercise, muscle perfusion increases from ~0.3 liters per minute at rest to ~10 liters per minute at peak exercise (Saltin *et al.* 1998).

Hussain *et al.* (1996) demonstrated that during a 30-second Wingate Test mean femoral artery blood flow and heart rate increased seven- and two-fold, respectively, and remained elevated above baseline values at 60 minutes post-exercise. In addition to changes in blood flow, marked increases in cardiac output have been observed in response to high-intensity exercise. Studies that have examined changes in ventricular function have demonstrated a progressive increase in left ventricular (LV) ejection fraction during incremental exercise. Foster and colleagues (1999) reported significant increases in LV ejection

fraction during 60 seconds of intense cycling, and suggested that enhanced LV emptying was one of the mechanisms for increasing cardiac output during high-intensity exercise. The precise mechanisms that regulate the cardiovascular system during intense exercise are multi-factorial. Although a wealth of information that describes changes in cardiovascular regulation during steady-state exercise exists, additional research is warranted to clarify the precise nature of the cardiovascular response to maximal intermittent exercise.

1.3 ADAPTATIONS TO HIGH-INTENSITY SPRINT INTERVAL TRAINING

1.3.1 Performance adaptations

Regular ET performed over several weeks elicits marked improvements in endurance capacity and aerobic exercise performance. For example, repeated sessions of high-volume endurance exercise result in an increased ability to sustain a given submaximal workload for a longer period of time, or to achieve a higher power output over a fixed distance (Hawley 2002). In contrast to traditional ET, high-intensity interval training is often employed to improve sprint ability and anaerobic performance. Several studies have demonstrated increases in peak and mean power output during single or repeated 30-second Wingate Test(s), and improvements in supramaximal tests to exhaustion after six to eight weeks of SIT (Barnett *et al.* 2004; Harmer *et al.* 2000; MacDougall *et al.* 1998; Parra *et al.* 2000; Stathis *et al.* 1994). In addition to improvements in anaerobic performance, increases in maximal oxygen uptake have been reported after two to eight weeks

of intense interval training (Barnett *et al.* 2004; Dawson *et al.* 1998; Gorostiaga *et al.* 1991; MacDougall *et al.* 1998; Rodas *et al.* 2000; Talanian *et al.* 2007). Aside from changes in VO_{2peak} , no studies have examined sprint training-induced changes in the ability to perform exercise that is primarily “aerobic” in nature. Additional studies that incorporate tests of volitional exercise capacity such as an endurance test to fatigue or a time-trial performed at a fixed submaximal workload are warranted to determine the effect of SIT on aerobic exercise capacity.

Recent work has emphasized the importance of recovery in facilitating performance adaptations. Rodas and colleagues (2000) examined skeletal muscle metabolic and performance adaptations to two distinct sprint training protocols in which the training volume was the same (14 sessions), however, the distribution of recovery was varied. Significant improvements in peak and mean power output elicited during a Wingate Test were reported after six weeks of SIT that permitted 1-2 days of rest between training sessions, whereas power output was unchanged after 14 consecutive days of sprint exercise (Parra *et al.* 2000). These findings highlight the importance of adequate rest periods between training sessions in order to facilitate performance adaptations and avoid muscle fatigue.

1.3.2 Skeletal muscle metabolic adaptations

Adaptations in skeletal muscle metabolism following repeated sessions of sprint exercise appear to be dependent on many factors. Training-induced

alterations in skeletal muscle vary depending on the training status of the individual, the experimental protocol employed (e.g., mode, intensity, frequency) (Ross & Leveritt 2001), and the distribution of recovery periods during consecutive exercise bouts (Parra *et al.* 2000). Changes in enzyme activity and substrate utilization represent a major adaptation to training and result in improvements in exercise capacity. For example, an upregulation of key regulatory enzymes and an increase in muscle substrate storage improve the *rate* of ATP resynthesis during exercise and increase *total* energy production in skeletal muscle, respectively (Ross & Leveritt 2001).

Given the large energy production from non-oxidative sources during high-intensity exercise, increases in glycolytic enzyme activity following repeated sessions of sprint exercise represent an important adaptation to training (Kubukeli *et al.* 2002). Higher rates of glycolysis are expected to provide a corresponding improvement in anaerobic performance by enhancing peak rates of energy production from non-oxidative sources. Several studies have reported marked increases in glycolytic enzyme activity, including hexokinase (MacDougall *et al.* 1998), phosphofructokinase (Linossier *et al.* 1993; MacDougall *et al.* 1998), glycogen phosphorylase (Dawson *et al.* 1998; Linossier *et al.* 1997) and lactate dehydrogenase (Linossier *et al.* 1993; Linossier *et al.* 1997) after a prolonged period of SIT (i.e. \geq six weeks).

In contrast to glycolytic adaptations, it has been suggested that high-intensity interval training has less of an effect on skeletal muscle oxidative

capacity (Gleeson 2000; Kubukeli *et al.* 2002), however, studies that have examined changes in mitochondrial enzymes after SIT are equivocal. For example, increases in the maximal activity of citrate synthase have been reported after six to eight weeks of interval training that consisted of repeated sprint bouts lasting 15-30 seconds (Henriksson & Reitman 1976; Jacobs *et al.* 1987; MacDougall *et al.* 1998). In contrast, studies that have failed to observe an increase in citrate synthase incorporated short sprints lasting less than 10 seconds (Dawson *et al.* 1998; Linossier *et al.* 1993), or sprints that were not performed at maximal effort (Gorostiaga *et al.* 1991). Interestingly, recent work has demonstrated marked increases in citrate synthase maximal activity and peak oxygen uptake after only two weeks of daily interval training (Parra *et al.* 2000; Rodas *et al.* 2000), suggesting that these adaptations occur quite quickly.

An increase in the amount of skeletal muscle metabolic substrate may improve the ability to maintain high rates of energy production during intense exercise (Ross & Leveritt 2001). Several researchers have investigated the effect of SIT on resting levels of ATP, PCr, and muscle glycogen, however the results are equivocal. For example, long-term (i.e., > six weeks) sprint training protocols have been reported to cause no change (Barnett *et al.* 2004; Dawson *et al.* 1998; Nevill *et al.* 1989), or a decrease in the resting concentration of ATP and PCr (Stathis *et al.* 1994), whereas short-term training (i.e. two weeks) has been shown to increase PCr content (Parra *et al.* 2000; Rodas *et al.* 2000). Thus, it has been suggested that changes in ATP and PCr levels may be affected by the

frequency and volume of training (Ross & Leveritt 2001). Consistent with changes in muscle phosphagen stores, the effect of sprint training on resting muscle glycogen content is equivocal with some studies demonstrating an increase in glycogen (Barnett *et al.* 2004; Parra *et al.* 2000; Rodas *et al.* 2000), whereas others have not (Dawson *et al.* 1998; Linossier *et al.* 1997; Nevill *et al.* 1989; Stathis *et al.* 1994; Talanian *et al.* 2007). One reason for the lack of congruity among studies may be the absence of strict dietary controls prior to the skeletal muscle biopsy procedure. In order to clearly establish the effect of sprint training on skeletal muscle metabolite concentrations, careful control of nutritional intake must be considered.

In contrast to data regarding adaptations in resting muscle, much less is known about the effect of SIT on skeletal muscle metabolism during an acute bout of exercise. The few investigations that have examined metabolic adaptations during exercise have suggested that sprint training either increases or does not change glycogenolytic rate during brief “all-out” challenges to exhaustion (e.g., Barnett *et al.* 2004; Linossier *et al.* 1993; Nevill *et al.* 1989; Stathis *et al.* 1994). A limitation of these studies is that power output differs between the pre- and post-training trials and thus training-induced changes in skeletal muscle metabolism are difficult to establish. More recently, Harmer and colleagues (2000) demonstrated that seven weeks of SIT reduced glycogen utilization and lactate accumulation during a 30-second matched-worked exercise challenge. These results contrasted from other studies that used non

matched-work trials and implied that the contribution from aerobic metabolism was enhanced during exercise after sprint training. Although Harmer *et al.* (2000) did not specifically examine markers of oxidative metabolism, the authors hypothesized that sprint training might induce changes in the activation state of PDH. Other investigators have speculated on the potential importance of PDH in the muscle adaptive response to sprint training (MacDougall *et al.* 1998; Parra *et al.* 2000), noting that transient, repeated increases in PDH activity could serve as a stimulus for increasing mitochondrial capacity after SIT. Despite such speculations, the effect of sprint training on carbohydrate metabolism during an acute bout of submaximal exercise, in which the majority of energy is derived from oxidative metabolism, has not been determined. Thus, future studies are required to examine changes in the activity of key rate-determining enzymes (e.g., PDH) during matched-work exercise in order to determine the effect of sprint training on skeletal muscle metabolic control.

1.3.3 Other skeletal muscle adaptations

In addition to changes in substrate concentration and enzyme activity, high-intensity training has been shown to alter the protein content of metabolite transporters associated with lactate and H⁺ exchange (Bickham *et al.* 2006; Juel *et al.* 2004; Pilegaard *et al.* 1999). For example, two recent studies demonstrated significant increases in the membrane contents of the monocarboxylate transporters (MCT) MCT1 and MCT4 in human skeletal muscle after seven to eight weeks of intense one-legged knee-extensor exercise (Juel *et al.* 2004;

Pilegaard *et al.* 1999). More recently, Yoshida and colleagues (2005) demonstrated that three weeks of daily sprint-run training in rats increased the muscle content of both MCT1 and MCT4. Given the large accumulation of lactate and associated increase in H⁺ concentration during maximal-effort exercise, training-induced adaptations in skeletal muscle lactate/H⁺ transport systems are not surprising.

High-intensity interval training may induce changes in other metabolite transport proteins such as those associated with glucose and fatty acid metabolism; however, this has not been examined in humans. In a recent study, Terada and colleagues (2001) demonstrated that GLUT4 protein content in rat epitrocleolaris muscle was increased after a total of only 5 minutes of intense intermittent swim training over eight days. Thus it appears that high-intensity training elicits rapid increases in transport proteins of glucose metabolism in rat skeletal muscle, however, whether the response is similar in human skeletal muscle is unknown. Similarly, changes in fatty acid transport proteins in human skeletal muscle have not been described, although several weeks of SIT has been shown to increase the activity of enzymes related to lipid oxidation (e.g., 3-hydroxyacyl CoA dehydrogenase; HAD). Thus, SIT may increase the capacity for fat oxidation through changes in the skeletal muscle content of fatty acid transport proteins and/or the activity of key rate-determining enzymes of lipid oxidation (e.g., carnitine palmitoyl transferase). Future studies are required to examine changes in metabolite transport proteins of glucose and fatty acid

metabolism in human skeletal muscle in response to SIT, and the time-course for such adaptations.

1.3.4 Other physiological adaptations

The mechanisms that regulate endurance performance are multi-factorial and data from recent studies suggest that SIT may stimulate physiological adaptations that facilitate performance improvements aside from changes in mitochondrial potential. Recent work by Krstrup and colleagues (2004) examined the effect of seven weeks of intense one-legged knee-extensor training on the cardiovascular response to submaximal exercise of varying exercise intensities. The authors demonstrated that intense SIT enhanced thigh oxygen uptake at the onset of exercise due to both an increase in blood flow and oxygen extraction in the initial phase of exercise (Krstrup *et al.* 2004). More recently, Warburton *et al.* (2004) compared the effects of twelve weeks of continuous and interval training on cardiorespiratory function in sedentary men. Similar exercise-induced increases in cardiac output, stroke volume and end-diastolic volume were observed after six and twelve weeks of continuous and interval training. Preliminary work by our group has also demonstrated similar improvements in endothelial function following six weeks of high-volume ET or six weeks of low-volume SIT (Rakobowchuk *et al.* unpublished findings). Together, these results highlight the importance of cardiovascular adaptations in the facilitation of sprint-training induced changes in exercise capacity, and suggest that cardiovascular changes play a pivotal role in the adaptation to training.

1.4 COMPARISON OF PHYSIOLOGICAL ADAPTATIONS INDUCED BY HIGH-INTENSITY INTERVAL TRAINING VERSUS TRADITIONAL ENDURANCE TRAINING

Continuous low to moderate intensity exercise performed over several weeks elicits many physiological adaptations that contribute to improvements in exercise capacity. For example, changes in cardiovascular regulation, skeletal muscle composition and the metabolic response to exercise contribute to the training-induced improvement in aerobic capacity (Kubukeli *et al.* 2002). Increases in skeletal muscle oxidative potential, as reflected by changes in the maximal activities of mitochondrial enzymes, represent a classic adaptation to ET (Gollnick *et al.* 1973). Interestingly, similar increases in mitochondrial capacity have been reported after several weeks of SIT, suggesting that brief bouts of intense exercise may be just as effective as continuous exercise for increasing muscle oxidative potential (Henriksson & Reitman 1976; Saltin *et al.* 1976). Given the implications of these findings, it is surprising that very few studies have directly compared changes in mitochondrial enzymes following these two forms of training. For example, only a few human studies have compared SIT versus ET, and the results are equivocal with two studies reporting similar increases in the maximal activities of mitochondrial enzymes after interval and continuous training (Henriksson & Reitman 1976; Saltin *et al.* 1976), while two others reported increases after continuous training only (Fournier *et al.* 1982; Gorostiaga *et al.* 1991).

In terms of performance, only one previous study has examined changes in volitional exercise performance after high-intensity interval training and traditional ET. Eddy *et al.* (1977) employed matched-work training protocols in which subjects performed seven weeks of either moderate-intensity (70% of VO_{2peak}) or high-intensity exercise (repeated 1-minute bouts at 100% of VO_{2peak}). Following training, similar improvements in volitional performance were observed such that the time to exhaustion during a cycling challenge performed at 90% of VO_{2peak} increased to a similar extent in both the continuous and interval training groups (Eddy *et al.* 1977). In all of these human studies the total volume of work performed was similar between groups and thus the potential for low-volume SIT to elicit increases in oxidative capacity and aerobic performance that are comparable to high-volume ET is currently unknown.

Data from animal investigations has illustrated the potential for repeated bouts of intense exercise to induce changes in muscle oxidative capacity that are similar to prolonged bouts of ET (Dudley *et al.* 1982; Terada *et al.* 2001). For example, Dudley *et al.* (1982) reported similar increases in the maximal activity of cytochrome oxidase in rat skeletal muscle after eight weeks of training with either short bouts of running at high work intensities, or prolonged periods of continuous running at low work intensities. More recently, Terada and colleagues (2001) demonstrated that eight days of high-intensity swim training elicited similar increases in the maximal activity of citrate synthase as compared to eight days of high-volume ET in rat skeletal muscle. These results suggest that brief

bouts of sprint exercise are just as effective as prolonged bouts of ET for increasing muscle oxidative capacity in rat skeletal muscle; however, these diverse training approaches have not been studied in humans. Furthermore, previous studies have compared adaptations in resting skeletal muscle (Dudley *et al.* 1982; Eddy *et al.* 1977; Edge *et al.* 2006; Fournier *et al.* 1982; Gorostiaga *et al.* 1991; Henriksson & Reitman 1976; Saltin *et al.* 1976; Terada *et al.* 2001), and thus the potential for changes in substrate metabolism during exercise after both forms of training is unknown. Future work that directly compares interval versus continuous training using a research design that matches groups with respect to exercise mode (cycling) and training duration, but differs in terms of total training volume, is required to confirm speculations that SIT is indeed a time-efficient training strategy.

1.5 CONCLUSIONS

The general purpose of this thesis was to examine changes in exercise performance and skeletal muscle metabolism after short-term SIT, and to directly compare the adaptations induced by SIT versus traditional ET. The purpose of Study 1 was to examine changes in performance and resting skeletal muscle metabolism after two weeks of SIT that permitted 1-2 days of rest between training sessions to promote recovery and facilitate performance improvements. As previously discussed, repeated sessions of intense exercise elicit rapid improvements in energy metabolism. For example, two recent studies

demonstrated large increases in glycolytic and oxidative enzyme activity after only two weeks of daily sprint training (Parra *et al.* 2000; Rodas *et al.* 2000). However, despite marked improvements in energy metabolism, peak and mean power output elicited during a 30-second Wingate Test were unchanged, possibly due to chronic fatigue associated with daily sprint training (Parra *et al.* 2000; Rodas *et al.* 2000). Aside from changes in VO_{2peak} , no data suggests that sprint training leads to an increased ability to perform exercise that is primarily “aerobic” in nature; therefore we examined changes in time to exhaustion during an endurance test to fatigue at a fixed submaximal workload. We hypothesized that six sessions of SIT performed over fourteen days would increase muscle oxidative potential and endurance time to fatigue during cycling at ~80% of pre-training VO_{2peak} , despite a total exercise time of only 15 minutes.

In contrast to changes in resting skeletal muscle metabolism, much less is known about the effect of SIT on metabolic control during an acute bout of exercise. Furthermore, the few studies that have examined sprint training-induced changes in muscle metabolism have incorporated brief “all-out” exercise challenges to exhaustion (Linossier *et al.* 1993; Nevill *et al.* 1989; Stathis *et al.* 1994), and thus the interpretation of training on metabolic control is hampered by the fact that power output differs between the pre- and post-training tests. Therefore, the purpose of Study 2 was to examine the effect of two weeks of SIT on skeletal muscle metabolism during submaximal, matched-work exercise. In addition, we examined changes in 250 kJ cycling time-trial performance to

simulate a task in which athletes typically compete. The training protocol was identical to Study 1 and consisted of SIT performed over fourteen days with 1–2 days of recovery between training sessions. We hypothesized that two weeks of SIT would decrease muscle glycogenolysis and lactate accumulation during exercise and increase the capacity for carbohydrate oxidation through pyruvate dehydrogenase. We also hypothesized that short SIT would improve 250 kJ cycling time-trial performance, whereas exercise performance in a control group who would complete the time-trial two weeks apart with no training intervention would be unchanged.

Given the high rate of non-oxidative energy production during sprinting, the adaptive response to SIT may include changes in transport systems that regulate sarcolemmal lactate flux in skeletal muscle. For example, repeated sessions of brief, intense exercise have been shown to induce changes in MCT proteins that regulate lactate and H⁺ exchange in human skeletal muscle (Bickham *et al.* 2006; Juel *et al.* 2004; Pilegaard *et al.* 1999). Although the time-course for these changes are unknown, rapid increases in MCT proteins could contribute to reduced rates of muscle lactate accumulation during exercise, as demonstrated in Study 2. Similarly, SIT may induce changes in other metabolite transport proteins such as those associated with glucose and fatty acid metabolism, however, this has not been studied in humans. The purpose of Study 3 was to examine the time-course for changes in metabolite transport proteins in human skeletal muscle in response to six weeks of SIT. In addition,

changes in transport protein content were also examined after one and six weeks of de-training to examine changes following cessation of the SIT stimulus. We hypothesized that proteins associated with glucose and lactate transport would adapt more quickly than proteins associated with fatty acid transport. Specifically, we hypothesized that one week (3 sessions) of SIT would increase the muscle content of glucose transporter 4 (GLUT4), MCT1 and MCT4, as well as cytochrome c oxidase subunit 4 (COX4), which was used as a marker of oxidative capacity. In contrast, we hypothesized that changes in fatty acid translocase (FAT/CD36) and plasma membrane associated fatty acid binding protein (FABPpm) would occur more slowly and would not be evident until after six weeks of training.

Numerous studies have shown that high-intensity interval training elicits changes in skeletal muscle energy metabolism that resemble traditional ET (Henriksson & Reitman 1976; Saltin *et al.* 1976). However, the relatively few studies that have directly compared skeletal muscle metabolic adaptations to interval and continuous training have yielded equivocal results, and in all cases the total volume of work performed was similar between groups (Eddy *et al.* 1977; Edge *et al.* 2006; Fournier *et al.* 1982; Gorostiaga *et al.* 1991; Henriksson & Reitman 1976; Saltin *et al.* 1976). Moreover, these studies examined changes in resting biopsy samples, and thus provided limited insight regarding the potential for changes in substrate metabolism during exercise. The purpose of Study 4 was therefore to directly compare low-volume SIT versus high-volume

ET in a standardized manner with respect to changes in skeletal muscle metabolic control during exercise. By design, training volume and time commitment in the SIT group was ~90% lower compared to ET. We hypothesized that six weeks of SIT and ET would induce similar adaptations in muscle oxidative capacity and selected measures of whole-body and skeletal muscle substrate metabolism during exercise despite large differences in total training time commitment and exercise volume.

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CHAPTER 2

SIX SESSIONS OF SPRINT INTERVAL TRAINING INCREASES MUSCLE OXIDATIVE POTENTIAL AND CYCLE ENDURANCE CAPACITY IN HUMANS.

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2.1 INTRODUCTION

Performing repeated bouts of high-intensity “sprint”-type exercise over several weeks or months induces profound changes in skeletal muscle. A wide range of muscle metabolic and morphological adaptations have been described (MacDougall *et al.* 1998; Ross & Leveritt 2001), however, the magnitude and direction of change in many variables depends on the nature of the training protocol, i.e. the frequency, intensity and duration of sprint efforts as well as the recovery between bouts. Given the significant contribution from aerobic energy metabolism during repeated sprinting (Bogdanis *et al.* 1996; McKenna *et al.* 1997; Parolin *et al.* 1999; Trump *et al.* 1996), it is not surprising that an increase in muscle oxidative potential - as indicated by changes in the maximal activities of “marker” enzymes such as citrate synthase - has been reported after six to eight weeks of sprint training (Jacobs *et al.* 1987; MacDougall *et al.* 1998). Recently, Rodas and colleagues (2000) reported large increases in citrate synthase maximal activity, as well as peak oxygen uptake (VO_{2peak}), after only two weeks of daily sprint training. These data suggest that improvements in

aerobic energy metabolism can be rapidly stimulated by brief bouts of very intense exercise, however the effect of fewer sprint training sessions is not known. In addition, aside from changes in VO_{2peak} , we are aware of no data that suggests sprint training leads to an increased ability to perform exercise that is primarily “aerobic” in nature, e.g., an endurance test to fatigue at a fixed submaximal workload.

The primary purpose of the present study, therefore, was to examine the effect of six sessions of sprint interval training (SIT) on muscle oxidative potential, VO_{2peak} and endurance time to fatigue during cycling at an intensity equivalent to ~80% of pre-training VO_{2peak} . Based on pilot work in our laboratory that showed modest performance improvements after six consecutive days of SIT, we decided to employ a two-week training intervention, such that 1-2 days of rest was permitted between training sessions, in an effort to promote recovery and facilitate performance adaptations. The importance of rest days between sprint training sessions was emphasized in a recent study (Parra *et al.* 2000) that showed peak and mean power elicited during a Wingate Test were unchanged after 14 consecutive days of sprint training; however, when subjects performed the same number of training sessions over six weeks (i.e., with 1-2 days of rest between training sessions), power output improved significantly. While numerous mechanisms could potentially be involved, the importance of rest days between training sessions may be related in part to the fact that strenuous exercise leads to inactivation of muscle cation pumps (Leppik *et al.* 2004; Sandiford *et al.* 2004)

and it has been speculated that up to several days may be required for normalization of sarcoplasmic reticulum calcium pump function (Tupling 2004).

Thus, the mode and intensity of sprint efforts in the present study was similar to two recent studies that incorporated two-week training interventions (Parra *et al.* 2000; Rodas *et al.* 2000), however the overall volume was reduced by ~ two thirds and in total amounted to only ~15 minutes of exercise over two weeks. We hypothesized that our short sprint training protocol would increase muscle oxidative potential and cycle endurance capacity. We also measured resting muscle glycogen concentration since only a few sprint training studies have done so and these have yielded conflicting results (Harmer *et al.* 2000; Nevill *et al.* 1989; Ortenblad *et al.* 2000; Parra *et al.* 2000; Rodas *et al.* 2000). Our experimental design included a control group who completed the exercise performance tests two weeks apart with no training intervention, and all subjects performed extensive familiarization trials prior to baseline testing.

2.2 METHODS

2.2.1 Subjects

Sixteen healthy individuals volunteered to take part in the experiment (Table 2.1). Eight subjects (2 women) were assigned to a training group and performed exercise tests before and after a two-week sprint training intervention. Eight other men served as a control group and performed the exercise performance tests two weeks apart with no training intervention. We also

obtained needle biopsy samples from the training group in order to examine potential training-induced adaptations in resting skeletal muscle. We did not obtain biopsies from the control group for ethical reasons, since other studies have shown no change in resting muscle metabolite concentrations or the maximal activities of mitochondrial enzymes when control subjects are tested several weeks apart with no sprint training intervention (Barnett *et al.* 2004; Ortenblad *et al.* 2000). All subjects were recreationally active individuals from the McMaster University student population who participated in some form of exercise two to three times per week (e.g., jogging, cycling, aerobics), but none were engaged in any sort of structured training program. Following routine medical screening, the subjects were informed of the procedures to be employed in the study and associated risks, and all provided written informed consent. The experimental protocol was approved by the McMaster University and Hamilton Health Sciences Research Ethics Board.

2.2.2 Preexperimental procedures

Prior to baseline measurements, all subjects performed familiarization trials in order to become oriented with all testing procedures and training devices. Specifically, all subjects performed (i) a peak oxygen uptake test (VO_{2peak} test); (ii) a “practice ride” in order to establish a workload that elicited ~80% of pre-training VO_{2peak} ; and (iii) an endurance capacity test that consisted of cycling to volitional fatigue at ~80% of pre-training VO_{2peak} on two separate occasions.

Table 2.1 Subject characteristics.

	Training Group	Control Group
Age (yr)	22±1	25±2
Weight (kg)	83±5	79±2
Height (cm)	180±4	180±2
VO _{2peak} (ml.kg ⁻¹ .min ⁻¹)	44.6±3.2	46.4±1.4

All values are means±SE, n=8 per group.

2.2.3 Details of exercise performance tests

2.2.3.1 *VO_{2peak} test*

Subjects performed an incremental test to exhaustion on an electronically braked cycle ergometer (Lode BV, Excalibur Sport V2.0, The Netherlands) in order to determine VO_{2peak} using an online gas collection system (Moxus modular oxygen uptake system, AEI technologies, Pittsburg, PA). The initial three stages of the test consisted of 2-minute intervals at 50, 100, and 150 W, respectively, and the workload was then increased by 25 W every minute until volitional exhaustion. The value used for VO_{2peak} corresponded to the highest value achieved over a 30-second collection period.

2.2.3.2 *Cycle endurance capacity test*

Subjects cycled to volitional exhaustion on an electronically braked cycle ergometer (Lode BV) at a workload designed to elicit ~80% of pre-training VO_{2peak} . All performance trials were conducted in the absence of temporal, verbal or physiological feedback. The test was terminated when pedal cadence fell below 40 revolutions per minute (according to the manufacturer's specifications, the power output displayed may not have been valid below this cadence) and exercise duration was recorded. Expired breath samples for the determination of ventilation rate, VO_2 , VCO_2 and RER were collected and averaged over the 6-10 minute period of exercise.

2.2.3.3 *Reproducibility of exercise performance tests*

Eight individuals, who were not subjects in the present study, performed a VO_{2peak} test and cycle endurance capacity test on separate days at least one week apart, and method error reproducibility was calculated as described by Sale (1991). The coefficient of variation for the VO_{2peak} test and cycle endurance capacity test was 3.7% and 12.0%, respectively.

2.2.4 Experimental protocol

The experimental protocol consisted of (i) baseline testing (i.e., following familiarization procedures described above); (ii) a two-week sprint training intervention or similar period without sprint training (control group); and (iii) post-testing, as described further below.

2.2.4.1 *Baseline testing*

Baseline measurements for all subjects consisted of a VO_{2peak} test and a cycle endurance capacity test. Each baseline test was conducted on a separate day with 24 hours between tests. Subjects in the training group also underwent a muscle biopsy procedure three days following the baseline cycle endurance capacity test and several days prior to the start of the training intervention. For the biopsy procedure, the area over the lateral portion of one thigh was anesthetized (2% lidocaine; AstraZeneca Canada Inc., Ontario, Canada) and a small incision was made through the skin and underlying fascia in order to permit a tissue sample (50-100 mg) to be obtained from the vastus lateralis muscle (Bergström 1975). Details regarding the experimental protocol are summarized in

Figure 2.1.

2.2.4.2 *Training*

Training was initiated 3-5 days following the baseline muscle biopsy procedure and consisted of six sessions of sprint interval training spread over fourteen days. Each training session consisted of repeated 30-second “all out” efforts on an electronically braked cycle ergometer (Lode BV) against a resistance equivalent to $0.075 \text{ kg}\cdot\text{kg}^{-1}$ body mass (i.e., a Wingate Test). Subjects were instructed to begin pedaling as fast as possible as the appropriate load was applied by a computer interfaced with the ergometer and loaded with appropriate software (Wingate Software Version 1.11, Lode BV). Subjects were verbally encouraged to continue pedaling as fast as possible throughout the 30-second test. Peak power, mean power and fatigue index were subsequently determined using an on-line data acquisition system. During the 4-minute recovery period between tests, subjects remained on the bike and either rested or were permitted to cycle at a low cadence ($< 50 \text{ rpm}$) against a light resistance ($< 30 \text{ W}$) in order to reduce venous pooling in the lower extremities and minimize feelings of light-headedness or nausea. The training protocol consisted of exercise performed three times per week on alternate days (i.e., Monday, Wednesday, Friday) for two weeks. The number of Wingate Tests performed each day during training increased from four to seven over the first 5 training sessions, and on the final session subjects completed four intervals, as summarized in Figure 2.1.

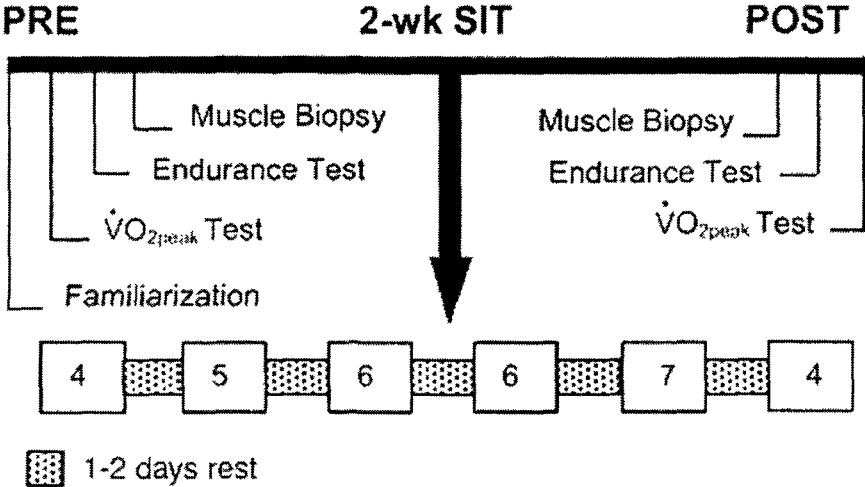
2.2.4.3 *Post-testing*

A second muscle biopsy sample was obtained three days following the final training session in order to examine training-induced changes in resting muscle, and a second battery of performance tests was initiated two days following the biopsy procedure (Figure 2.1). The control group performed a second set of tests two weeks following the baseline tests. The nature of the post-testing exercise performance measurements was identical in all respects to the baseline tests.

2.2.5 Dietary controls

In an attempt to minimize any potential diet-induced variability in exercise metabolism and the resting metabolic profile of skeletal muscle, subjects were instructed to consume the same types and quantities of food during the baseline and post-testing phases. The subjects in the training group were particularly encouraged to keep their diet as similar as possible during the 24 hours before the pre- and post-training biopsy procedures. Subjects were asked to record all food intake during these periods, and compliance was assessed by performing dietary analyses on the individual food records maintained by the subjects. Pre- and post-training food diaries were analyzed for total energy intake and the proportion of energy derived from carbohydrates, fats and protein (Nutritionist Five, First Data Bank Inc., San Bruno, California). These analyses confirmed that there was no difference between trials in the total amount of energy consumed or macronutrient proportions.

Figure 2.1 Overview of experimental protocol.



$\dot{V}O_{2peak}$, peak oxygen uptake; PRE, pre-training; POST, post-training; SIT, sprint interval training. Numbers in boxes denote number of Wingate Tests completed during each of six training sessions over a two-week period.

2.2.6 Muscle analyses

Upon removal from the leg, each muscle biopsy sample was immediately frozen by plunging the biopsy needle into liquid nitrogen. The samples were subsequently divided into two pieces while still frozen, and one piece was kept in liquid nitrogen for the determination of muscle enzyme activities. The remainder of each sample was freeze-dried, powdered and dissected free of blood and connective tissue, and stored at -86 °C prior to metabolite analyses.

2.2.6.1 *Citrate synthase*

Frozen wet muscle samples were initially homogenized using methods described by Henriksson and colleagues (1986) to a 50 times dilution. The maximal activity of citrate synthase was determined on a spectrophotometer (Ultrospec 3000 pro UV/Vis) using a method described by Carter *et al.* (2001). The intra-assay coefficient of variation for the citrate synthase assay, based on 10 repeats of the same sample, was 4.9%. Protein content of the homogenate was determined by the method of Bradford (1976) using a commercial assay kit (Quick Start, Bio-Rad Laboratories, Hercules, CA) and enzyme data are expressed as moles per kilogram of protein per hour.

2.2.6.2 *Metabolites*

An aliquot of freeze-dried muscle was extracted on ice using 0.5 M perchloric acid (containing 1 mM EDTA), neutralized with 2.2 M KHCO₃, and the resulting supernatant was used for the determination of all metabolites except

glycogen. ATP, phosphocreatine and creatine were measured using enzymatic assays adapted for fluorometry (Hitachi F-2500, Hitachi Instruments, Japan) (Harris *et al.* 1974; Passoneau & Lowry 1993). For glycogen analysis, a ~2 mg aliquot of freeze-dried muscle was incubated in 2.0 N HCl and heated for 2 hours at 100 °C to hydrolyse the glycogen to glucosyl units. The solution was subsequently neutralized with an equal volume of 2.0 N NaOH and analyzed for glucose using an enzymatic assay adapted for fluorometry (Passoneau & Lowry 1993). The intra-assay coefficient of variation for all muscle metabolite assays, based on 10 repeats of the same sample, ranged from 2-3%. All muscle metabolite measurements were corrected to the peak total creatine concentration for a given subject.

2.2.7 Statistical analyses

All exercise performance data were analyzed using a 2-factor repeated measures analysis of variance (ANOVA). For the single Wingate Test, endurance capacity test and VO_{2peak} , the factors were trial (pre, post) and condition (training, control). For the comparison of power output during the first versus last sprint training session (training group only), the factors were trial (pre, post) and sprint bout (1-4). All muscle data were analyzed using paired (2-tailed) t-tests. The level of significance for analyses was set at $P < 0.05$, and significant interactions and main effects were subsequently analyzed using Tukey's honestly significant difference post hoc test. All data are presented as means \pm SE.

2.3 RESULTS

2.3.1 Cycle endurance capacity

After training, the individual improvements in cycle endurance capacity ranged from 81-169% compared to baseline, with the exception of one subject (16% decrease) who upon completion of the study disclosed that he had sustained a minor ankle injury (unrelated to the experiment) on the day prior to his post-training ride. Even with the inclusion of this subject's data (Figure 2.2), the mean increase in cycle endurance time to fatigue for the training group (n=8) was 100% compared to baseline (51 ± 11 versus 26 ± 5 min, $P < 0.05$) and this was higher ($P < 0.05$) compared to the control group, who showed no change in performance (Figure 2.2). VO_2 during exercise was not different between the first and second rides in either group, however, expired ventilation (Post: 91 ± 7 versus Pre: 104 ± 9 L.min⁻¹) and RER (Post: 1.18 versus Pre: 1.24) were lower ($P < 0.05$) after training in the sprint-training group ($P < 0.05$). $\text{VO}_{2\text{peak}}$ did not change in either group over the course of the study.

2.3.2 Anaerobic work capacity

Peak power output during each of the 4 consecutive Wingate Tests performed during the last (sixth) training session was higher ($P < 0.05$) as compared to the first training session (Figure 2.3). However, fatigue index was also higher ($P < 0.05$) post-training, and thus there were no differences in mean power output for each of the 4 Wingate Tests during the first as compared to the last training session.

Figure 2.2 Cycle endurance time to fatigue before and after a two-week sprint training protocol (Training Group; SIT) or equivalent period without training (Control; CON). Values are means±SE (n=8 per group). Individual data are also plotted for all subjects in each group. * P<0.05.

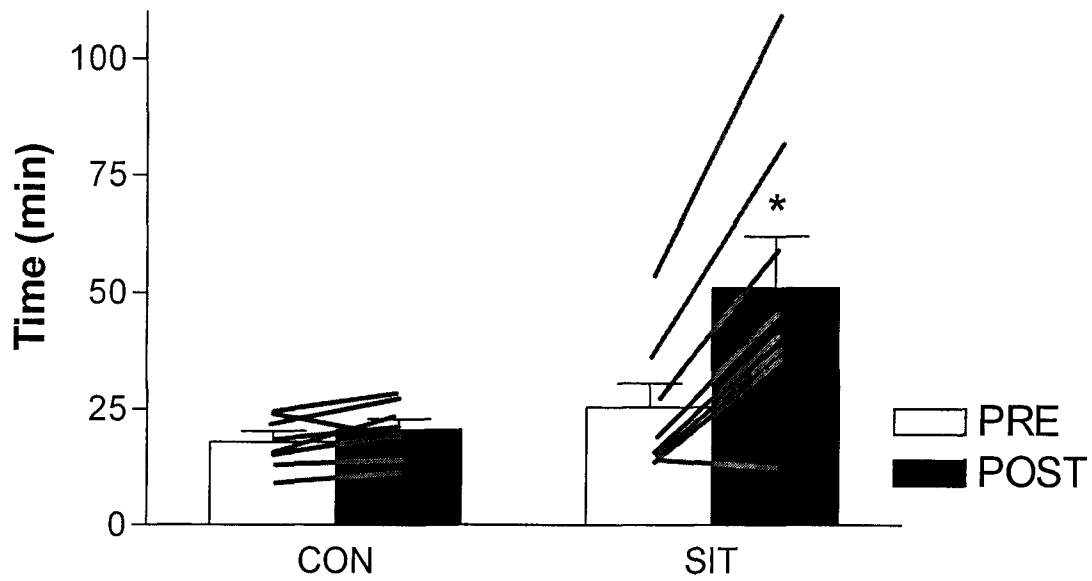
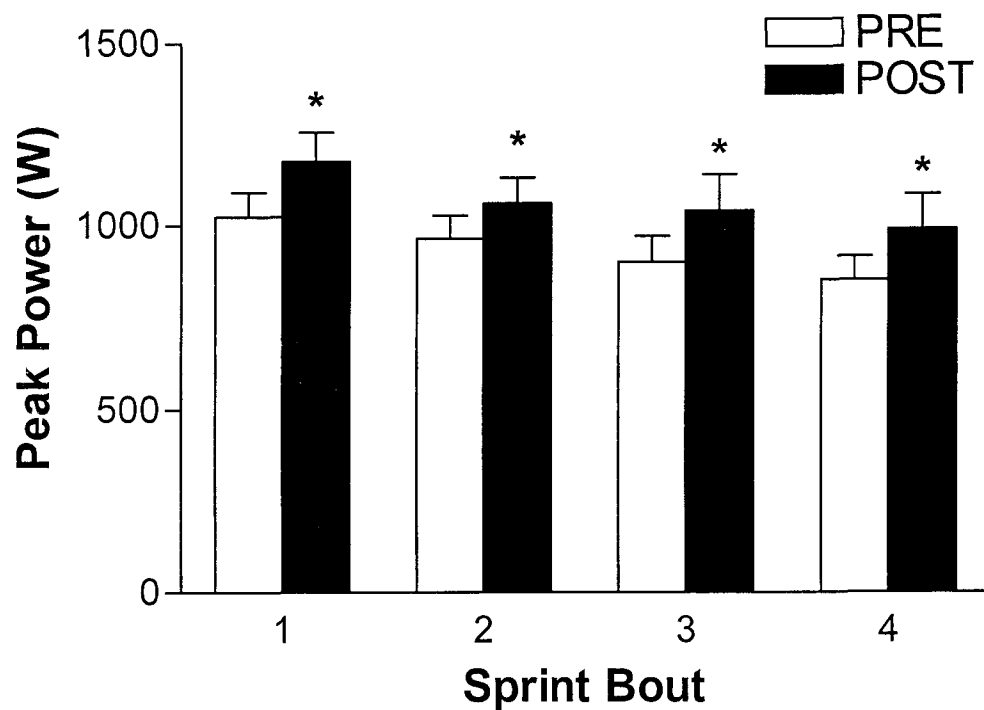


Figure 2.3 Peak anaerobic power elicited during four consecutive Wingate Tests performed during the first and last sprint training session. Values are means \pm SE (n=8). * P<0.05.



2.3.3 Citrate synthase activity and muscle metabolite concentrations

The maximal activity of citrate synthase increased ($P < 0.05$) by 38% after training (Figure 2.4). Resting muscle glycogen concentration increased ($P < 0.05$) by 26% after training (Figure 2.5), however there were no training-induced changes in the resting muscle concentrations of ATP, phosphocreatine or creatine (Table 2.2).

Figure 2.4 Maximal activity of citrate synthase (CS) measured in resting muscle biopsy samples obtained before and after a two-week sprint training protocol.

Values are means \pm SE (n=8) in mol.kg protein⁻¹.h⁻¹. * P<0.05.

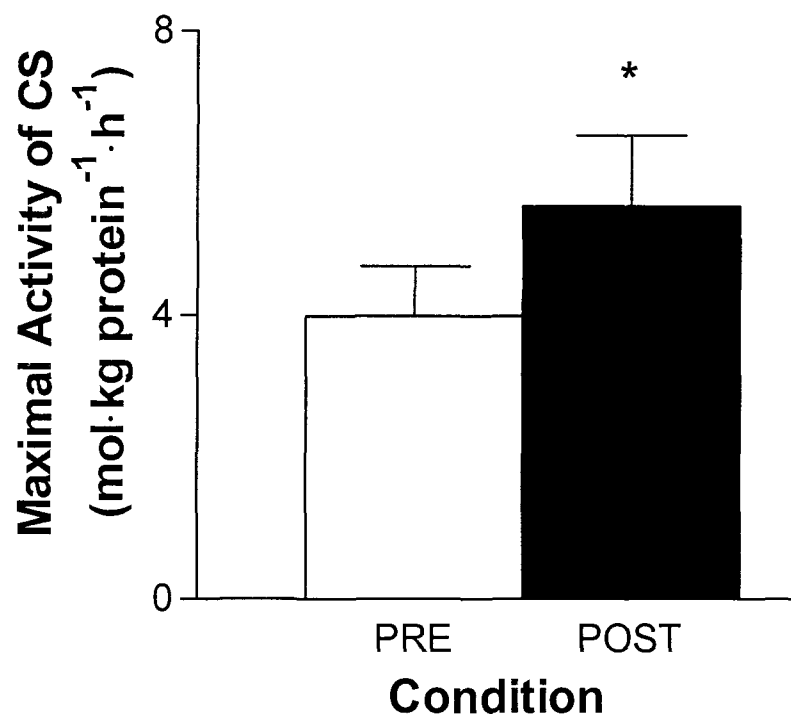


Figure 2.5 Muscle glycogen content measured in resting biopsy samples obtained before and after a two-week sprint training protocol. Values are means \pm SE (n=8) in mmol.kg⁻¹ dry wt. * P<0.05.

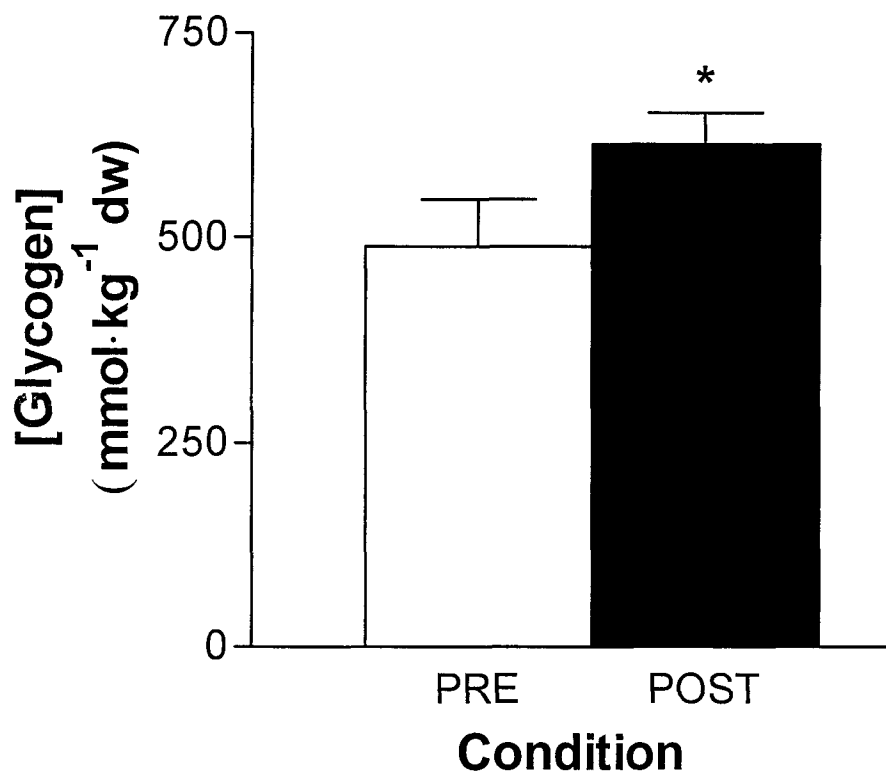


Table 2.2 Muscle metabolites before and after training.

	Pre-training	Post-training
ATP	24±1	24±2
Phosphocreatine	81±3	84±3
Creatine	30±2	27±3

All values are means±SE (n=8) in mmol.kg⁻¹ dry wt.

2.4 DISCUSSION

The primary novel finding from the present study was that six bouts of sprint interval training performed over fourteen days increased muscle oxidative potential and doubled endurance time to fatigue during cycling at ~80% of pre-training $\text{VO}_{2\text{peak}}$ in recreationally-active subjects. The validity of this latter observation is bolstered by the fact that all subjects performed extensive familiarization trials prior to testing and a control group showed no change in endurance performance when tested two weeks apart with no sprint training intervention. We also detected increases in resting muscle glycogen content after sprint training. The present data therefore demonstrate that short, repeated bouts of 30-second “all out” cycling efforts - amounting to ~15 minutes of total exercise over two weeks - dramatically increased cycle endurance capacity and favourably altered the resting metabolic profile of human skeletal muscle. While increases in citrate synthase activity and glycogen content have been previously reported after several weeks of sprint interval training; the data are equivocal and we show here that the total training volume necessary to stimulate these metabolic adaptations is substantially lower than previously suggested.

2.4.1 Muscle oxidative potential and glycogen content after short sprint interval training

We measured the maximal activity of citrate synthase in resting muscle biopsies before and after training, since this is arguably the most commonly used “marker” of muscle oxidative potential and other investigators have justified their

selection of this enzyme since it exists in constant proportion with other mitochondrial enzymes (e.g., Green *et al.* 1999). There are equivocal data regarding the effect of sprint training on the maximal activity of this enzyme, however studies that have failed to observe an increase in citrate synthase generally used very short sprints lasting less than 10 seconds (Dawson *et al.* 1998; Linossier *et al.* 1993) or sprints that were not “all out” maximal efforts (Gorostiaga *et al.* 1991). In contrast, all studies that have reported increases in citrate synthase activity incorporated maximal effort sprint bouts that lasted 15-30 seconds (Jacobs *et al.* 1987; MacDougall *et al.* 1998; Parra *et al.* 2000; Rodas *et al.* 2000). Another relevant consideration is the fact that acute exercise *per se* may elevate citrate synthase activity, potentially confounding the interpretation of training-induced effects, and thus the timing of muscle sampling relative to the last exercise session is critical when measuring the activity of this enzyme (Leek *et al.* 2001; Tonkonogi *et al.* 1997). In the present study, we allowed 72 hours of recovery prior to any biopsy sampling procedure (i.e., after baseline testing and following the final training session) in order to minimize the potential confounding effects of acute prior exercise on citrate synthase activity (Leek *et al.* 2001). Our data clearly show that the maximal activity of citrate synthase was increased after only six sessions of sprint interval training. Notably, the magnitude of the increase was similar to that reported in other studies that incorporated a substantially greater number of sprint training bouts (Jacobs *et al.* 1987; MacDougall *et al.* 1998; Parra *et al.* 2000; Rodas *et al.* 2000). Moreover, the

increase in citrate synthase activity in the present study is comparable to that reported by some authors after six to seven days of traditional endurance exercise training (i.e., 2 hours per day at ~65% VO_{2peak}) (Chesley *et al.* 1996; Spina *et al.* 1996), whereas others have reported no change in muscle oxidative potential after short endurance training (e.g., Green *et al.* 1995). The present data do not explain the mechanism for the upregulation of citrate synthase activity and additional work is warranted in this regard. Finally, while there are limited and equivocal data regarding the effect of sprint interval training on resting muscle glycogen stores (Ross & Leveritt 2001), our results are consistent with two recent studies that reported increased muscle glycogen content after fourteen sessions of SIT (Parra *et al.* 2000; Rodas *et al.* 2000). One particularly novel aspect of our data is that the magnitude of the increase in muscle glycogen was comparable to what has been reported after five to seven sessions of traditional endurance exercise training (average increase: ~20% range: 13-35%; e.g., Chesley *et al.* 1996; Green *et al.* 1992; Putman *et al.* 1998).

2.4.2 The effect of sprint interval training on endurance performance

Several studies have reported increases in VO_{2peak} after 14-24 SIT sessions performed over two to eight weeks (Dawson *et al.* 1998; Gorostiaga *et al.* 1991; MacDougall *et al.* 1998; McKenna *et al.* 1997). Aside from these observations, however, we are aware of no data that suggests sprint training leads to an increased capacity to perform exercise that is primarily aerobic in nature. Thus, in the present study we decided to employ an endurance capacity

test in the form of cycling at ~80% of pre-training $\text{VO}_{2\text{peak}}$, a task in which the vast majority of energy is supplied from oxidative metabolism. Our data show that aerobic endurance capacity was dramatically improved after only six sessions of SIT, despite the fact that $\text{VO}_{2\text{peak}}$ remained unchanged. Indeed, exercise time to exhaustion more than doubled in 6 of 8 subjects who performed the training intervention (see individual data in Figure 2.2) and the mean performance improvement was 100%. It seems unlikely that this finding is a spurious result, given that the mean improvement was substantially higher than the day-to-day variability for this test in our laboratory (coefficient of variation = 12%) and a control group showed no change in cycle endurance capacity when tested two weeks apart with no sprint training intervention. To our knowledge this is the first study to show that short SIT dramatically improves endurance capacity during a fixed workload test in which the majority of cellular energy is derived from aerobic metabolism.

We can only speculate on potential mechanisms responsible for the dramatic improvement in cycle endurance capacity, but it is plausible that a training-induced increase in mitochondrial potential - as measured by citrate synthase maximal activity - improved respiratory control sensitivity during exercise as classically proposed (Holloszy & Coyle 1984). However, the precise mechanisms that regulate endurance performance are multi-factorial and extremely complicated (Fluck & Hoppeler 2003) and data from other studies suggest that sprint training can stimulate a range of adaptations that might

facilitate performance aside from changes in mitochondrial potential. For example, recent investigations have shown that five to eight weeks of SIT increases skeletal muscle blood flow and vascular conductance (Krustrup *et al.* 2004), lactate transport capacity and H⁺ release from active muscle (Juel *et al.* 2004), ionic regulation (Harmer *et al.* 2000) and sarcoplasmic reticulum function (Ortenblad *et al.* 2000). While the time-course for these adaptations is unknown, other studies have reported similar adaptations after only five to seven days of aerobic-based training, including changes in blood flow kinetics (Shoemaker *et al.* 1996), lactate extrusion from exercising muscle (Bonen 1998) and cation pump activity (Green *et al.* 2004). We hope that the present observations will stimulate additional research in order to clarify the precise nature, time-course and significance of the physiological adaptations induced by short SIT.

In summary, the results from the present study demonstrate that 6 bouts of sprint interval training performed over two weeks (a total of ~15 minutes of very intense exercise) increased citrate synthase maximal activity and doubled endurance capacity during cycling exercise at ~80% of pre-training VO_{2peak} in recreationally active subjects. The validity of this latter observation is bolstered by the fact that all subjects performed extensive familiarization trials prior to testing and a control group showed no change in cycle endurance capacity when tested two weeks apart without any sprint training intervention. To our knowledge this is the first study to show that sprint training dramatically improves endurance capacity during a fixed workload test in which the majority of cellular energy is

derived from aerobic metabolism. These data demonstrate that brief repeated bouts of very intense exercise can rapidly stimulate improvements in muscle oxidative potential that are comparable to or higher than previously reported aerobic-based training studies of similar duration.

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CHAPTER 3

EFFECT OF SHORT-TERM SPRINT INTERVAL TRAINING ON HUMAN SKELETAL MUSCLE CARBOHYDRATE METABOLISM DURING EXERCISE AND TIME-TRIAL PERFORMANCE.

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3.1 INTRODUCTION

Sprint interval training (SIT), which is characterized by recurring sessions of brief, repeated bouts of very intense exercise, is a potent stimulus for inducing metabolic adaptations in human skeletal muscle (Kubukeli *et al.* 2002; Ross & Leveritt 2001). With respect to carbohydrate (CHO) metabolism, a wide range of adaptations have been described, including an increase in resting glycogen content (Harmer *et al.* 2000; Parra *et al.* 2000), increases in the maximal activities of various enzymes involved in glycolytic (Jacobs *et al.* 1987; MacDougall *et al.* 1998) and oxidative energy provision (Jacobs *et al.* 1987; MacDougall *et al.* 1998), and increases in lactate transport capacity (Juel *et al.* 2004; Pilegaard *et al.* 1999). Many of these adaptations occur very quickly after a surprisingly small volume of intense exercise training (Burgomaster *et al.* 2005; Parra *et al.* 2000; Rodas *et al.* 2000); for example, we recently reported increases in resting glycogen content and citrate synthase maximal activity after only six sessions of SIT performed over two weeks (Burgomaster *et al.* 2005).

In contrast to the wealth of data regarding adaptations in resting muscle,

much less is known about the effect of sprint training on CHO metabolism during an acute bout of exercise. Several investigators have suggested that SIT either increases or does not change muscle glycogenolytic rate and non-oxidative ATP provision during exercise, based on research designs that incorporated brief “all out” exercise challenges to exhaustion (e.g., Linossier *et al.* 1993; Nevill *et al.* 1989; Stathis *et al.* 1994). While this type of approach is certainly valid, the interpretation of training per se on metabolic control is hampered by the fact that power output differs between the pre and post-training tests. Recently, a unique study by Harmer *et al.* (2000) employed a matched-work exercise comparison to investigate the effect of sprint training on metabolic perturbations in human muscle. The authors demonstrated that seven weeks of SIT reduced non-oxidative ATP generation during intense exercise, as evidenced by lower muscle glycogen degradation and lactate accumulation after 30 seconds of cycling at 130% of pre-training peak oxygen uptake (VO_{2peak}). These findings thus contrasted sharply from other studies that used non matched-work exercise challenges (Linossier *et al.* 1993; Nevill *et al.* 1989; Stathis *et al.* 1994) and implied that the contribution from aerobic metabolism was enhanced during intense exercise after sprint training, as previously suggested by others (McKenna *et al.* 1997b; McKenna *et al.* 1997a). Harmer *et al.* (2000) did not specifically examine markers of muscle oxidative metabolism, but they hypothesized that sprint training might increase the activity of pyruvate dehydrogenase (PDH) and thus the capacity for mitochondrial pyruvate

oxidation. Other investigators have also speculated on the potential importance of PDH in the muscle adaptive response to sprint training (MacDougall *et al.* 1998; Parra *et al.* 2000) but to date no study has directly examined whether sprint training alters PDH activity during exercise.

The primary purpose of the present study was to examine the effect of two weeks of SIT on skeletal muscle CHO metabolism during submaximal, matched-work exercise. The training protocol was identical to that described in our previous study (Burgomaster *et al.* 2005) and consisted of six sessions of brief, repeated maximal cycling efforts, performed over fourteen days with 1-2 days of recovery between training sessions. We hypothesized that short-term SIT would decrease muscle glycogenolysis and lactate accumulation during exercise and increase the capacity for CHO oxidation through PDH. In addition to the matched-work exercise test that was used to assess muscle metabolic adaptations, our design included a separate test of volitional exercise performance. Given that many events require athletes to complete a fixed amount of work in as short a time as possible (i.e., a race), we tested the hypothesis that short SIT would improve 250 kJ cycling time-trial performance. As in our previous study (Burgomaster *et al.* 2005), we included a control group who completed the exercise performance test two weeks apart with no training intervention, and all subjects were thoroughly familiarized with all experimental procedures prior to baseline testing.

3.2 METHODS

3.2.1 Subjects

Sixteen young healthy men volunteered to participate in the experiment (Table 3.1). All subjects were drawn from the same subject population, namely young active students at McMaster University who took part in some form of recreational exercise two to three times per week (e.g., jogging, cycling, etc). None of the subjects were specifically engaged in training for a particular sporting event, although one was a varsity runner who was out of season at the time of the experiment. Eight of the subjects served as a training group that performed exercise performance tests before and after a two-week sprint training intervention. The other eight subjects served as a control group that completed exercise performance tests two weeks apart with no training intervention. The control group was older ($P=0.03$) however there were no differences between groups in peak oxygen uptake or any other descriptive characteristic (Table 3.1). In addition to the exercise performance tests, the training group also performed a separate matched-work exercise test before and after training, and we obtained muscle biopsy samples at rest and during exercise in order to examine potential adaptations in metabolic regulation. We did not obtain biopsies from the control group for ethical reasons, because other studies have shown no change in resting muscle metabolites or the maximal activities of various enzymes when control subjects are tested several weeks apart with no sprint training intervention (Barnett *et al.* 2004; Ortenblad *et al.* 2000). Following routine

medical screening, subjects were advised of the purpose of the study and associated risks, and all provided written informed consent. The experimental protocol was approved by the McMaster University and Hamilton Health Sciences Research Ethics Board.

3.2.2 Preexperimental procedures

Prior to baseline measurements, subjects made several familiarization visits to the laboratory in order to become oriented with the testing procedures and training devices. During one of these visits, subjects performed an incremental test to exhaustion on an electronically braked cycle ergometer (Lode Excalibur Sport V2.0, Groningen, The Netherlands) to determine VO_{2peak} using an online gas collection system (Moxus modular oxygen uptake system, AEI technologies, Pittsburgh, PA). The initial three stages of the test consisted of 2-minute intervals at 50, 100, and 150 W, respectively, and the workload was increased by 25 W every minute until volitional exhaustion. The value used for VO_{2peak} corresponded to the highest value achieved over a 30-second collection period. All subjects also performed a familiarization time-trial. Subjects in the training group only also performed a familiarization Wingate Test and an incremental exercise test to establish workloads that were used during the main experimental trials. Details regarding all experimental exercise tests are described below.

Table 3.1 Subject characteristics.

Variable	Training group	Control group
Age (yr)	21±1	25±1*
Height (cm)	181±2	180±2
Weight (kg)	78±5	76±3
VO _{2peak} (L.min ⁻¹)	3.8±0.2	3.9±0.2

Values are means±SE, n=8 per group. VO_{2peak} = peak oxygen uptake. * P<0.05 versus Training group.

3.2.3 Details of experimental tests

3.2.3.1 *Time-trial*

Subjects were instructed to complete a 250 kJ self-paced laboratory time-trial on an electronically braked cycle ergometer (Lode) as quickly as possible with no temporal, verbal, and physiological feedback. The only feedback that subjects received during the test was work done, which was presented as “distance covered” on a computer monitor (i.e., 250 kJ was equated to 10 km, such that visual feedback at any point during the ride was presented in units of distance rather than work done). Time required to complete the test and average power output were recorded upon completion of each test. Method error reproducibility for the time-trial (coefficient of variation, determined using the method described by Sale 1991) was 2.6% when eight individuals were tested one week apart with no sprint training intervention (Day 1: 17.1 ± 1.0 versus Day 2: 17.2 ± 0.8 min).

3.2.3.2 *Wingate Test*

Subjects performed a 30-second “all-out” effort on a mechanically-braked cycle ergometer (Monark Bicycle Ergometer; Model 814E, Sweden) against a resistance equivalent to $0.075 \text{ kg} \cdot \text{kg}^{-1}$ body mass. Subjects were instructed to begin pedaling as fast as possible against the ergometer’s inertial resistance and then the appropriate load was manually applied. Subjects were verbally encouraged to continue pedaling as fast as possible throughout the 30-second

test. Peak power, mean power and fatigue index were subsequently determined using an online data acquisition system.

3.2.3.3 *Exercise metabolism test*

Subjects reported to the laboratory and rested in the supine position while the area over one thigh was anesthetized (1% w/v lidocaine hydrochloride, AstraZeneca Canada Inc, Mississauga, ON) and prepared for the extraction of needle biopsy samples from the vastus lateralis muscle (Bergström 1975). Three separate incisions were made through the skin and underlying fascia, approximately 2 cm apart, and a needle biopsy sample was obtained through one of the incision sites. The three sites were covered with sterile gauze and surgical tape, and the subject climbed onto an electronically braked cycle ergometer (Lode) and commenced cycling at a work intensity that elicited ~60% $\text{VO}_{2\text{peak}}$. After 10 minutes of exercise, the subject stopped cycling and a second biopsy sample was obtained as quickly as possible through the second incision site while the subject remained seated on the ergometer. The time delay between cessation of exercise and muscle excision was 10 to 20 seconds. After the muscle sample was removed from the leg, the area over the biopsy site was recovered with sterile gauze and surgical tape. Sixty seconds after the cessation of exercise at the first workload, subjects resumed cycling at a higher work intensity that elicited ~90% $\text{VO}_{2\text{peak}}$. The time delay for the biopsy procedure between workloads was standardized in an effort to reduce potential variability that may have been induced by differing amounts of recovery. After 10 minutes

of exercise at the second workload, the subject stopped cycling and a third and final biopsy was obtained from the third incision site while the subject remained seated on the ergometer. All biopsy samples were immediately frozen in liquid nitrogen after the needle was removed from the leg. Cardiorespiratory data were collected and averaged over the 6-9 minute period of exercise at each workload.

3.2.4 Experimental protocol

After the familiarization procedures, the experimental protocol consisted of 1) baseline testing; 2) a two-week sprint training intervention or similar period without sprint training (control group); and 3) post-testing, as described in detail below.

3.2.4.1 *Baseline testing*

Baseline measurements for all subjects consisted of a 250 kJ laboratory time-trial. Subjects in the training group also performed a Wingate Test and an invasive exercise metabolism test (see above), with at least 2 days of recovery between tests.

3.2.4.2 *Training*

The sprint training protocol was identical to that described in our previous study (Burgomaster *et al.* 2005). Training was initiated three days after the exercise metabolism test and consisted of six sessions of sprint interval exercise spread over fourteen days. Each training session consisted of repeated 30-second "all out" cycling efforts (Wingate Tests) with 4 minutes of recovery

between tests. During the 4-minute recovery interval, subjects remained on the bike and either rested or cycled at a low cadence (< 50 rpm) against a light resistance (< 30 W) to reduce venous pooling in the lower extremities and minimize feelings of light-headedness or nausea. The training protocol consisted of exercise performed three times per week on alternate days (i.e., Monday, Wednesday, Friday) for two weeks. The number of Wingate Tests performed on each training day increased from four to seven over the first five training sessions, and on the final session subjects completed four intervals.

3.2.4.3 *Post-testing*

All subjects performed a second series of experimental tests that were identical in all respects to the baseline tests. The training group performed the exercise metabolism test (using the same absolute workloads as during the baseline test) 3 days after the final sprint training session, followed 2 days later by a time-trial, 1 day later by a VO_{2peak} test and 1 day later by a Wingate Test. Subjects in the control group performed a second time-trial two weeks after the baseline test with no sprint training intervention.

3.2.5 Physical activity and nutritional controls

All subjects were instructed to continue their normal dietary and physical activity practices throughout the experimental period. Subjects were also specifically instructed to refrain from any exercise aside from activities of daily living for 2 days prior to all pre- and post-training exercise tests. In order to minimize diet-induced variability in muscle metabolism, subjects were instructed

to consume the same types and quantities of food for 2 days prior to the time-trial and exercise metabolism test. Subjects were also required to maintain food diaries prior to the baseline exercise metabolism test, which were then collected, photocopied and returned to the subjects before the post-training test. Subjects were asked to replicate their individual pattern of food intake and to highlight any deviations in the types or amounts of food consumed. Subsequent dietary analyses (Nutritionist Five, First Data Bank Inc., San Bruno, CA) revealed no difference ($P=0.2$) in total daily energy intake or the relative macronutrient consumption prior to the experimental trials before (9.36 ± 0.71 MJ; $57\pm 3\%$ CHO, $30\pm 3\%$ fat, $13\pm 1\%$ protein) or after training (8.44 ± 0.70 MJ, $57\pm 3\%$ CHO, $29\pm 3\%$ fat, $14\pm 1\%$ protein).

3.2.6 Muscle analyses

One piece of frozen wet muscle (~ 10 - 15 mg) from all samples was chipped under liquid nitrogen and used for the determination of the active fraction of PDH (PDHa) using the method described by Constantin-Teodosiu *et al.* (1991) as modified by Putman *et al.* (1993). PDHa values were adjusted to the highest total creatine content for a given subject in order to account for differences in blood or connective tissue between samples. A second piece of frozen wet muscle (~ 10 - 15 mg) from the resting samples only was chipped under liquid nitrogen and homogenized using the method described by Henriksson and colleagues (1986) to a 50 times dilution. The homogenate was subsequently analyzed to determine the maximal activity of citrate synthase (CS) on a

spectrophotometer (Ultrospec 3000 pro UV/Vis) using a method described by Carter et al. (2001), and the maximal activity of 3-hydroxyacyl CoA dehydrogenase (HAD) on a fluorometer (Hitachi F-2500, Hitachi Instruments, Japan) using a method described by Chi and colleagues (1983). Protein content of the muscle homogenate was determined by the method of Bradford (1976) using a commercial assay kit (Quick Start, Bio-Rad Laboratories, Hercules, CA), and enzyme data are expressed as moles per kilogram of protein per hour.

The remainder of each muscle sample was freeze-dried, powdered, and dissected free of all non-muscle elements and stored at -80 °C. Aliquots of freeze-dried muscle were extracted with 0.5 M perchloric acid, neutralized with 2.2 M KHCO₃ and assayed for lactate, ATP, PCr and creatine using standard enzymatic methods (Harris *et al.* 1974; Passoneau & Lowry 1993) adapted for fluorometry (Hitachi F-2500, Hitachi Instruments, Japan). For glycogen analysis, a ~2 mg aliquot of freeze-dried muscle was incubated in 2.0 N HCl and heated for 2 hours at 100 °C to hydrolyse the glycogen to glucosyl units. The solution was subsequently neutralized with an equal volume of 2.0 N NaOH and analyzed for glucose using an enzymatic assay adapted for fluorometry (Passoneau & Lowry 1993).

3.2.7 Statistical analyses

Time-trial performance data were analyzed using a 2-factor mixed analysis of variance (ANOVA), with the between factor “group” (training, control) and repeated factor “trial” (pre, post). Muscle metabolite and PDHa data were

analyzed using a 2-factor repeated measures ANOVA with the factors “trial” (pre, post) and “time” (0, 10, and 20 minutes). Data from the VO_{2peak} and Wingate Tests, the maximal activities of citrate synthase and HAD and net changes in muscle glycogen and lactate during exercise were analyzed using paired t-tests (pre- versus post-training). Significant interactions or main effects were subsequently analyzed using a Tukey’s honestly significant difference post hoc test, and the level of significance for all analyses was set at $P < 0.05$. All data are presented as means \pm SE based on $n=8$ per group, except for the cardiorespiratory data, which are based on a mean of $n=6$ due to technical problems during some of the post-training exercise metabolism tests.

3.3 RESULTS

3.3.1 Time-trial performance

Time required to complete the 250 kJ time-trial decreased ($P=0.004$) by 9.6% after training (Figure 3.1) and this was reflected by an increase in average power output from 247 ± 37 to 272 ± 24 W ($P=0.004$). The effect of training was also evidenced by the fact that peak and mean power output elicited during a 30-second Wingate Test increased after training by 5.4% (Post: 1016 ± 97 versus Pre: 964 ± 88 W, $P=0.04$) and 8.7% (Post: 854 ± 86 versus Pre: 786 ± 68 W, $P=0.02$), respectively, and percent fatigue was reduced by 17.9% (Post: 28 ± 2 versus $35 \pm 3\%$, $P=0.002$). The control group showed no change in time-trial performance ($P=0.74$) when tested two weeks apart with no training intervention

(Figure 3.1) and average power output was similarly unchanged (Post: 229 ± 14 versus Pre: 231 ± 15 W, $P=0.37$).

3.3.2 Maximal activities of mitochondrial enzymes

The maximal activity of CS increased ($P=0.04$) by 11% after training (Figure 3.2), but the maximal activity of HAD was unchanged (5.1 ± 0.7 versus Pre: 4.9 ± 0.6 mol.kg protein⁻¹.h⁻¹, $P=0.76$).

3.3.3 Muscle metabolic and cardiorespiratory data during matched-work exercise

PDHa was higher after training (main effect, $P=0.04$) but there was no interaction between trials (Figure 3.3). Muscle glycogen content was higher after training (main effect, $P=0.0001$; Figure 3.4) and while there was no interaction effect ($P=0.06$), net muscle glycogenolysis during exercise was reduced after training (Post: 100 ± 16 versus Pre: 139 ± 11 mmol.kg⁻¹ dry wt, $P=0.03$). Muscle lactate content was lower after training (main effect, $P=0.02$; Figure 3.5) and while there was no interaction effect ($P=0.07$), net lactate accumulation during exercise was reduced after training (Post: 55 ± 2 versus Pre: 63 ± 1 mmol.kg⁻¹ dry wt, $P=0.03$). The muscle contents of creatine and ATP were lower after training (main effects, $P=0.002$ and $P=0.007$, respectively) but phosphocreatine content was not different ($P=0.09$) (Table 3.2). VO_{2peak} was not different after training (Post: 51.6 ± 2.1 versus Pre: 48.9 ± 2.1 ml.kg⁻¹.min⁻¹, $P=0.13$), and there were no training-induced changes in mean exercise VO_2 , RER or heart rate during the exercise metabolism test (Table 3.2).

Figure 3.1 Time required to complete a 250 kJ cycling time-trial before (PRE) and after (POST) two weeks of sprint interval training (SIT) or equivalent period without training (Control; CON). All values are means \pm SE (n=8 per group). * P<0.05 versus PRE. Individual data are plotted for all subjects in each group.

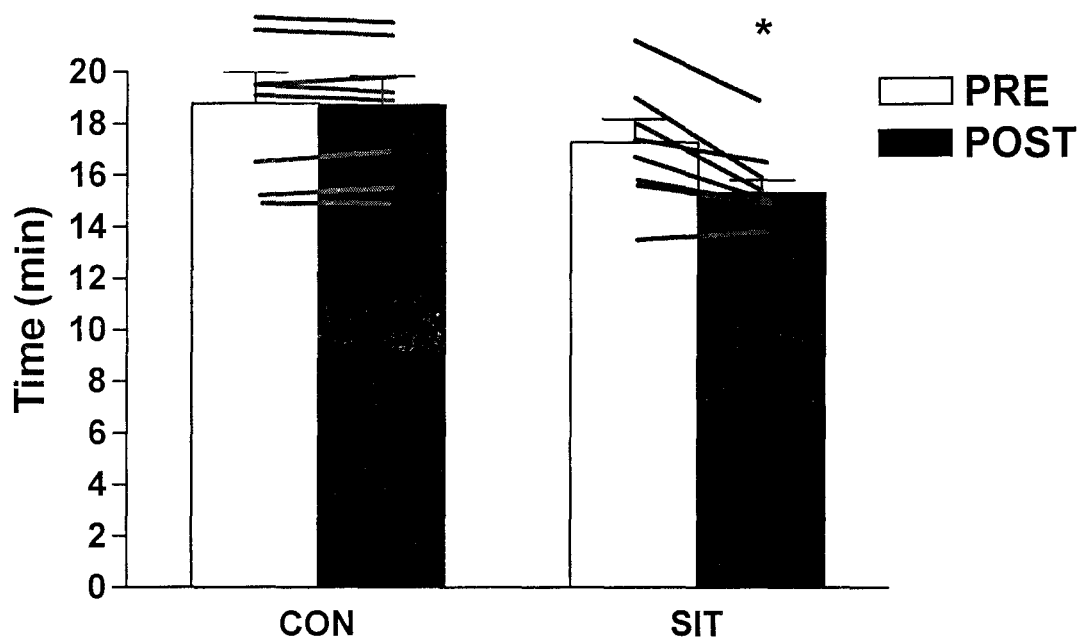


Figure 3.2 Maximal activity of citrate synthase (CS) measured in resting muscle biopsy samples before (PRE) and after (POST) two weeks of sprint interval training. All values are means \pm SE (n=8). * P<0.05 versus PRE.

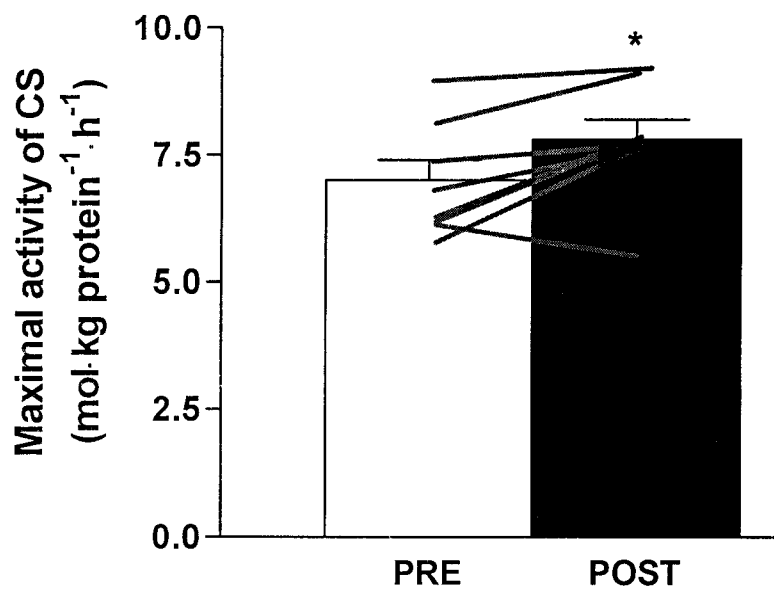


Figure 3.3 Muscle pyruvate dehydrogenase activity (PDHa) measured at rest and after each stage of a matched-work exercise bout that consisted of 10 minutes at $\sim 60\%$ VO_{2peak} followed by 10 minutes at $\sim 90\%$ VO_{2peak} before (PRE) and after (POST) two weeks of sprint interval training. All values are means \pm SE (n=8), WW, wet weight. * Main effect for trial (P=0.04), such that POST > PRE.

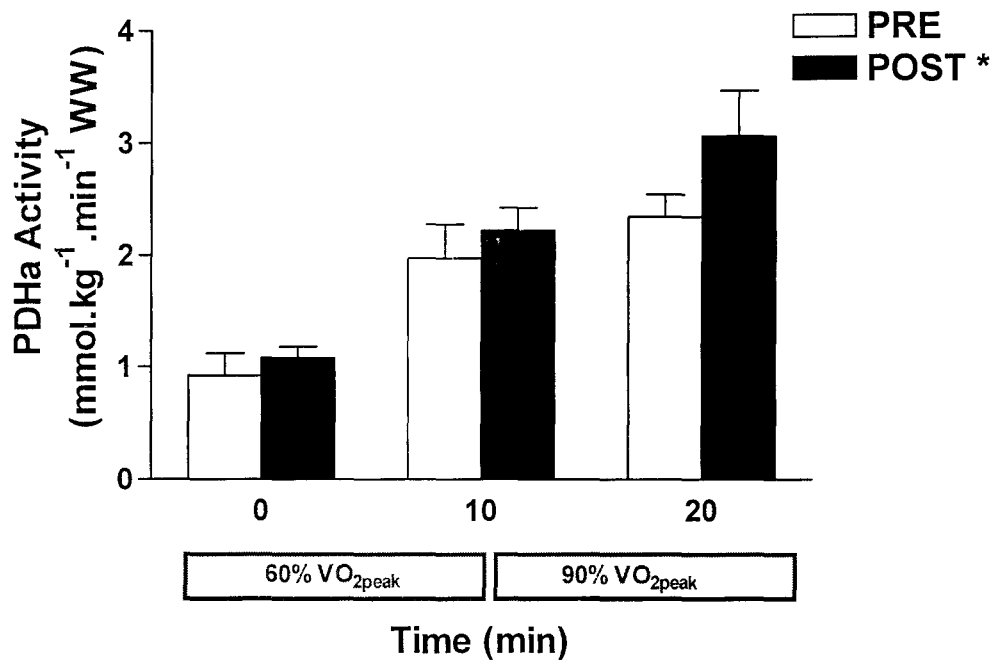


Figure 3.4 Muscle glycogen content measured at rest and after each stage of a matched-work exercise bout that consisted of 10 minutes at $\sim 60\%$ VO_{2peak} followed by 10 minutes at $\sim 90\%$ VO_{2peak} before (PRE) and after (POST) two weeks of sprint interval training. Values are means \pm SE (n=8), DW, dry weight. * Main effect for trial (P=0.03), such that POST>PRE. There was no condition x time interaction (P=0.06), however net muscle glycogenolysis during the exercise bout was lower POST versus PRE (P=0.03).

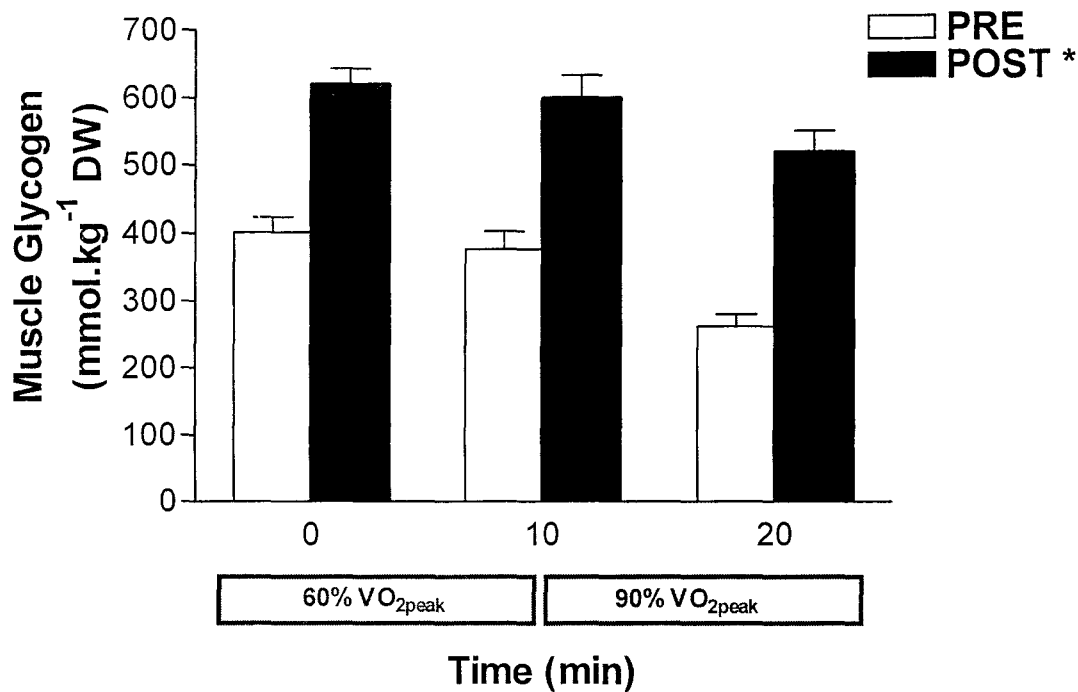


Figure 3.5 Muscle lactate content measured at rest and after each stage of a matched-work exercise bout that consisted of 10 minutes at ~60% VO_{2peak} followed by 10 minutes at ~90% VO_{2peak} before (PRE) and after (POST) two weeks of sprint interval training. Values are means \pm SE (n=8), DW, dry weight. * Main effect for trial ($P<0.05$), such that $POST<PRE$. There was no condition x time interaction ($P=0.07$), however net muscle lactate accumulation during the exercise bout was lower POST versus PRE ($P=0.03$).

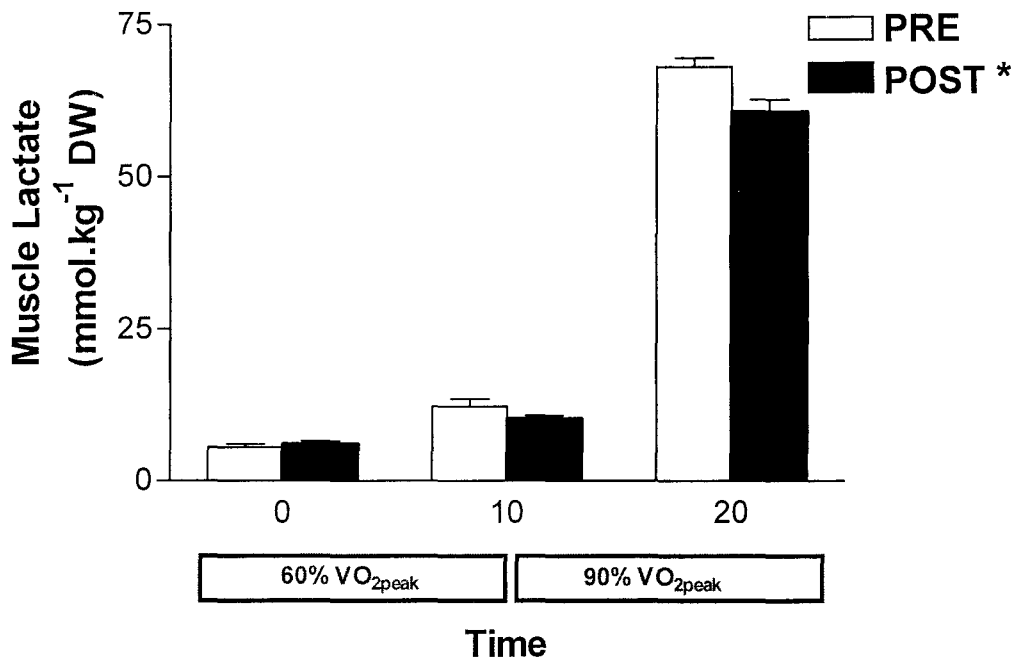


Table 3.2 Cardiorespiratory and muscle metabolite data during a two-stage matched-work exercise test before and after training.

Variable	Condition	Rest	60% VO _{2peak}	90% VO _{2peak}
VO ₂	Pre	ND	2.33±0.09	3.72±0.11
(L.min ⁻¹)	Post	ND	2.32±0.10	3.66±0.16
Heart rate	Pre	ND	132±2	178±2
(beats.min ⁻¹)	Post	ND	132±3	175±2
RER	Pre	ND	0.96±0.03	1.17±0.05
	Post	ND	0.96±0.03	1.15±0.03
Creatine *	Pre	31±2	52±3	112±3
(mmol.kg ⁻¹ dry wt)	Post	24±2	38±2	104±5
Phosphocreatine	Pre	98±4	69±3	15±2
(mmol.kg ⁻¹ dry wt)	Post	99±2	78±4	20±4
ATP *	Pre	24±1	25±1	23±1
(mmol.kg ⁻¹ dry wt)	Post	20±1	22±2	21±1

All values are means±SE, n=8 for metabolite data and n=6 for cardiorespiratory data. ND = Not determined. RER = Respiratory exchange ratio. VO_{2peak} = peak oxygen uptake. * Main effect for trial (P<0.05), post-training versus pre-training.

3.4 DISCUSSION

The main finding from the present study was that short-term SIT decreased net muscle glycogenolysis and lactate accumulation during matched-work exercise and increased the capacity for pyruvate oxidation through PDH. We also showed that the time required to complete a 250 kJ cycling time-trial decreased by 10% and to our knowledge this is the first demonstration that sprint training improves performance during an aerobic-based task that simulates the manner in which athletes typically complete. Finally, the present study confirms our previous finding that muscle oxidative capacity can be enhanced by only a few brief sessions of very intense exercise training in young active individuals (Burgomaster *et al.* 2005).

It is well established that as little as three to ten consecutive days of exercise training for 2 hours per day at 60-70% of VO_{2peak} reduces muscle glycogenolysis and lactate accumulation compared to pre-training at the same absolute workload (Chesley *et al.* 1996; Green *et al.* 1992; Putman *et al.* 1998; Spriet & Heigenhauser 2002). However, it remains controversial whether these adaptations precede (Green *et al.* 1992; Putman *et al.* 1998) or are attributable to an increase in mitochondrial capacity (Chesley *et al.* 1996; Spriet & Heigenhauser 2002), as judged by changes in the maximal activity of citrate synthase. In the present study, we found that six sessions of SIT over two weeks, or a total of only 15 minutes of very intense exercise, decreased net muscle glycogenolysis and lactate accumulation during a matched-work exercise test.

These changes were accompanied by an increase in citrate synthase maximal activity, although the relative increase was smaller than observed in our previous study (Burgomaster *et al.* 2005), despite an identical training stimulus. The difference in response magnitude could be related in part to genetic differences in the adaptive response to training (Timmons *et al.* 2005) or the fact that subjects in the present study were more fit to begin with, as evidenced by a higher mean $\text{VO}_{2\text{peak}}$ and citrate synthase maximal activity. There was no change in HAD in the present study, which suggests that short-term SIT does not stimulate a coordinated increase in all mitochondrial enzymes. This finding is supported by work from MacDougall *et al.* (1998) who reported large increases in the maximal activities of several tricarboxylic acid cycle enzymes, including citrate synthase, after seven weeks of Wingate-based training but no significant change in HAD. Other studies have suggested that sprint training increases HAD (e.g., Parra *et al.* 2000) and given these conflicting data, future studies should directly evaluate whether SIT increases the maximal capacity for lipid oxidation in skeletal muscle, for example, by using stable isotopic tracers or by measuring the maximal activity of the rate-determining enzyme carnitine palmitoyl transferase (Achten & Jeukendrup 2004).

PDHa was higher after training and thus the present data confirm previous speculations by others (Harmer *et al.* 2000; MacDougall *et al.* 1998; Parra *et al.* 2000) that suggested sprint training may increase the maximum capacity to oxidize pyruvate. For example, Harmer *et al.* (2000) proposed that “brief, intense

exercise ... may result in greater PDHa during exercise (and) a slower rate of pyruvate presentation would probably permit a greater proportion to be oxidized, thus constituting a considerable energetic advantage after training." Although the measured active form of PDH has been shown to closely match estimated PDH flux in vivo (Spriet & Heigenhauser 2002), there were no training-induced changes in whole-body gas exchange during exercise in the present study (Table 3.2). Theoretically, an increase in PDH flux should result in an increase in active muscle VO_2 if the pyruvate-derived acetyl units are consumed in the process of oxidative phosphorylation. However, it is unlikely that the small increase in carbohydrate oxidation needed to offset the reduction in non-oxidative ATP provision in the present study could have been detected at the whole-body level.

The reason for the higher PDHa after training is unclear, but given the complex regulation of this multi-enzyme complex, the adaptive response could involve changes in PDH itself or the associated regulatory enzymes PDH phosphatase (PDP) or PDH kinase (PDK), which serve to activate and inhibit the enzyme complex, respectively (Peters 2003). Training could have induced either acute changes in intramitochondrial effectors of PDH (i.e., signals that sense muscle contractile state, cellular energy charge, and substrate/product availability), or stable changes in total protein content or intrinsic activities of PDH, PDP and PDK. With respect to acute regulators, it is traditionally believed that calcium stimulation of PDP is the initial and most powerful signal that activates PDH at the start of exercise (Spriet & Heigenhauser 2002). Ortenblad

et al. (2000) showed that high-intensity intermittent cycle training enhanced peak sarcoplasmic calcium release and thus it is possible that the higher PDHa after training in the present study was due in part to transient alterations in calcium handling that increased PDP. This interpretation is supported by data from Ward and colleagues (1986), who showed that resistance training increased the active form of PDH even though total PDH activity was unchanged. The authors of that study noted that “the type of strength training used in the present study resembles ‘sprint’ training” and attributed the higher PDH activity to training-induced changes in PDP sensitivity to calcium.

The training-induced increase in PDHa in the present study may have also been related to an increase in total PDH activity and/or stable changes in PDP or PDK. Two studies (LeBlanc *et al.* 2004a; Putman *et al.* 1998) have reported no change in total PDH activity after five to seven consecutive days of aerobic-based exercise and concluded that any regulation of PDH with short-term training would be through acute regulators acting on PDP and PDK. However, given that high-intensity exercise results in rapid, maximal conversion of PDH to its active form (Parolin *et al.* 1999; Putman *et al.* 1995), it is possible that the time-course for changes in total PDH after sprint training may differ from traditional endurance training. Clearly, it is possible to increase the maximal activity of some mitochondrial enzymes such as citrate synthase after short-term SIT (present data and Ref. Burgomaster *et al.* 2005), whereas the effect of short-term endurance training on mitochondrial capacity is equivocal (Chesley *et al.* 1996;

Green *et al.* 1992; Putman *et al.* 1998; Spriet & Heigenhauser 2002). Total PDH activity in skeletal muscle is increased after a longer period of endurance training (eight weeks), as recently demonstrated by LeBlanc *et al.* (2004) for the first time in humans (LeBlanc *et al.* 2004b). However, endurance training also increased PDK2 (LeBlanc *et al.* 2004b), which the authors proposed would increase metabolic control sensitivity to pyruvate and reduced PDHa during submaximal exercise, as shown in a separate study (LeBlanc *et al.* 2004a). Additional work is warranted to clarify the specific factors responsible for changes in PDHa after short-term sprint training, and to determine the effect of long-term sprint training on skeletal muscle fuel metabolism.

A final observation with respect to metabolic changes is that we measured a 20% decrease in muscle ATP content after training in the present study, which differs from the results of our previous study (Burgomaster *et al.* 2005), but is comparable to the 19% decrease previously reported by Stathis *et al.* (1994) after a seven-week sprint training program. The discrepancy between studies is likely related in part to individual differences in purine nucleotide metabolism during intense exercise and recovery. During strenuous exercise, AMP produced from ATP hydrolysis can be deaminated by AMP deaminase, resulting in the formation of inosine 5' mono-phosphate (IMP) and ammonia, and subsequent breakdown of IMP to inosine and hypoxanthine results in a loss of adenine nucleotides from the muscle (Hellsten *et al.* 1998). Replacement of purine nucleotides lost from the muscle is a relatively slow, energy-consuming process

and appears to continue for several days after intense exercise (Hellsten *et al.* 1998). Thus, the lower ATP content measured after training in the present study may have been due to the stress of chronic training, or the acute residual effects of the final training bout, which was performed 72 hours before tissue extraction.

With respect to exercise performance, we recently showed that six sessions of SIT performed over two weeks dramatically improved cycle endurance capacity, such that the mean time to exhaustion during cycling at ~80% VO_{2peak} increased from 25 to 51 minutes (Burgomaster *et al.* 2005). Because many athletic events require athletes to complete a fixed amount of work in as short a time as possible (i.e., a race), in the present study we incorporated a time-trial to evaluate potential changes in volitional exercise capacity. We found that time-trial performance improved by 9.6% after only two weeks of sprint training, despite no change in VO_{2peak} . Thus, the physiological adaptations conferred by a short period of SIT not only increase aerobic endurance capacity (Burgomaster *et al.* 2005) but also increase the mean power output than can be sustained during a fixed work bout that is dominated by aerobic metabolism. As in our previous study (Burgomaster *et al.* 2005), the validity of our performance data is bolstered by the fact that all subjects performed extensive familiarization trials prior to the experiment, and a control group showed no change in performance when tested two weeks apart with no training intervention (Figure 3.1). The training-induced improvement in time-trial performance is noteworthy considering that our subjects were active individuals

who were already quite fit at the start of the study (the mean VO_{2peak} at baseline for the trained group was within the 80th percentile for this age group [Balady *et al.* 2000]). Nonetheless, while seven of eight subjects in the trained group showed similar improvements in time-trial performance (Figure 3.1), the one non-responder was the individual who posted the fastest time-trial at the beginning of the study. Additional studies are warranted to evaluate the adaptations induced by short-term SIT in very fit subjects, but other investigators have shown that highly trained athletes can benefit from a period of intensified training that is characterized by short bouts of intense exercise (Westgarth-Taylor *et al.* 1997).

In summary, the results from the present study demonstrate that six sessions of SIT decreased net muscle glycogenolysis and lactate accumulation during submaximal exercise and increased the activity of PDHa. The net result was consistent with a closer matching between muscle pyruvate production and oxidation. This is also the first study to show that sprint training improves aerobic exercise performance during a laboratory time-trial that closely simulates the way in which athletes typically compete. Finally, the present data confirm the novel results from our recent study (Burgomaster *et al.* 2005) that showed skeletal muscle oxidative capacity can be enhanced by a brief two-week period of SIT, equivalent to only 15 minutes of very intense exercise spread over a total time commitment of ~2.5 hours.

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CHAPTER 4

DIVERGENT RESPONSE OF METABOLITE TRANSPORT PROTEINS IN HUMAN SKELETAL MUSCLE AFTER SPRINT INTERVAL TRAINING AND DETRAINING.

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4.1 INTRODUCTION

In order to generate the high power outputs achieved during short bursts of “all out” maximal exercise, skeletal muscle primarily relies on muscle glycogen for energy provision. For example, a single 30-second bout of maximal cycling exercise can utilize almost one-quarter of the glycogen content in muscle, the majority of which is converted to lactate, to fuel non-oxidative ATP provision (McCartney *et al.* 1986; Parolin *et al.* 1999; Putman *et al.* 1995). Although the rate of muscle glycogenolysis is reduced during repeated sprinting, large amounts of lactate accumulate and the associated increase in hydrogen ion concentration ($[H^+]$) poses a challenge to systems that regulate muscle pH (Juel 2006). It is therefore not surprising that high-intensity sprint interval training (SIT), or repeated sessions of brief, intense exercise for several weeks, induces changes in monocarboxylate transporter (MCT) proteins that regulate lactate and H^+ exchange in human skeletal muscle (Bickham *et al.* 2006; Juel *et al.* 2004; Pilegaard *et al.* 1999). Although the early time course is unknown, relatively rapid increases in MCT1 and MCT4 could contribute to the reduced rate of muscle

lactate accumulation and improved high-intensity exercise performance that have been documented after short-term (i.e., two weeks) SIT (Burgomaster *et al.* 2006; Burgomaster *et al.* 2005; Gibala *et al.* 2006).

SIT may induce changes in other metabolite transport proteins, such as those associated with glucose and fatty acid metabolism, however this has not been studied in humans. Although the contribution from blood glucose during sprint exercise is modest (Juel *et al.* 2004), an increased GLUT4 protein content could facilitate higher glucose uptake during recovery and explain in part the higher muscle glycogen content observed after SIT (Burgomaster *et al.* 2005; Burgomaster *et al.* 2006; Gibala *et al.* 2006). Similarly, while extramuscular lipid is not a major source of energy during sprint exercise (Jones *et al.* 1980), several weeks of high-intensity training may increase the capacity for skeletal muscle lipid oxidation, as evidenced by an increased maximal activity of 3-hydroxyacyl CoA dehydrogenase (HAD) (Parra *et al.* 2000; Rodas *et al.* 2000; Simoneau *et al.* 1987). Other studies have reported no change in HAD after short-term SIT (Burgomaster *et al.* 2006), which suggests pathways associated with fatty acid metabolism may adapt more slowly than those involved in carbohydrate metabolism.

The primary purpose of the present study was to examine the early time-course for changes in metabolite transport proteins in human skeletal muscle in response to SIT. We speculated that proteins associated with glucose and lactate transport would adapt more quickly than proteins associated with fatty

acid transport. Specifically, we hypothesized that one week (3 sessions) of SIT would increase the muscle content of GLUT4, MCT1 and MCT4, as well as COX4, which was used as a marker of oxidative capacity. In contrast, we hypothesized that changes in fatty acid translocase (FAT/CD36) and plasma membrane associated fatty acid binding protein (FABPpm) would occur more slowly and not be evident until after six weeks of training. Muscle tissue was also harvested after one and six weeks of detraining to examine the time-course for changes in metabolite transport proteins upon cessation of the SIT stimulus.

4.2 METHODS

4.2.1 Subjects and general design

Eight healthy men (22 ± 1 yr, 176 ± 3 cm, 80 ± 4 kg) volunteered to take part in the main experiment. The subjects were active students at McMaster University who participated in some form of physical activity several times per week. The specific activities included jogging, bicycling for transportation and intramural sports such as soccer and water polo. None of the subjects were specifically engaged in a regular program of exercise training for a particular sport or event. The study design consisted of a six-week SIT program followed by a six-week period of detraining. Given the generally active lifestyle of the subjects, this meant that the detraining phase of the study was characterized by loss of the SIT stimulus but not complete sedentariness. Subjects were tested prior to training, after one and six weeks of training, and one and six weeks of

detraining. As described further below, measurements included a resting needle biopsy sample for the determination of metabolite transport proteins, and a 250 kJ cycling time-trial to assess changes in exercise capacity. In order to more rigorously evaluate the validity of the performance data, eight other men drawn from the same population (26 ± 1 yr, 180 ± 2 cm, 76 ± 3 kg) served as a control group. The control group performed a series of time-trials at intervals equivalent to the SIT group, but without any training intervention or muscle biopsy sampling. Following routine medical screening and explanation of potential risks, all subjects provided written informed consent prior to their participation. The study was approved by the McMaster University and Hamilton Health Sciences Research Ethics Board.

4.2.2 Experimental protocol

Subjects initially made several visits to the laboratory in order to become oriented with the testing procedures and training devices. All subjects performed a peak oxygen uptake (VO_{2peak}) test and a familiarization 250 kJ time-trial, and subjects in the training group also performed a Wingate Test (see below). Approximately one week following the familiarization procedures, all subjects performed a baseline (pre-training) time-trial. Subjects in the training group also underwent a needle biopsy procedure, which was always performed 1 hour prior to the time-trial. All subsequent time-trials for a given subject were performed at the same time of day in order to minimize potential diurnal variations in exercise performance. The sprint training protocol consisted of three sessions per week

on alternate days (i.e., Monday, Wednesday, Friday) over six weeks. Each training session consisted of 4-6 x 30-second Wingate Tests separated by 4 minutes of recovery. Recovery consisted of cycling at 30 W in order to reduce venous pooling in the legs and potential feelings of dizziness or nausea. The number of intervals performed during each training session increased from 4 during the first two weeks of training, to 5 during the middle two weeks, and finally 6 intervals were performed per session during the final two weeks of training. Subjects in the training group underwent a muscle biopsy procedure and performed a time-trial after one and six weeks of training. The biopsy was obtained ~72 hours following the previous training session, and was followed 1 hour later by the time-trial to match the timing of measurements performed during baseline testing. In order to obtain the one-week data, the normal Monday training session was eliminated and replaced by the testing procedures. Subjects also underwent a muscle biopsy procedure and performed a time-trial after one and six weeks of detraining. As previously indicated, subjects in the control group performed a series of time-trials over the course of the study that coincided with the timing of exercise tests performed in the training group.

4.2.3 Details of experimental procedures

4.2.3.1 *VO_{2peak} test*

Subjects performed an incremental test to exhaustion on an electronically braked cycle ergometer (Lode Excalibur Sport V2.0, Groningen, The Netherlands) to determine VO_{2peak} using an online gas collection system (Moxus

modular oxygen uptake system, AEI technologies, Pittsburgh, PA). The initial workload was set at 50 W and was increased by 1 W every 2 seconds until fatigue. The value used for $\text{VO}_{2\text{peak}}$ corresponded to the highest value achieved over a 30-second collection period.

4.2.3.2 *Time-trial*

Subjects were instructed to complete a 250 kJ self-paced laboratory time-trial on an electronically braked cycle ergometer (Lode) as quickly as possible with no temporal, verbal, and physiological feedback. Time required to complete the test and average power output were recorded upon completion of each test. Method error reproducibility for the time-trial (coefficient of variation) was 2.6% when eight individuals were tested one week apart with no sprint training intervention (Burgomaster *et al.* 2006).

4.2.3.3 *Wingate Test*

This test consisted of a 30-second “all-out” sprint on an electronically braked cycle ergometer (Lode) against a resistance set at $0.075 \text{ kg}\cdot\text{kg}^{-1}$ body mass. Subjects were instructed to begin cycling as fast as possible and the application of resistance was immediately applied by a computer interfaced with the ergometer and loaded with appropriate software (Wingate software version 1.11, Lode).

4.2.4 Muscle biopsy

The area over one thigh was prepared for the extraction of a resting

biopsy sample from the vastus lateralis muscle (Bergström 1975). An incision was made through the skin and underlying fascia under local anesthetic (1% w/v lidocaine hydrochloride, AstraZeneca Canada Inc, Mississauga, ON) and the biopsy sample was immediately frozen in liquid nitrogen after excision.

4.2.5 Dietary controls

In order to minimize any potential diet-induced variability in measurements subjects were instructed to document their dietary intake and refrain from using alcohol and caffeine for 48 hours prior to the baseline muscle biopsy procedure and time-trial. Dietary records were subsequently duplicated and returned to the subjects 2 days prior to each subsequent testing session, and subjects were instructed to replicate their individual patterns of food intake. Subsequent dietary analyses (Nutritionist Five, First Data Bank Inc., San Bruno, CA) of the food records revealed no differences in total energy intake or macronutrient composition between testing sessions (data not shown).

4.2.6 Muscle analyses

Frozen muscle samples were initially homogenized and protein concentrations were determined using a BCA assay (Sigma, St. Louis, MO). Proteins were separated using SDS-PAGE followed by Western blotting to detect the presence of GLUT4, MCT1, MCT4, COX4, FABPpm and FAT/CD36 using routine procedures that we have previously described (Bonen *et al.* 2000; Bonen *et al.* 2004). Equal quantities of protein were loaded into each lane and a common standard was included in all blots. Blots were detected using enhanced

chemiluminescence (Perkin Elmer Life Science, Boston, MA) and subsequently quantified using Gene Tool densitometry (SynGene, ChemiGenius2, Perkin-Elmer, Woodbridge, ON). Commercial suppliers were used for GLUT4 (Chemicon, Temecula, CA) and COX4 (Invitrogen Canada Inc., Burlington, ON). The other antibodies were gifts from Dr. H. Hatta, University of Tokyo, Tokyo, Japan (MCT1 and MCT4), Dr. Calles-Escandon, Wake Forest School of Medicine, Winston-Salem, NC (FABPpm) and Dr. N. Tandon, Thrombosis Research Laboratory, Otsuka Maryland Medicinal Laboratories, Rockville, MD (FAT/CD36).

4.2.7 Statistical analyses

All muscle data were analyzed using a 1-factor (time) repeated measures analysis of variance (ANOVA). Time-trial performance data were analyzed using a 2-factor mixed ANOVA with the between factor “group” and the repeated factor “time”. The relationship between each muscle protein and time-trial performance was analyzed using Pearson’s Product Moment correlation. The level of significance for all analyses was set at $P < 0.05$ and significant main effects were subsequently analyzed using a Fisher’s least squares difference test. All values are reported as means \pm SE. Performance data are based on $n=8$ per group, and muscle data for the training group are based on $n=6$ due to inadequate muscle biopsy samples which precluded a complete data set for 2 subjects.

4.3 RESULTS

4.3.1 GLUT4

Muscle GLUT4 content was higher at all time points during training and detraining compared to Pre ($P < 0.05$) (Figure 4.1). GLUT4 content was lower after six weeks of detraining compared to six weeks of training ($P < 0.05$).

4.3.2 COX4

Muscle COX4 content increased after one week of training and remained higher at all other time points during training and detraining compared to Pre ($P < 0.05$) (Figure 4.2).

4.3.3 MCT1 AND MCT4

The muscle content of MCT1 was higher after six weeks of training and remained elevated compared to Pre after one week of detraining ($P < 0.05$) (Figure 4.3). MCT4 protein content was elevated above Pre after one and six weeks of training ($P < 0.05$) but was not different after one and six weeks of detraining (Figure 4.3). MCT4 content after six weeks of detraining was lower ($P < 0.05$) compared to six weeks of training.

4.3.4 FABPpm and FAT/CD36

The muscle content of the two measured fatty acid transport proteins remained unchanged during training and detraining (Table 4.1).

Figure 4.1 GLUT4 protein content at baseline (Pre) and after one (TR-1) and six weeks (TR-6) of sprint interval training, and one (DeTR-1) and six weeks (DeTR-6) of detraining. Values are means \pm SE (n=6). * versus Pre, † versus TR-6, both P<0.05.

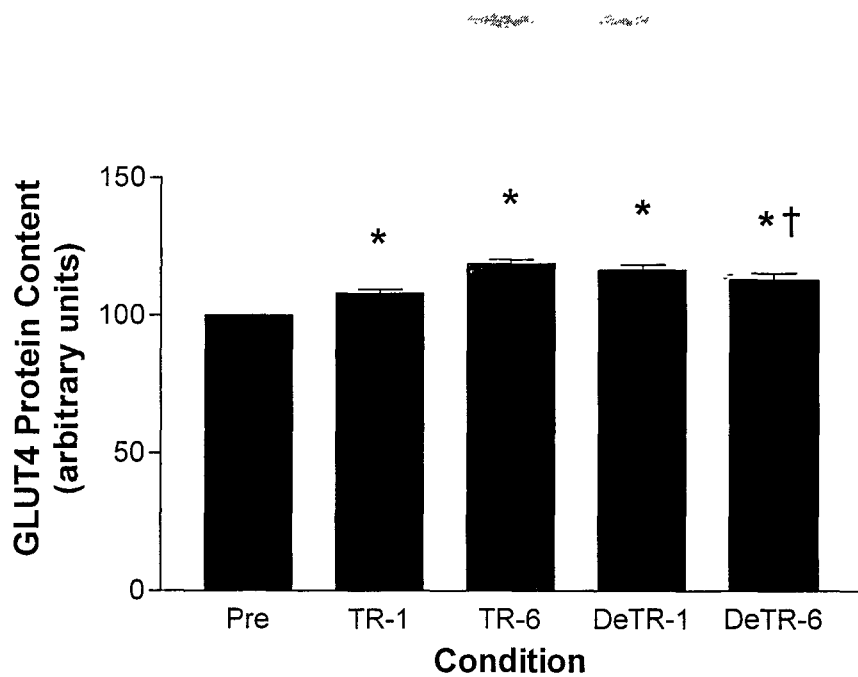


Figure 4.2 COX4 protein content at baseline (Pre) and after one (TR-1) and six weeks (TR-6) of sprint interval training, and one (DeTR-1) and six weeks (DeTR-6) of detraining. Values are means \pm SE (n=6). * P<0.05 versus Pre.

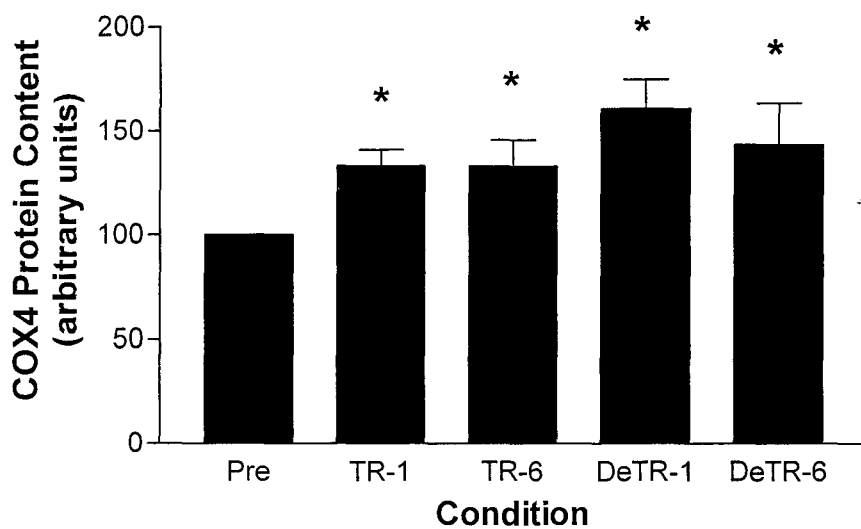


Figure 4.3 Protein content of MCT1 (top panel) and MCT4 (bottom panel) at baseline (Pre) and after one (TR-1) and six weeks (TR-6) of sprint interval training, and one (DeTR-1) and six weeks (DeTR-6) of detraining. Values are means±SE (n=6). * versus Pre, † versus TR-6, both P<0.05.

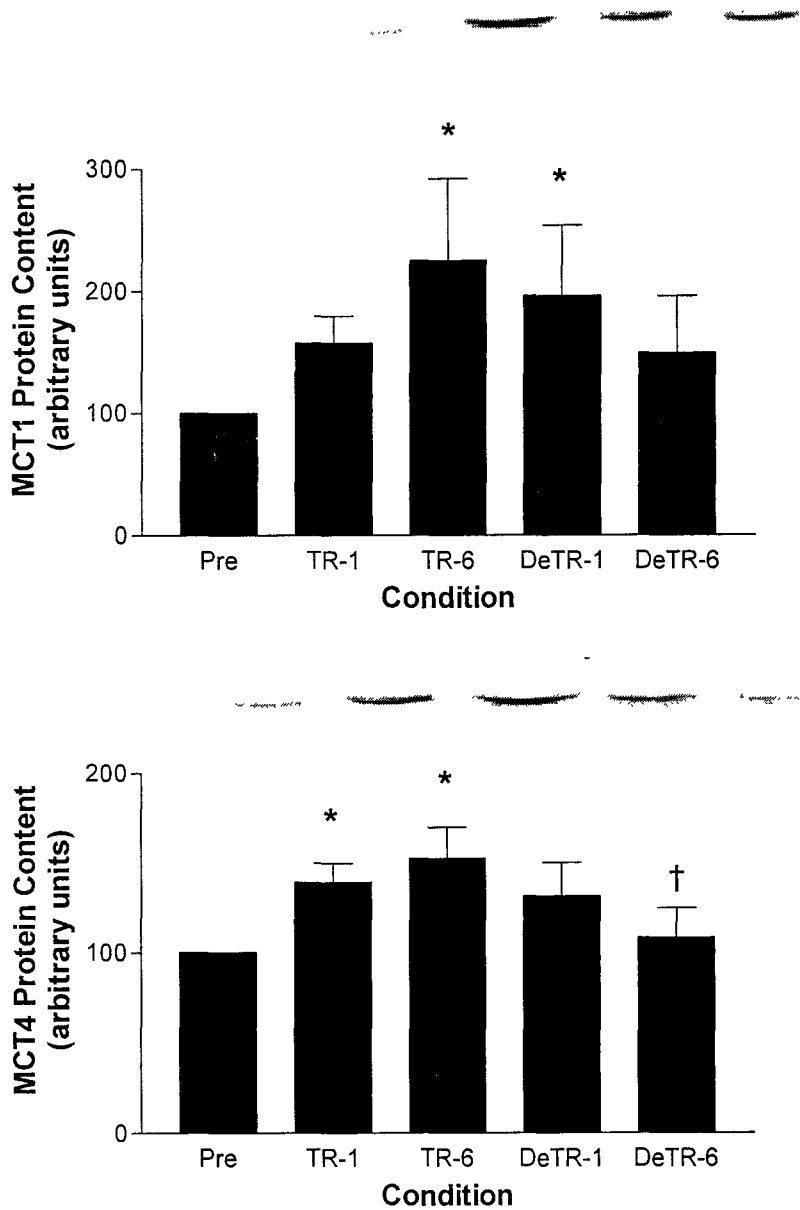


Table 4.1 Fatty acid transport proteins.

Protein	Pre	TR-1	TR-6	DeTR-1	DeTR-6
FAT/CD36	100	106±12	109±9	100±8	127±12
FABPpm	100	86±8	87±5	101±9	91±6

All values are means±SE (n=6), expressed in arbitrary units relative to a baseline (Pre) value of 100. TR-1 and TR-6 refer to one and six weeks of training, respectively. DeTR-1 and DeTR-6 refer to one and six weeks of detraining, respectively.

4.3.5 Exercise Performance

There was no difference between the training and control groups at baseline (Pre) in the time required to complete the 250 kJ cycling test (Figure 4.4). However, in the SIT group, time-trial performance was improved after six weeks of training and after one and six weeks of detraining compared to Pre ($P<0.05$). The control group showed no change in performance over the course of the study (Figure 4.4) and their time was slower versus the training group after six weeks of training and one and six weeks of detraining ($P<0.05$). A regression analyses based on individual data at all time points revealed that the change in time-trial performance from baseline was correlated with the change in the GLUT4 ($R=0.70$; $P<0.05$) and MCT4 protein content ($R=0.39$; $P<0.05$) (Figure 4.5) and also tended to correlate with MCT1 protein content ($P=0.07$).

Figure 4.4 Time to complete a 250 kJ cycling time-trial before (Pre) and after one (TR-1) and six weeks of sprint interval training (TR-6) and after one (DeTR-1) and six weeks of detraining (DeTR-6). A control group (CON) performed the time-trial on several occasions over the course of the study with no training intervention. Values are means \pm SE (n=8 per group) for the SIT and CON groups. ^a P<0.05 versus Pre. ^b P<0.05 versus CON group at same time point. ^c P<0.05 versus TR-1.

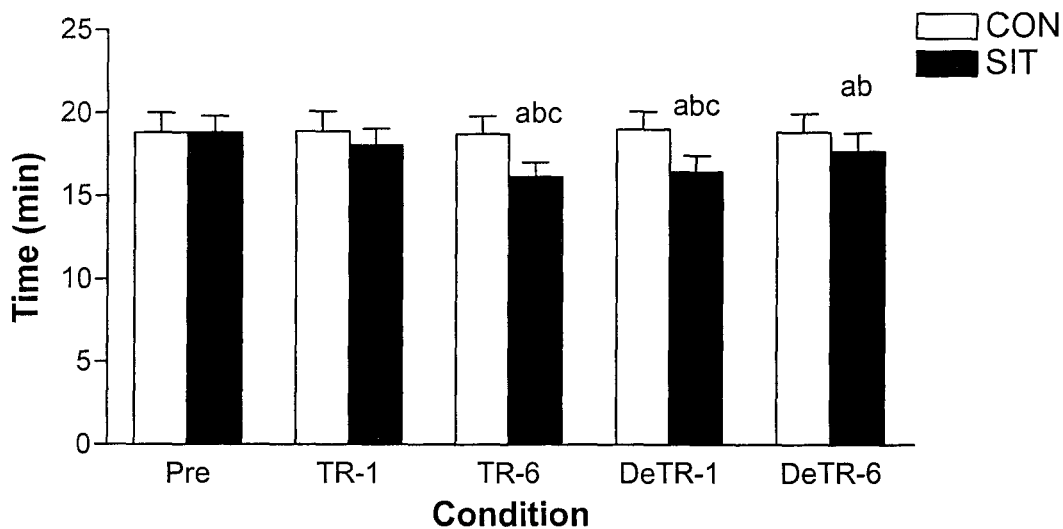
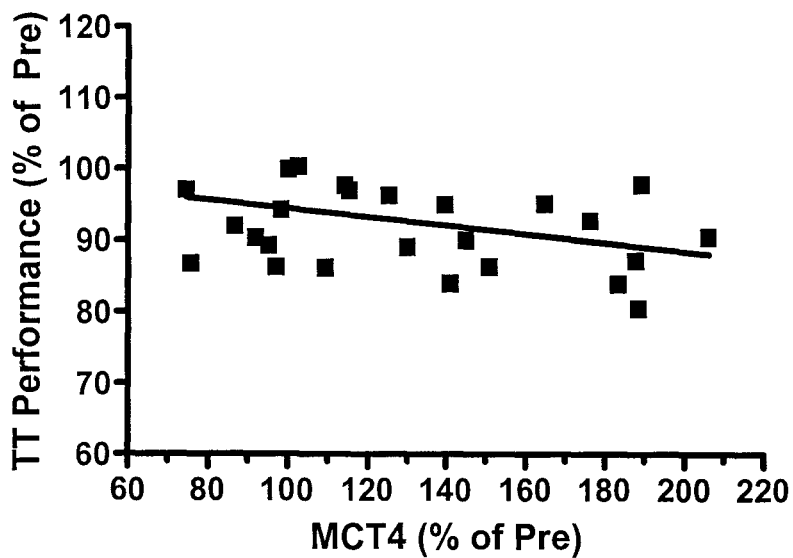
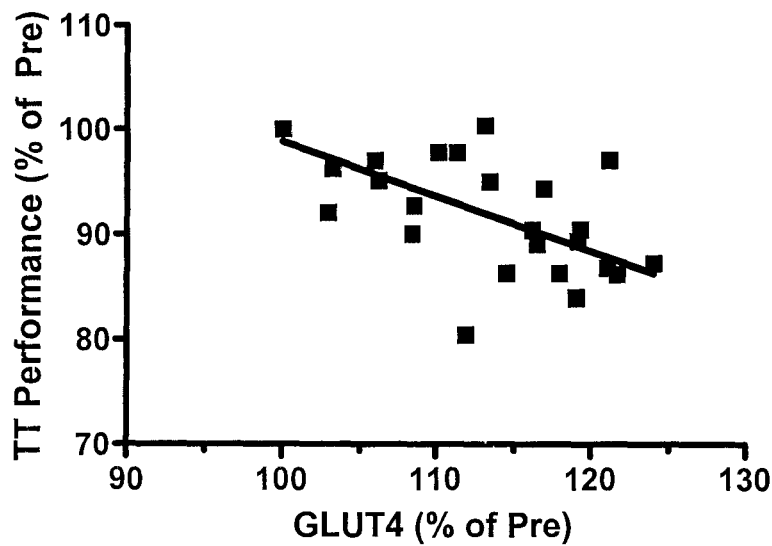


Figure 4.5 Relationship between the change in time-trial performance and the muscle content of GLUT4 (R=0.70; top panel) and MCT4 (R=0.39; bottom panel). For all variables, data was expressed relative to the pre-training value normalized to 100%.



4.4 DISCUSSION

This was the first study to examine the effect of SIT and detraining on metabolite transport proteins in human skeletal muscle. Muscle GLUT4 content, as well as the content of the mitochondrial marker COX4, were increased after only three sessions of SIT, or a total of 6 minutes of very intense exercise over one week. There was no further increase in GLUT4 and COX4 after six weeks of SIT, but surprisingly, the training-induced increases in these proteins persisted for six weeks after cessation of the SIT stimulus. The muscle content of MCT4 increased after one week of training, whereas the increase in MCT1 content was more variable and not significant until after six weeks. Both MCT1 and MCT4 declined upon cessation of the SIT stimulus and returned to baseline values after six weeks of detraining. The muscle contents of the fatty acid transport proteins FATCD/36 and FABPpm were unchanged by training and detraining.

4.4.1 GLUT4

This is the first study to demonstrate that brief, intense interval training increases the protein content of GLUT4 in human skeletal muscle. Our data are supported by work from Terada and colleagues (2001), who showed that GLUT4 protein content in rat epitroclearis muscle was increased after a total of only 5 minutes of intense intermittent swim training over eight days. While the training-induced increase in GLUT4 content was consistent across subjects in the present study, the magnitude of the change was modest, and peaked at ~25% above baseline values after six weeks of SIT. In contrast, GLUT4 protein content

can be increased by $\geq 100\%$ after five to seven consecutive days of traditional endurance training, i.e., 1-2 hours per day at 60-75% VO_{2peak} (Houmard *et al.* 1995; Gulve & Spina 1995; Phillips *et al.* 1996). It has also been reported that a single bout of moderate-intensity exercise can increase GLUT4 protein content 8-22 hours following the bout (Greiwe *et al.* 2000). It is therefore possible that the “training”-induced increases in GLUT4 reported in the present study were due in part by residual “acute” effects from the previous exercise bout. We feel that any residual acute effect from the previous exercise bout was likely small given the 72 hour period between the training session and the biopsy procedure. However, even if we assume that the increase in GLUT4 after one week of training was solely attributable to the acute effect of the previous high-intensity interval session, this suggests that a total of 2 minutes of “all out” cycling is sufficient to increase GLUT4 protein content in human skeletal muscle. Regardless of the time course for changes in GLUT4 after SIT, the present data confirm that exercise modes other than traditional moderate-intensity training can increase muscle GLUT4 content, as previously shown for resistance exercise (Holten *et al.* 2004; Tabata *et al.* 1999).

One of the most interesting findings from the present study was that the training-induced increase in GLUT4 persisted for six weeks after the cessation of SIT. Several studies have reported that GLUT4 content decreases relatively quickly (within one to two weeks) when previously endurance-trained individuals stop training (McCoy *et al.* 1994; Vukovich *et al.* 1996), although this is not a

universal finding (Houmard *et al.* 1993). Houmard *et al.* (1996) showed that the training-induced increase in GLUT4 content was maintained for two weeks in subjects who reduced their training volume by 50%, whereas the gains were lost if subjects stopped training completely. While speculative, it is possible that the persistent elevation in GLUT4 during detraining in the present study was related to our subjects' return to their previously "active" lifestyle (i.e., which consisted of a few low to moderate intensity sessions of physical activity per week as opposed to complete sedentariness). Alternatively, a recent study showed that long-term high-intensity exercise training provided more enduring benefits to insulin action compared with moderate- or low-intensity exercise (DiPietro *et al.* 2006), which lends credence to the notion that SIT may induce unique adaptations with respect to muscle GLUT4.

4.4.2 COX4

Another major finding from the present study was that muscle oxidative capacity, as reflected by the muscle content of COX4, was increased after only three sessions of SIT over one week. Previously, we have reported increases in mitochondrial enzymes after six sessions of SIT over two weeks (Burgomaster *et al.* 2005; Burgomaster *et al.* 2006), and Gibala *et al.* (2006) recently showed that the increase in citrate synthase maximal activity after this protocol was comparable to a group who performed the same number of endurance training sessions (90-120 minutes of cycling at 65% VO_{2peak} per day). The potency of high-intensity exercise is also supported by work in animals that showed rapid

increases in citrate synthase maximal activity after short-term swim training (eight days of 15 x 20-second intervals with 10 seconds of rest) (Terada *et al.* 2001). The potency of SIT to elicit rapid changes in skeletal muscle phenotype is likely related to its high level of muscle fibre recruitment, and the potential to stress type II fibres in particular (Gollnick *et al.* 1973). Animal studies have shown that differences in contraction intensity result in selective activation of specific intracellular signalling pathways, which may determine the specific adaptations induced by different forms of exercise training (Nader & Esser 2001; Terada *et al.* 2005). In this regard, Terada and colleagues have shown rapid increases in mitochondrial biogenesis after short-term high-intensity swim training in rats, which the authors attributed to exercise-induced changes in the expression of peroxisome proliferator-activated receptor co-activator-1 (Terada *et al.* 2001; Terada *et al.* 2005).

Similar to the change in GLUT4, the training-induced increase in COX4 persisted after six weeks of detraining in the present study. It is believed that mitochondrial capacity decreases relatively quickly after cessation of endurance training (Coyle 1984; Henriksson & Reitman 1977) but data regarding changes in muscle oxidative capacity after high-intensity intermittent training and detraining are limited and equivocal (Fournier *et al.* 1982; Linossier *et al.* 1997; Simoneau *et al.* 1987). The estimated rate of change in muscle oxidative capacity during detraining may depend on the specific mitochondrial enzyme that is measured. For example, Simoneau *et al.* (1987) reported that oxoglutarate dehydrogenase

remained elevated seven weeks after the cessation of a fifteen-week SIT program, whereas HAD returned to pre-training levels. Persistent increases in muscle oxidative capacity have also been reported several weeks after the cessation of aerobic exercise training (Wibom *et al.* 1992), specifically the maximal activity of COX, a subunit of which was measured in the present study. Thus, the elevated muscle COX4 content observed during detraining in the present study may be specific to that protein or could represent a unique adaptation induced by the SIT stimulus, influenced in part by the habitual activity of the subjects.

4.4.3 MCT1 and 4

The present data are consistent with the findings of Pilegaard *et al.* (1999) and indicate that several weeks of intense, interval-based training can increase both MCT1 and MCT4 content in human muscle. A novel finding from the present work was that the increase in MCT4 occurred relatively quickly after only three sessions of SIT. This observation is supported by a preliminary report by Yoshida and colleagues (2005) that showed three weeks of daily sprint-run training in rats increased the muscle content of both MCT1 and MCT4. Although the mean changes during training were larger for MCT1 compared to MCT4 in the present study, the individual responses were more variable. Compared to baseline, peak individual increases over the six-week training period ranged from ~30 to 530% for MCT1 and ~15 to 200% for MCT4. We attribute this mainly to individual differences in the response to the SIT stimulus (i.e., physiological variability)

since previous work from our laboratory has shown less variable changes in MCTs after electrical stimulation in animals (Baker *et al.* 1998) and aerobic-based training in humans (Bonen *et al.* 1998). For example, seven consecutive days of aerobic-based training increased MCT1 content by 18% in human skeletal muscle however the range (0 to 62%) was considerably smaller than in the present study (Bonen *et al.* 1998). Other groups have also reported larger mean changes and higher variability for MCT1 as compared to MCT4 after high-intensity exercise training (Pilegaard *et al.* 1999). Muscle fibre composition has been shown to influence the adaptive response of MCT1 to exercise training in rodents (Baker *et al.* 1998), and this may explain in part the variability associated with the human adaptive response to SIT.

4.4.4 Fatty acid transporters

This is the first study to examine the effect of SIT on fatty acid transporters in human skeletal muscle, and in contrast to our hypothesis, we found no effect of training or detraining on the muscle protein content of FABPpm or FAT/CD36. Our hypothesis was based on the fact that several weeks of SIT have been shown to increase the maximal activity of HAD, which is a commonly-used marker for fat oxidation in muscle (Parra *et al.* 2000; Rodas *et al.* 2000). However, the relationship between fatty acid transporters and the maximal capacity for fatty acid oxidation in skeletal muscle is unclear. Aerobic-based training increases the maximal capacity for fat oxidation (Achten & Jeukendrup 2004), however there are limited and conflicting data regarding the effect of this

type of training on muscle FABPpm and FAT/CD36 content (Kiens *et al.* 1997; Tunstall *et al.* 2002). For example, Tunstall *et al.* (2002) reported the muscle content of FAT/CD36, but not FABPpm, was increased by short-term aerobic training (1 hour at 63% VO_{2peak} per day for nine days), whereas Kiens and colleagues (1997) showed that three weeks of endurance training increased muscle FABPpm. One of the rate-determining steps in skeletal muscle lipid oxidation appears to be the activity of carnitine palmitoyl transferase (Achten & Jeukendrup 2004), and thus it is possible that SIT increases the maximal capacity for lipid oxidation during exercise however an increased skeletal muscle content of fatty acid transport proteins is not obligatory for this to occur. Additional work is warranted to clarify the effect of interval-based training on skeletal muscle fat metabolism and the regulatory factors involved.

4.4.5 Relation between training-induced changes in exercise performance and transport proteins

Finally, while the major focus of this study was metabolite transport proteins, we also rigorously evaluated the effect of sprint training and detraining on exercise performance using a task that closely resembles athletic competition. It is unlikely that the observed changes in performance were a spurious result or related to a “learning effect”, since the control group showed no change in performance over the course of the study. Obviously, the factors responsible for training-induced improvements in performance are extremely complex and determined by numerous physiological (e.g., cardiovascular, muscle metabolic,

neural, respiratory, thermoregulatory) and psychological attributes (e.g., mood, motivation, perception of effort). The change in time-trial performance from baseline (Pre) was correlated with the change in MCT4 protein content and also tended to correlate with the change in MCT1 protein content ($P=0.07$). These data support previous research that linked exercise tolerance to the content of MCTs in human muscle (Thomas *et al.* 2005) and suggest that changes in lactate/H⁺ transport capacity may contribute to performance improvements induced by SIT. These findings are also supported by a recent study that showed changes in time-trial performance after short-term SIT were accompanied by an increased muscle buffering capacity (Gibala *et al.* 2006). The strongest correlation in the present study was between time-trial performance and muscle GLUT4 content. Given that blood glucose likely contributed $\leq 10\%$ of total energy provision during the relatively intense exercise protocol in the present study (McCartney *et al.* 1986; van Loon *et al.* 2001), the physiological significance of the training-induced changes in muscle GLUT4 may be related more to the post-exercise period and explain in part the higher resting muscle glycogen content observed after SIT (Burgomaster *et al.* 2005; Burgomaster *et al.* 2006; Gibala *et al.* 2006).

4.4.6 Conclusion

In summary, the results from the present study demonstrate that SIT rapidly increased muscle oxidative potential and the proteins associated with glucose and lactate/H⁺ transport. Upon cessation of the training stimulus, the

muscle content of MCT1 and MCT4 declined to pre-training levels, however the training-induced increases in GLUT4 and COX4 persisted after six weeks of detraining. The muscle content of fatty acid transport proteins was unchanged throughout the study, which suggests proteins associated with carbohydrate metabolism are more responsive to the initiation and cessation of SIT in humans. Future studies should examine the signalling pathways and regulatory mechanisms responsible for the training-induced increases in muscle oxidative capacity and CHO transport proteins, as well as the physiological/health-related consequences of these adaptations. The present data add novel insights to the growing body of evidence that shows the potency of SIT to elicit rapid metabolic adaptations in human skeletal muscle, despite a relatively small training time commitment and total exercise volume.

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CHAPTER 5**SIMILAR METABOLIC ADAPTATIONS DURING EXERCISE AFTER LOW VOLUME SPRINT
INTERVAL AND TRADITIONAL ENDURANCE TRAINING IN HUMANS.**

(*J.Physiol.* In press [EPub], November 8, 2007)

5.1 INTRODUCTION

Prolonged sessions of moderate-intensity exercise (e.g., ≥ 1 hour at $\sim 65\%$ of VO_{2peak}), performed repeatedly for at least several weeks, increases skeletal muscle oxidative capacity and alters substrate utilization during matched-work exercise, resulting in improved endurance capacity (Gollnick *et al.* 1973). While less widely appreciated, numerous studies have shown that brief, repeated sessions of “all out” sprint-type or high-intensity interval exercise elicit changes in skeletal muscle energy metabolism that resemble traditional endurance training (Henriksson & Reitman 1976; Saltin *et al.* 1976; Gibala *et al.* 2006). The relatively few studies that have directly compared skeletal muscle metabolic adaptations to interval and continuous training have yielded equivocal results, and in all cases the total volume of work performed was similar between groups (Henriksson & Reitman 1976; Saltin *et al.* 1976; Eddy *et al.* 1977; Fournier *et al.* 1982; Gorostiaga *et al.* 1991; Edge *et al.* 2006). Recently, our group (Gibala *et al.* 2006) examined molecular and cellular adaptations in resting human skeletal muscle after six sessions of sprint interval (SIT) or traditional endurance training

(ET) performed over two weeks. By design, total training time commitment and exercise volume was markedly lower in the SIT group, yet we found similar improvements in the maximal activity of cytochrome c oxidase (COX) and protein content of COX subunits II and IV after training in both groups. While previously speculated by others (Coyle *et al.* 2005), to our knowledge this was the first study to demonstrate that SIT was indeed a very “time-efficient” strategy to improve skeletal muscle oxidative capacity and exercise performance (Gibala *et al.* 2006).

In the present study, we sought to confirm and extend the findings of our previous work that showed similar increases in muscle oxidative capacity after two weeks of SIT or ET (Gibala *et al.* 2006). Our previous study (Gibala *et al.* 2006) was limited in that the duration of training was relatively short and it can be argued that the very intense nature of SIT might stimulate rapid skeletal muscle remodelling (possibly due to altered fibre recruitment), whereas adaptations to lower-intensity ET accrue more slowly. Second, our previous study involved a single marker of muscle oxidative capacity in resting biopsy samples, and thus provided limited insight regarding the potential for changes in substrate metabolism during exercise. In consideration of these issues, the unique purpose of the present study was to compare adaptations in markers of skeletal muscle carbohydrate (CHO) and lipid metabolism and metabolic control during matched-work exercise, before and after six weeks of low-volume SIT or high-volume ET. The SIT protocol was modeled after previous work in our lab and consisted of four to six 30-second “all out” cycling tasks performed three

times per week for six weeks (Burgomaster *et al.* 2006; Gibala *et al.* 2006). In contrast, the ET protocol was modeled after public health guidelines (American College of Sports Medicine, 1998) and consisted of 40-60 minutes of continuous cycling at $\sim 65\%$ VO_{2peak} five days per week for six weeks. Whereas previous studies have examined adaptations in resting skeletal muscle after matched-work interval and continuous training (Henriksson & Reitman 1976; Saltin *et al.* 1976; Eddy *et al.* 1977; Fournier *et al.* 1982; Gorostiaga *et al.* 1991; Edge *et al.* 2006), the present study was unique in that we assessed changes during exercise and by design, the total weekly exercise volume was $\sim 90\%$ lower in the SIT group (i.e., 225 versus 2250 $\text{kJ}\cdot\text{wk}^{-1}$ in the SIT and ET group, respectively). We hypothesized that six weeks of SIT and ET would induce similar adaptations in muscle oxidative capacity and selected measures of whole-body and skeletal muscle substrate metabolism during exercise despite large differences in total training time commitment and exercise volume.

5.2 METHODS

5.2.1 Subjects

Twenty young healthy men and women (n=5 men and 5 women per group) volunteered for the study (Table 5.1). A preliminary screening process was employed to establish that subjects were (a) free of risk factors associated with cardiovascular, pulmonary or metabolic disease; (b) deemed safe to begin a physical activity program; and (c) aside from activities of daily living, not engaged

in a regular exercise training program (i.e., ≤ 2 sessions per week and ≤ 30 minutes per session, for at least one year prior to the study). The experimental procedures and potential risks were fully explained to the subjects prior to the study, and all subjects provided written, informed consent. The experimental protocol was approved by the McMaster University and Hamilton Health Sciences Research Ethics Board.

5.2.2 Preexperimental procedures

Subjects initially performed a progressive exercise test (increasing 1 W every 2 seconds) on an electronically braked cycle ergometer (Lode BV, Excalibur Sport V2.0, The Netherlands) in order to determine their peak oxygen uptake (VO_{2peak}) using an on-line gas collection system (Moxus Modular VO_2 System, AEI Technologies, Inc., Pittsburgh, PA). The value used for VO_{2peak} corresponded to the highest value achieved over a 30-second collection period. Subjects subsequently performed a familiarization ride in order to determine the workload that elicited $\sim 65\%$ of VO_{2peak} . All subjects also performed a 30-second all-out effort (Wingate Test) on the same cycle ergometer against a resistance equivalent to $0.075 \text{ kg}\cdot\text{kg}^{-1}$ body mass. After the familiarization procedures, subjects were assigned to either a sprint training group or an endurance training group in a matched fashion based on sex and VO_{2peak} (Table 5.1).

Table 5.1 Subject characteristics and peak oxygen uptake data before and after three and six weeks of exercise training.

	SIT Group			ET Group		
	PRE	3 WK	6 WK	PRE	3 WK	6 WK
Age (yr)	24±1			23±1		
Height (cm)	171±2			175±4		
Weight (kg)	69±3	69±3	68±3	75±4	75±4	75±4
VO _{2peak} (ml.kg ⁻¹ .min ⁻¹)*	41±2	44±2	44±2	41±2	42±2	45±2
VO _{2peak} (L.min ⁻¹)*	2.8±1	3.0±1	3.0±1	3.0±1	3.2±1	3.2±1

Values are means±SE, n=10 per group. *P<0.05, main effect for training (3 WK = 6 WK > PRE).

5.2.3 Experimental protocol

Each subject served as their own control and performed two experimental trials, before and after a six-week exercise training intervention (see below). Upon arrival at the laboratory, the lateral portion of one thigh was prepared for the extraction of needle biopsy samples from the vastus lateralis muscle (Bergström 1975). Two small incisions were made in the skin and underlying fascia after injection of a local anesthetic (2% lidocaine). A biopsy was obtained at rest, and then subjects commenced cycling for 60 minutes on an electronically braked cycle ergometer (Lode BV) at a workload designed to elicit ~65% of pre-training VO_{2peak} . Heart rate was determined using telemetry (Polar Electro, Woodbury, NY) and expired gases were collected for the determination of VO_2 , VCO_2 and RER using a metabolic cart (Moxus Modular VO_2 System, AEI Technologies, Inc., Pittsburgh, PA) and used to estimate rates of whole body fat and carbohydrate oxidation (Peronnet & Massicotte 1991). A second muscle biopsy was obtained immediately following exercise. The second experimental trial was performed 96 hours following the final exercise training session and was identical in all respects to the first experimental trial, including power output which was set at the same absolute workload (i.e., 65% of pre-training VO_{2peak}).

5.2.4 Training protocol

The training protocols were initiated several days after the first experimental trial (Table 5.2). Endurance training consisted of continuous cycling on an ergometer (Lode), five days per week (Monday-Friday) for six weeks, at a power

output corresponding to ~65% of VO_{2peak} . Subjects performed 40 minutes of exercise per training session for the first two weeks. Exercise time was increased to 50 minutes per session during weeks three and four, and during the final two weeks subjects performed 60 minutes of exercise per session. VO_{2peak} tests were re-administered after three weeks of training and training loads were adjusted in order to maintain a training intensity equivalent to 65% VO_{2peak} . Sprint training consisted of repeated Wingate Tests on an ergometer (Lode) three days per week (Monday, Wednesday, Friday) for six weeks. The number of Wingate Tests performed during each training session increased from four during weeks 1-2, to five during weeks 3-4, and finally to six during weeks 5-6. For all training sessions, the recovery interval between Wingate Tests was fixed at 4.5 minutes, during which time subjects cycled at a low cadence (< 50 rpm) against a light resistance (30 W) to reduce venous pooling in the lower extremities and minimize feelings of light-headedness or nausea. The ET program was based on general guidelines recommended by leading public health agencies (American College of Sports Medicine 1998) whereas the SIT program was modeled after recent studies conducted in our laboratory that have examined metabolic and performance adaptations to low-volume high-intensity interval training (Burgomaster *et al.* 2005; Burgomaster *et al.* 2006; Burgomaster *et al.* 2007; Gibala *et al.* 2006). By design, the protocols differed substantially in terms of total exercise training volume and time commitment in order to evaluate adaptations in skeletal muscle metabolism to two diverse training impulses.

Table 5.2 Summary of sprint interval training (SIT) and endurance training (ET) protocols.

Variable	SIT Group (n=10)	ET Group (n=10)
Protocol	30 s x 4-6 repeats, 4.5 min rest (3 x per wk)	40-60 min cycling (5 x per wk)
Training intensity (workload)	“All out” maximal effort (~700 W)	65% of VO_{2peak} (~175 W)
Weekly training time commitment	~10 min (~1.5 h including rest)	~4.5 h
Weekly training volume	~225 kJ	~2250 kJ

VO_{2peak} , peak oxygen uptake.

5.2.5 Dietary controls

Subjects were instructed to continue their normal dietary and physical activity practices throughout the experiment but to refrain from alcohol and exercise for 48 hours prior to each trial. Exercise was performed 3 hours following a standardized meal. Subjects recorded their dietary intake for 24 hours prior to the pre-trial so that their individual pattern of food intake could be replicated in the 24 hours before the post-trial. Subsequent dietary analyses (Nutritionist Five, First Data Bank, San Bruno, CA) revealed no differences in total energy intake or macronutrient composition prior to the two trials and no differences between groups (Table 5.3).

Table 5.3 Nutritional data.

	SIT Group		ET Group	
	PRE	POST	PRE	POST
Energy intake, MJ	11±1	10±1	11±1	10±1
Carbohydrate, %	60±2	59±2	56±3	55±4
Fat, %	23±2	24±2	26±2	26±2
Protein, %	17±1	17±1	18±2	19±2

Data collected for 24 hours prior to each experimental trial. Values are means±SE. No difference between trials or groups.

5.2.6 Muscle analyses

Muscle samples were initially sectioned into several pieces under liquid nitrogen and one of the pieces was freeze-dried, powdered, and dissected free of all non-muscle elements. The samples were stored at -80 °C prior to analyses.

5.2.3.1 *Enzyme activities*

One piece of frozen wet muscle was chipped from the resting samples only and homogenized to a 50 times dilution as described by Henriksson *et al.* (1986). The homogenate was subsequently analyzed to determine the maximal activity of CS on a spectrophotometer (Ultrospec 3000 pro UV/Vis) using a method described by Carter *et al.* (2001). The remaining homogenate was further diluted to 150 times (Henriksson *et al.* 1976) and analyzed for the maximal activity of HAD on a fluorometer (Hitachi F-2500, Hitachi Instruments, Tokyo, Japan) using a method described by Chi *et al.* (1983). Total protein content of all homogenates was determined by the method of Bradford (1976) and enzyme activities are expressed as moles per kilogram of protein per hour.

5.2.3.2 *Immunoblotting*

An aliquot of freeze-dried muscle was added to 250 µl of homogenizing buffer containing 50mM Tris, pH 7.5, 1mM EDTA, 1mM EGTA, 10% glycerol, 1% Triton X-100, 50mM NaF, 5mM Na pyrophosphatase, 1mM DTT and 2µl of protease inhibitor cocktail. The sample was homogenized on ice and subsequently centrifuged at 13,000 rpm for 5 minutes. The supernatant was

collected and the protein content was determined (Pierce BCA Protein Assay Reagent). The sample was subsequently diluted with homogenizing buffer to $3\text{mg}\cdot\text{ml}^{-1}$ protein. A $200\mu\text{l}$ aliquot of sample was mixed with $50\mu\text{l}$ of loading buffer and heated at $100\text{ }^{\circ}\text{C}$ for 5 minutes. For each blot, a standard and a positive control was loaded along with $40\mu\text{l}$ of each sample onto a 5% polyacrylamide stacking gel and separated using a 10% polyacrylamide separating gel of 1.5mm thickness at 180 V with a running time of ~45 minutes in Tris-glycine electrophoresis buffer. The gels were electroblotted onto nitrocellulose membranes in transfer buffer (37mM Tris base, 140mM glycine, 20% methanol) for 90 minutes at 90 V in a cold room. Membranes were then incubated in Tris-buffered saline Tween (TBST; 10 mM Tris base, 150 mM NaCl, 0.05% Tween-20) with 5% skim milk for 1 hour, and washed twice with TBST for 3 minutes. The membranes were subsequently incubated overnight at $4\text{ }^{\circ}\text{C}$ in PBS containing 1% BSA and primary antibodies against the E1 α subunit of PDH (Molecular Probes, Eugene, OR) or PGC-1 α (Chemicon, Temecula, CA). Following incubation with the primary antibody, the membranes were rewashed with TBST and exposed to appropriate anti-species horseradish peroxidase-conjugated secondary antibodies (Cell Signaling Technology, Danvers, MA) at a 1:10,000 dilution in blocking buffer for 60 minutes at room temperature. Membranes were then washed again with TBST before being exposed to a chemiluminescent liquid (Immuno-Star HRP Substrate Kit, Bio-Rad) for 2 minutes. Membranes were exposed using a Biorad Chemi-Doc System for 5 minutes and the density of the

bands were determined using associated image-analysis software.

5.2.3.3 *Metabolites*

An aliquot of freeze-dried muscle was extracted on ice using 0.5 M PCA containing 1 mM EDTA, neutralized with 2.2 M KHCO_3 , and the resulting supernatant was analyzed for creatine, PCr, ATP, and lactate using enzymatic assays adapted for fluorometry (Hitachi). Another aliquot of freeze-dried muscle was incubated in 2.0 N HCl and heated for 2 hours at 100 °C to hydrolyse the glycogen to glucosyl units. The solution was neutralized with an equal volume of 2.0 N NaOH and analyzed for glucose using an enzymatic assay adapted for fluorometry (Passoneau & Lowry 1993).

5.2.7 Statistical analyses

Exercise capacity data, whole-body substrate oxidation and cardiorespiratory data and enzyme data were analyzed by using a 2-factor mixed ANOVA, with the between factor “group” (SIT versus ET) and repeated factor “condition” (pre-training versus post-training). Muscle metabolite data were analyzed by using a 3-factor mixed ANOVA, with the factors “group” (SIT versus ET), “condition” (pre-training versus post-training) and “time” (0, 60 minutes). The level of significance for analyses was set at $P < 0.05$ and significant interactions or main effects were determined using Tukey’s honestly significant difference *post hoc* test. All data are presented as means \pm SE based on 10 subjects per group.

5.3 RESULTS

5.3.1 Exercise capacity and cardiorespiratory data

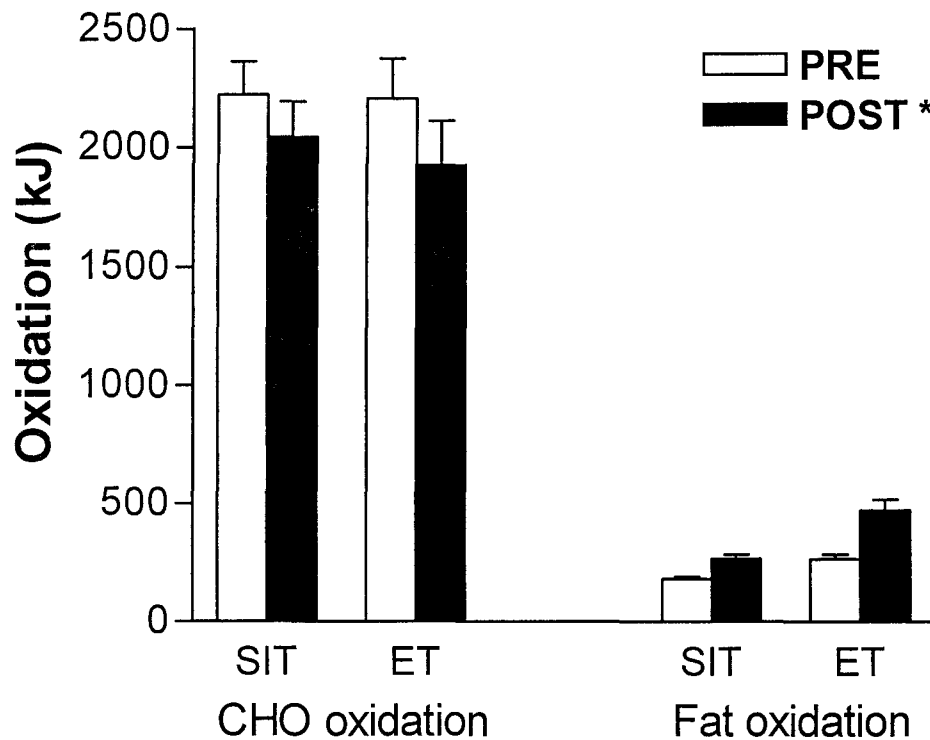
VO_{2peak} increased after training, with no difference between groups (main effect for condition, $P < 0.05$; Table 5.1). Peak power output elicited during the Wingate Test increased by 17 and 7% in the SIT and ET groups, respectively, with no difference between groups (main effect for condition, $P < 0.05$), whereas, mean power output was increased by 7% in the SIT group only ($P < 0.05$). Oxygen uptake during the constant-load cycling challenge was similar before and after training, however mean heart rate and ventilation were reduced (main effects, $P < 0.05$; Table 5.4). Mean respiratory exchange ratio during exercise was reduced after training (main effect, $P < 0.05$; Table 5.4) and calculated rates of whole-body fat and carbohydrate oxidation were increased and decreased, respectively, with no difference between groups (main effects, $P < 0.05$; Figure 5.1).

Table 5.4 Cardiorespiratory data during cycling exercise at 65% $\text{VO}_{2\text{peak}}$ before and after six weeks of sprint interval training (SIT) or six weeks of endurance training (ET).

	SIT Group		ET Group	
	PRE	POST	PRE	POST
Heart rate, $\text{beats}\cdot\text{min}^{-1}$ *	160±5	151±6	157±5	144±5
RER*	0.977±0.01	0.965±0.01	0.967±0.01	0.941±0.01
Ventilation, $\text{l}\cdot\text{min}^{-1}$ *	48±3	42±3	47±5	42±4
VO_2 , $\text{ml}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$	27.7±1.0	26.6±1.0	26.1±1.1	25.3±1.8

Values are means±SE (n=10 per group). RER, respiratory exchange ratio; VO_2 , oxygen uptake. * versus Post-training (main effect for condition, such that Pre-training ≠ Post-training, no difference between groups), $P < 0.05$.

Figure 5.1 Whole body carbohydrate and fat oxidation during exercise that consisted of 60 minutes at 65% VO_{2peak} before (PRE) and after (POST) six weeks of sprint interval training (SIT) or six weeks of endurance training (ET). Values are means \pm SE (n=10 per group). * Main effect for condition (P<0.05), such that carbohydrate and fat oxidation PRE and POST are different.



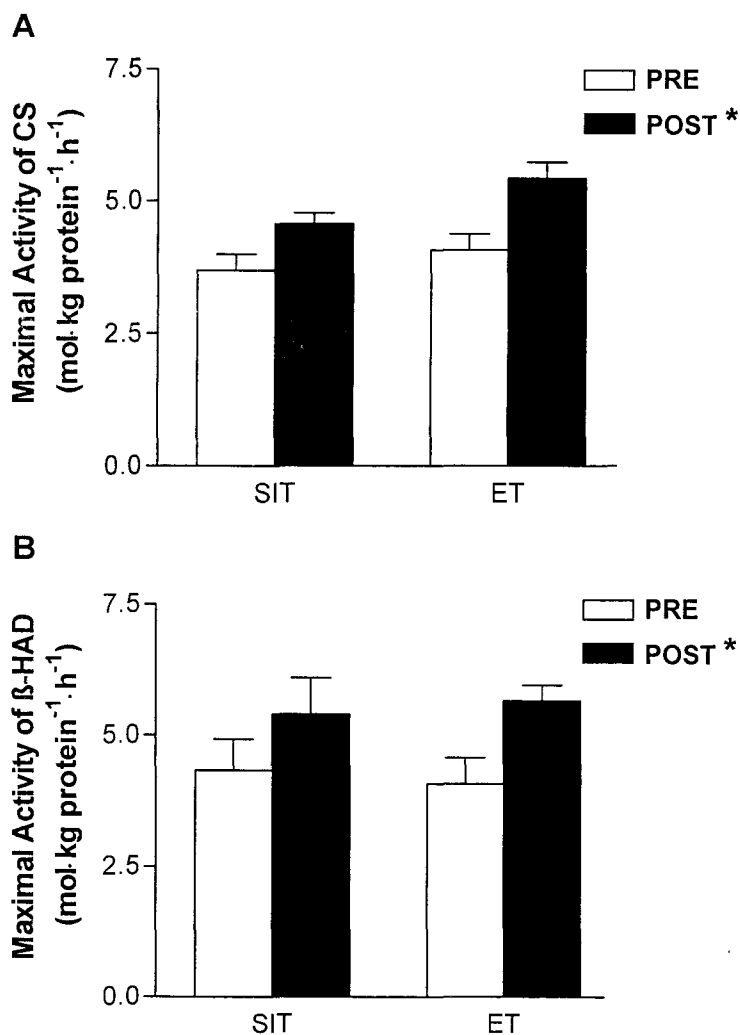
5.3.2 Skeletal muscle mitochondrial enzymes and PGC1- α

The maximal activities of CS and HAD and the total protein content of PDH were increased after training, but there were no differences between groups (main effect for condition, $P < 0.05$; Figure 5.2). Similarly, PGC-1 α protein content increased after training but there was no difference between groups (main effect for condition, $P < 0.05$; Figure 5.3).

5.3.3 Skeletal muscle metabolites

Muscle glycogen content was higher at 60 minutes of exercise post-training compared to pre-training, with no difference between groups (condition x time interaction, $P < 0.05$; Figure 5.4). Net muscle glycogenolysis during exercise was also reduced after SIT (POST: 166 ± 20 versus PRE: 283 ± 28 mmol.kg⁻¹ dry wt; $P < 0.05$) and ET (POST: 154 ± 25 versus PRE: 226 ± 15 mmol.kg⁻¹ dry wt; $P < 0.05$), with no difference between groups (main effect for condition, $P < 0.05$). Muscle PCr content was higher at 60 minutes of exercise post-training compared to pre-training with no difference between groups (condition x time interaction, $P < 0.05$; Figure 5), whereas creatine showed a reciprocal change and was lower after 60 minutes of exercise post-training compared to pre-training (condition x time interaction, $P < 0.05$; Table 5.5). Muscle ATP was unchanged by acute exercise, however, ATP was reduced after six weeks of SIT compared to ET (group x condition interaction, $P < 0.05$; Table 5.5). Lactate accumulation was reduced after training, however the relative exercise-induced changes were modest and overall there were no significant effects (Table 5.5).

Figure 5.2 Maximal activity or total protein content of mitochondrial enzymes citrate synthase (CS; Panel A), 3-hydroxyacyl CoA dehydrogenase (β -HAD; Panel B) and pyruvate dehydrogenase (PDH; Panel C) measured in biopsy samples obtained before (PRE) and after (POST) six weeks of sprint interval training (SIT) or six weeks of endurance training (ET). Values are means \pm SE (n=10 per group). * Main effect for condition (P<0.05), such that POST > PRE.



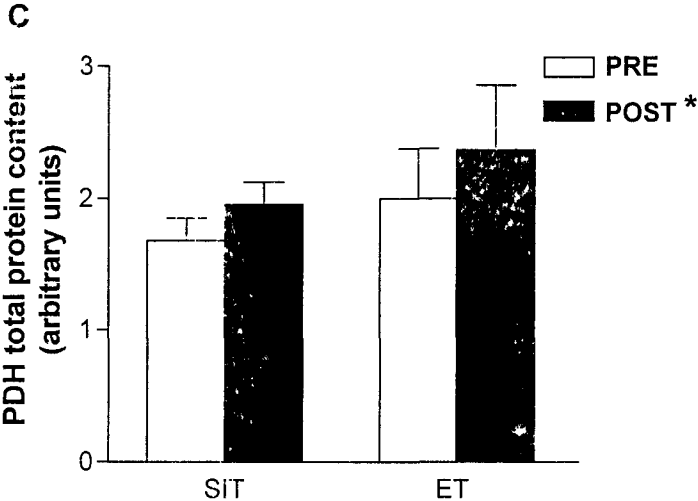


Figure 5.3 Total protein content of PGC-1 α measured in biopsy samples obtained before (PRE) and after (POST) six weeks of sprint interval training (SIT) or six weeks of endurance training (ET). Values are means \pm SE (n=10 per group).

* Main effect for condition (P<0.05), such that POST > PRE.

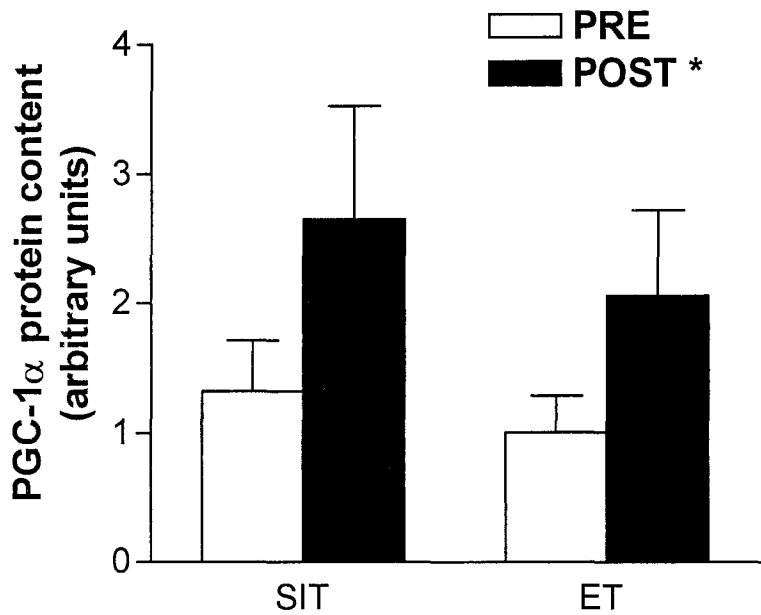


Figure 5.4 Muscle glycogen content measured at rest and during cycling exercise that consisted of 60 minutes at 65% VO_{2peak} before (PRE) and after (POST) six weeks of sprint interval training (SIT) or six weeks of endurance training (ET). Values are means \pm SE (n=10 per group); DW, dry weight. * Main effect for condition ($P<0.05$), such that POST > PRE. † Condition (PRE, POST) x time (0, 60 minutes) interaction ($P<0.05$), such that POST 60 minutes > PRE 60 minutes in both groups.

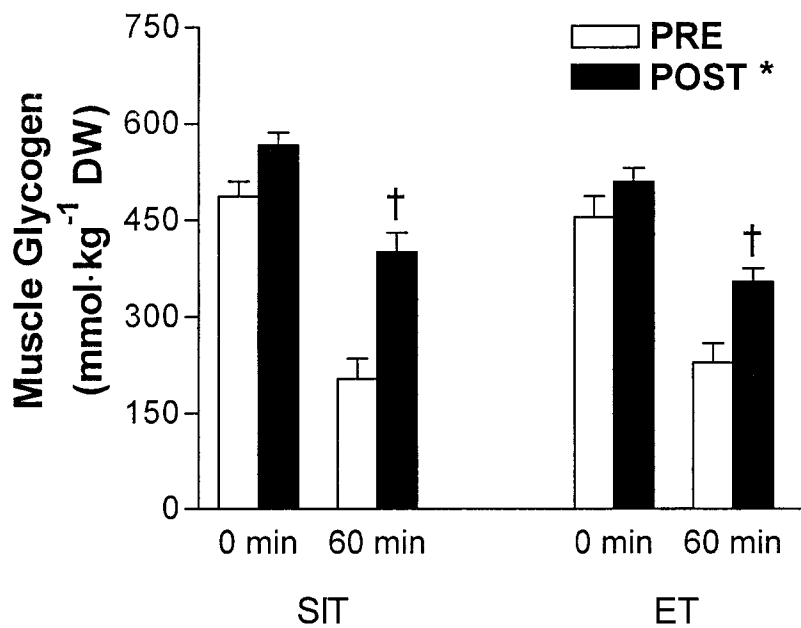


Figure 5.5 Muscle phosphocreatine (PCr) content measured at rest and during cycling exercise that consisted of 60 minutes at 65% VO_{2peak} before (PRE) and after (POST) six weeks of sprint interval training (SIT) or six weeks of endurance training (ET). Values are means \pm SE (n=10 per group); DW, dry weight. * Main effect for condition ($P<0.05$), such that POST > PRE. † Condition (PRE, POST) x time (0, 60 minutes) interaction ($P<0.05$), such that POST 60 minutes > PRE 60 minutes in both groups.

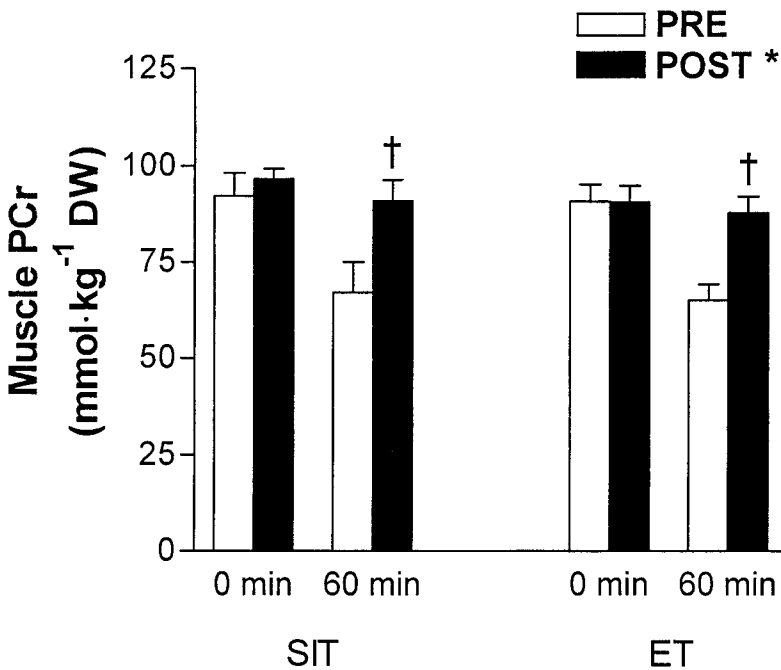


Table 5.5 Muscle metabolites at rest and following 60 minutes of exercise at 65% $\text{VO}_{2\text{peak}}$ before and after six weeks of sprint interval training (SIT) or six weeks of endurance training (ET).

		SIT Group		ET Group	
		Rest	60 min	Rest	60 min
Cr	PRE	54±6	79±8	51±4	77±4
	POST	49±5	55±5†	64±6	54±4†
ATP	PRE	22±1	24±1	22±1	22±1
	POST	19±1#	20±1#	23±1	25±1
Lactate	PRE	13±2	36±9	20±2	37±4
	POST	15±2	30±5	20±2	27±3

Values are means±SE (n=10 per group). Metabolite data expressed in $\text{mmol}\cdot\text{kg}^{-1}$ dry wt. Cr, Creatine; † Condition x time interaction ($P<0.05$) for Creatine, such that POST 60 minutes > PRE 60 minutes in both groups. # Group x Condition interaction ($P<0.05$) for ATP, such that POST < PRE in SIT versus ET.

5.4 DISCUSSION

The major novel finding from the present study was that six weeks of SIT elicited adaptations in selected markers of skeletal muscle CHO and lipid metabolism and metabolic control during exercise that were comparable to ET, despite a much lower training volume and time commitment. By design, weekly training volume was ~90% lower in the SIT group (~225 versus ~2250 kJ.wk⁻¹ for ET) which necessitated a training time commitment that was only ~ one third of the ET group (~1.5 versus 4.5 hours). Most of the training time in the SIT group was spent in recovery between short, intense bursts of all out cycling and actual weekly exercise time was only ~10 minutes, as compared to ~4.5 hours of continuous moderate-intensity cycling in the ET group. The present results confirm and extend the findings from a recent study from our laboratory (Gibala *et al.* 2006) that showed similar increases in muscle oxidative capacity and volitional exercise performance after six sessions of SIT or ET over two weeks. To our knowledge, the present study is the first to directly compare SIT versus ET in a standardized manner with respect to changes in skeletal muscle metabolism during matched-work exercise.

5.4.1 Skeletal muscle oxidative capacity

Similar to our previous investigation that assessed changes after two weeks of training (Gibala *et al.* 2006), in the present study we found similar increases in muscle oxidative capacity after six weeks of SIT and ET despite large differences in training volume. Interestingly, the relative increase in citrate synthase maximal

activity after six weeks in the present study (~25% in both groups) was similar to that observed for cytochrome oxidase (COX) after two weeks in our previous study (Gibala *et al.* 2006). Collectively, these findings suggest that much of the increase in mitochondrial content occurs relatively “early” in the training process, which is supported by another recent study that showed increased COX protein content after one week (3 sessions) of SIT, yet no further increase after six weeks of training (Burgomaster *et al.* 2007). Data from animal investigations also illustrates the potential for brief bouts of intense interval exercise to elicit rapid changes in muscle oxidative capacity. For example, Terada *et al.* (2001) showed that eight days of high-intensity, intermittent swim training (lasting less than 5 minutes per day) increased citrate synthase maximal activity in rat skeletal muscle to a level similar to that induced by six hours of daily low-intensity training.

In terms of comparing SIT versus ET, a novel aspect of the present study was the measurement of other mitochondrial enzymes that are commonly used to reflect the maximal capacity for skeletal muscle CHO (PDH) and lipid oxidation (HAD). While previously speculated by others (Harmer *et al.* 2000), this is the first study to show that SIT increases the total protein content of PDH (E1 α subunit) in human skeletal muscle, the magnitude of which was similar to that previously reported for ET (LeBlanc *et al.* 2004). The training-induced increase in HAD maximal activity in the present study is in contrast to a previous report from our laboratory (Burgomaster *et al.* 2006) that showed no change after two weeks

of SIT. These data suggest that a minimum volume of intense, interval-based exercise training may be necessary in order to elicit changes in the maximal capacity for lipid oxidation in skeletal muscle. Consistent with this interpretation, Talanian and coworkers (2007) recently reported that seven sessions of high-intensity interval training over two weeks increased the maximal activity of HAD and protein content of FABPpm in human skeletal muscle. Each training session in that study (Talanian *et al.* 2007) consisted of ten 4-minute bouts of cycling at 90% of $\dot{V}O_{2peak}$ with 2 minutes of rest between intervals. The total training time commitment (~5 hours) and exercise volume (~3000 kJ) over the two-week training period was thus substantially higher than in our recent short-term studies that have employed Wingate-based exercise training (Burgomaster *et al.* 2005; Burgomaster *et al.* 2006; Burgomaster *et al.* 2007).

5.4.2 Skeletal muscle metabolism during exercise

Training-induced shifts in substrate utilization during exercise have been well-documented after several weeks of ET. In contrast to traditional ET, very few studies have examined changes in substrate utilization in working human skeletal muscle after SIT (Nevill *et al.* 1989; Harmer *et al.* 2000; Barnett *et al.* 2004; Burgomaster *et al.* 2006). Furthermore, the few studies that have examined sprint training-induced changes in skeletal muscle metabolism during exercise have incorporated brief “supramaximal” exercise challenges in which the power output differs markedly between the pre and post-training trials (Nevill *et al.* 1989; Parra *et al.* 2000; Barnett *et al.* 2004). We therefore compared changes in

skeletal muscle metabolism during a prolonged bout of fixed-load exercise (1 hour of cycling at 65% of pre-training $\text{VO}_{2\text{peak}}$) before and after six weeks of either SIT or ET. Consistent with our hypothesis, we observed similar reductions in net muscle glycogenolysis and PCr degradation during matched-work exercise after both forms of training, despite a marked decrease in training volume in the SIT group. Although reductions in glycogen utilization after several weeks of ET have been well-established, the effect of interval training on muscle glycogenolysis is equivocal with most (Harmer *et al.* 2000; Parra *et al.* 2000; Burgomaster *et al.* 2006), but not all studies (Nevill *et al.* 1989; Barnett *et al.* 2004) showing a decrease in glycogen utilization during exercise. Similarly, reductions in PCr utilization during exercise have been reported after some (Parra *et al.* 2000), but not all (Nevill *et al.* 1989; Stathis *et al.* 1994; Harmer *et al.* 2000; Rodas *et al.* 2000; Barnett *et al.*, 2004) sprint training studies.

A final observation with respect to skeletal muscle metabolic changes is that we observed a reduction in ATP content at rest and during exercise following SIT but not ET. This finding is consistent with a previous study from our laboratory that investigated metabolic adaptations after two weeks of SIT (Burgomaster *et al.* 2006) and work by Stathis and colleagues (1994) who reported a reduction in resting muscle ATP content after seven weeks of SIT. During strenuous exercise, AMP produced from ATP hydrolysis can be deaminated by AMP deaminase, resulting in the formation of IMP and ammonia, and subsequent breakdown of IMP to inosine and hypoxanthine resulting in a

loss of adenine nucleotides from the muscle (Hellsten *et al.* 1998). Replacement of purine nucleotides lost from the muscle is a relatively slow, energy-consuming process and appears to continue for several days after intense exercise (Hellsten *et al.* 1998). Thus the lower ATP content measured after training in the present study may have been due to the stress of chronic training or the acute residual effects of the final training bout, which was performed approximately 72-96 hours before tissue extraction.

5.4.3 Potential mechanisms involved in skeletal muscle remodeling after SIT

While the present study demonstrates the potency of SIT to elicit changes in muscle oxidative capacity and selected metabolic adjustments during exercise that resemble ET, the underlying mechanisms are unclear. From a cell signalling perspective, exercise is typically classified as either “strength” or “endurance”, with short-duration, high-intensity work usually associated with increased skeletal muscle mass, and prolonged, low- to moderate-intensity work associated with increased mitochondrial mass and oxidative enzyme activity (Baar 2006). Given the oxidative phenotype that is rapidly upregulated by SIT, it is possible that metabolic adaptations to this type of exercise could be mediated in part through signalling pathways normally associated with traditional ET. A key regulator of oxidative enzyme expression in a number of cell types, including skeletal muscle, is PGC-1 α (Koulmann & Bigard 2006). PGC-1 α coordinates mitochondrial biogenesis by interacting with various nuclear genes encoding for mitochondrial

proteins. Acute exercise induces transient transcriptional activation of the PGC-1 α gene in human skeletal muscle (Pilegaard *et al.* 2003) but to our knowledge only one previous human study has examined the effect of prolonged exercise on PGC-1 α protein content. Russell *et al.* (2003) reported that endurance training for six weeks (three days per week) increased PGC-1 α protein content in Type I, IIa and IIx fibers, which is consistent with data obtained in rats (Taylor *et al.* 2005). The present study is the first to show that low-volume SIT increases PGC-1 α protein content similar to high-volume ET in human skeletal muscle. The potency of interval-based training in this regard is supported by the work of Terada *et al.* (2005), who showed increased skeletal muscle PGC-1 α protein content after a single bout of high-intensity intermittent swim exercise in rats. While the precise molecular events are unclear, increases in the AMP-activated protein kinase (AMPK), mitogen-activated protein kinase (MAPK) and calcium-signaling mechanisms appear to be important in the regulation of PGC-1 α expression and activity (Koulmann & Bigard 2006). Consistent with this interpretation, a preliminary report by our group showed activation of AMPK and p38 MAPK in response to an acute session of Wingate-based exercise training (Gibala *et al.* 2006).

In summary, the results of the present study demonstrate that low-volume SIT is a time-efficient strategy to induce changes in selected markers of whole-body and skeletal muscle CHO and lipid metabolism during exercise that are comparable to traditional high-volume ET. Skeletal muscle remodelling after SIT

may be mediated in part through signalling pathways normally associated with traditional ET, but additional research is warranted to clarify the molecular mechanisms responsible for metabolic adaptations induced by these different acute exercise 'impulses'. It is also important to stress that the relatively limited array of metabolic measurements performed in the present study may not be representative of other physiological adaptations normally associated with ET. For example, SIT may differ from ET with respect to changes induced in the cardiovascular and respiratory systems, metabolic control in other organs (liver, adipose tissue, etc.) and protection from various factors associated with chronic inactivity (insulin resistance, lipid dysregulation, etc).

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CHAPTER 6

GENERAL CONCLUSIONS

6.1 INTRODUCTION

Classic training studies have demonstrated that several weeks of SIT elicits marked changes in resting human skeletal muscle, including an increase in muscle oxidative capacity (Saltin *et al.* 1976; Henriksson & Reitman 1976; Jacobs *et al.* 1987). Thus, the potential for SIT to induce adaptations in skeletal muscle has been known for over three decades. However, in contrast to data regarding adaptations in resting muscle, much less is known about the early time-course for adaptations (*i.e.*, over the first one to two weeks) or the effect of SIT on skeletal muscle metabolism during an acute bout of exercise. The few investigations that have examined metabolic adaptations during exercise have incorporated brief “all-out” challenges to exhaustion in which the power output differs between the pre- and post-training trials (Barnett *et al.* 2004; Linossier *et al.* 1993; Nevill *et al.* 1989; Stathis *et al.* 1994). Furthermore, despite earlier work that demonstrated increased muscle oxidative capacity after SIT, no study has examined sprint training-induced changes in the ability to perform exercise primarily “aerobic” in nature, aside from tests of maximal oxygen uptake. The studies outlined in the proceeding chapters add novel insight regarding skeletal muscle metabolic and performance adaptations to SIT, and represent the first work to examine the potential for low-volume SIT to elicit alterations in human

skeletal muscle that are comparable to high-volume ET.

The first two studies (Chapter 2 and 3) examined the effect of six sessions of SIT performed over fourteen days on aerobic exercise capacity and skeletal muscle metabolism at rest (Chapter 2) and during exercise (Chapter 3). The results of these studies demonstrated that ~15 minutes of very intense exercise increased skeletal muscle oxidative capacity and resulted in a closer matching between glycogenolytic flux and pyruvate oxidation during submaximal exercise. This novel work emphasized the potency of SIT to elicit rapid adaptations in skeletal muscle metabolism and endurance capacity and suggested that brief bouts of intense exercise may be just as effective as continuous training for improving the metabolic profile of skeletal muscle.

The third study (Chapter 4) examined the early time-course for changes in transport proteins associated with glucose, lactate, and fatty acid metabolism in human skeletal muscle in response to SIT. The results of this work demonstrated the potency of SIT to elicit rapid increases in metabolite transport proteins associated with carbohydrate (CHO) metabolism, as demonstrated by an increase in GLUT4, COX4 and MCT4 after only three sessions of SIT. In contrast, six weeks of SIT had no effect on fatty acid transport which suggests that proteins associated with fatty acid metabolism may adapt more slowly than proteins of CHO metabolism.

The final study (Chapter 5) directly compared adaptations in skeletal muscle CHO and lipid metabolism, and metabolic control during exercise after six

weeks of low-volume SIT or six weeks of high-volume ET. Despite marked differences in training volume both protocols induced similar increases in mitochondrial markers for skeletal muscle CHO and lipid metabolism, and reduced glycogen and PCr utilization during exercise. These findings demonstrate that the total volume necessary to stimulate skeletal muscle metabolic adaptations is substantially lower than previously suggested.

The present chapter collectively summarizes the important findings from these studies and provides novel insight regarding performance adaptations and the regulation of skeletal muscle energy provision after SIT. In addition, potential signalling mechanisms involved in the adaptation to SIT are highlighted. Finally, the implications and limitations of this work are discussed with recommendations for future research.

6.2 NEW INSIGHTS REGARDING PERFORMANCE IMPROVEMENTS AND THE REGULATION OF SKELETAL MUSCLE ENERGY PROVISION AFTER SIT

One of the most striking findings from this thesis work was that six sessions of SIT, amounting to only ~15 minutes of total exercise over two weeks, markedly improved exercise performance during tasks that rely mainly on aerobic energy provision. In Study 1 (Chapter 2) cycle endurance time to fatigue at ~80% of pre-training VO_{2peak} increased by 100% after only six sessions of SIT. Subsequent to this, Study 2 (Chapter 3) revealed marked improvements in 250 kJ cycling time-trial performance thus demonstrating that short-term SIT improves performance

during aerobic-based tasks in which athletes typically compete. Our work also demonstrated progressive increases in 250 kJ time-trial performance over six weeks of SIT, and these improvements remained elevated above baseline values following six weeks of detraining (Study 3; Chapter 4). The validity of these findings are bolstered by the fact that all subjects performed extensive familiarization trials in order to eliminate any potential “learning effect”, and two separate control groups showed no change in time to fatigue during cycling at 80% of VO_{2peak} and 250 kJ time-trial performance when tested two weeks apart with no training intervention.

In addition to rapid improvements in cycle endurance capacity, our findings demonstrated that brief repeated bouts of very intense exercise rapidly increased skeletal muscle oxidative potential. We have consistently found an increased muscle oxidative capacity ranging from 10 to 38% in select markers of mitochondrial potential after two to six weeks of SIT, including the maximal activity or protein content of citrate synthase, PDH, and COX4. Interestingly however, increases in the maximal activity of HAD were not observed until after six weeks of SIT (Study 4; Chapter 5) suggesting that changes in the capacity for skeletal muscle lipid oxidation may accrue more slowly. In fact, six weeks of SIT had no effect on the muscle content of fatty acid translocase (FAT/CD36) or the plasma membrane associated fatty acid binding protein (FABPpm) (Study 3; Chapter 4). Contrary to these findings, Talanian and colleagues (2007) recently demonstrated that two weeks of high-intensity interval training increased the

maximal activity of HAD and the muscle protein content of FABPpm in young women. However, in that study each training session consisted of ten four-minute bouts of cycling at 90% of VO_{2peak} , and thus the total training volume (~3000 kJ) and exercise time commitment (~280 minutes) was substantially higher than in our short-term studies (~600 kJ and ~15 minutes, respectively).

Although it has been suggested that interval training has little effect on muscle oxidative capacity, and endurance performance (Gleeson 2000; Kubukeli *et al.* 2002), numerous studies have shown that brief, repeated sessions of high-intensity exercise elicit changes in skeletal muscle that resemble traditional ET (Henriksson & Reitman 1976; Saltin *et al.* 1976). In the studies that have compared metabolic adaptations to these two forms of training the total volume of work performed was similar in the SIT and ET groups (Henriksson & Reitman 1976; Saltin *et al.*, 1976; Eddy *et al.*, 1977; Fournier *et al.*, 1982; Gorostiaga *et al.*, 1991; Edge *et al.*, 2006). Our work demonstrates that the total volume of exercise necessary to induce changes in skeletal muscle oxidative capacity and endurance performance is substantially lower than previously suggested. The results from Study 4 (Chapter 5) are unique because, by design, weekly time commitment (~1.5 versus ~4.5 hours) and total training volume (~225 versus ~2250 kJ.wk⁻¹) was substantially lower in the SIT versus ET group. Despite marked differences in training volume, both protocols induced similar increases in mitochondrial capacity and reductions in glycogen and PCr utilization during exercise. Given the large difference in training volume, these data demonstrate

that SIT is a time-efficient strategy to induce adaptations in skeletal muscle and exercise performance that are comparable to ET. The results of this work confirm and extend the findings of our previous work that showed similar improvements in the maximal activity of COX and the protein content of COX subunits II and IV after two weeks of low-volume SIT or two weeks of high-volume ET (Gibala *et al.* 2006b). Together, this work raises fundamental questions regarding the nature of the contractile “impulse” necessary to induce skeletal muscle remodelling (i.e., through activation of various signalling cascades).

6.3 POTENTIAL SIGNALLING MECHANISMS INVOLVED IN SKELETAL MUSCLE

Although it is known that high-intensity exercise activates signalling cascades in skeletal muscle, relatively little is known regarding the intracellular events that mediate skeletal muscle remodelling in response to SIT. Contraction-induced disturbances in cellular homeostasis rapidly activate signalling cascades in an attempt to maintain skeletal muscle ATP concentration during exercise. Several putative signals, including changes in intracellular calcium concentrations and alterations in the energetic (i.e., ATP/ADP ratio) and redox (i.e., NAD/NADH ratio) state of the cell activate kinases involved in signal transduction (Hawley *et al.* 2006). For example, exercise has been shown to activate AMP-activated protein kinase (AMPK), mitogen-activated protein kinase (MAPK) and calcium/calmodulin-dependent protein kinase (CaMK), which promote the transcription of the peroxisome proliferator-activated receptor γ co-

activator 1α (PGC- 1α), a critical regulator of genes controlling oxidative phosphorylation. Given our findings of a rapid increase in oxidative capacity after brief bouts of intense exercise, it is possible that the metabolic adaptations to this type of exercise could be mediated in part through such signalling pathways. Consistent with this interpretation, the results of a preliminary report (Gibala *et al.* 2006a) demonstrated rapid activation of AMPK and p38 MAPK in response to an acute session of Wingate-based exercise training. Interestingly, the magnitude of the exercise-induced response in signal transduction appears to be intensity-dependent, with greater activation observed during very high-intensity exercise (e.g., ~100% of VO_{2peak}) compared to moderate-intensity exercise (e.g., ~70% of VO_{2peak}) (Hawley *et al.* 2006). Together, these results suggest that high-intensity training elicits marked signalling events in human skeletal muscle, and the activation of AMPK and p38 MAPK may be obligatory in the promotion of mitochondrial biogenesis after SIT. Additional research is warranted to clarify the effect of different acute exercise 'impulses' on molecular signalling events in human skeletal muscle and the precise time course and mechanisms responsible for adaptations induced by SIT.

6.4 IMPLICATIONS

Physical inactivity represents one of the major threats to public health in Canada and places an annual economic burden of 5.7 billion dollars on the health care system (Health Canada). A sedentary lifestyle is a significant risk

factor for cardiovascular disease (CVD) which is the leading cause of premature death among Canadian men and women. Health Canada recommends that every adult should perform 30 to 60 minutes of physical activity each day in order to improve health; however, few Canadians follow this guideline and over 50% of the population is sedentary and at higher risk for inactivity-related disorders including CVD, type 2 diabetes, hypertension and certain types of cancers. One of the most commonly cited barriers to physical activity is lack of time (Godin *et al.* 1994) and thus there is an urgent need to develop evidence-based physical activity programs that yield health benefits with minimal time commitments.

The results of my thesis work suggest that brief bouts of intense exercise induce changes in the metabolic profile of skeletal muscle that are similar to repeated sessions of continuous training. For example, our studies demonstrated that SIT increased the protein content of GLUT4 and glycogen concentration, thereby outlining important ramifications for persons with diabetes. Furthermore, our work revealed rapid sprint training-induced increases in skeletal muscle oxidative capacity and thus populations with "compromised" skeletal muscle function (e.g. the elderly, persons with a spinal cord injury) who exhibit skeletal muscle disuse-atrophy and a corresponding loss of muscle strength and oxidative capacity may benefit from a period of interval training. In fact, there is growing appreciation of the potential for SIT to stimulate improvements in health and fitness in a range of populations, including persons with various disease conditions. For example, interval-based training has been shown to improve

cardiorespiratory fitness, peak work capacity, and the morphological and biochemical characteristics of skeletal muscle in a wide range of populations, such as those with advanced chronic obstructive pulmonary disease (Vogiatzis *et al.* 2005) and coronary artery disease (Rognmo *et al.* 2004; Warburton *et al.* 2005).

6.5 LIMITATIONS AND FUTURE WORK

Although our findings suggest that low-volume interval-training elicits skeletal muscle metabolic adaptations normally associated with traditional high-volume ET, these studies did not examine the potential for SIT to induce similar adaptations in other major physiological systems (e.g., the cardiovascular and/or respiratory system). Additional research is required to examine whether SIT induces other physiological adaptations that are similar to ET, including changes in cardiovascular regulation and health-related measures such as glucose tolerance and insulin sensitivity. Furthermore, additional studies are necessary to establish the optimal frequency, intensity and duration of SIT to induce physiological adaptations normally associated with ET. Our previous studies are limited in that the duration of training has been relatively short (i.e., two to six weeks), and whether the adaptations are similar after many months of low-volume interval and high-volume continuous training remains to be determined. It is possible that the time-course for physiological adjustments over several months differs between training protocols. It can be argued that the very intense

nature of SIT might stimulate rapid changes in skeletal muscle, whereas the adaptations to low-intensity ET may accrue more slowly.

Given the extremely high intensity at which sprint exercise bouts are performed, interval-based training programs are less widely adopted by the general public. High-intensity SIT requires an extremely high level of dedication and motivation, and thus a Wingate-based model may not be a practical method of training for the general population. In contrast to continuous endurance exercise, high-intensity interval training is often performed on a specialized ergometer equipped with an online training program that is both expensive and laborious. More recently, studies have begun to examine physiological adaptations to 'modified' interval training on standard stationary bicycles in which the intensity of exercise is slightly lower than that elicited during a typical Wingate Test (Talanian *et al.* 2007). Future studies are required to examine 'modified' interval-based approaches to identify the optimal combination of training intensity and volume necessary to induce such adaptations in a practical and time-efficient manner.

Given that lack of time is such a common barrier to exercise participation, interval training may represent a time-efficient strategy for improving skeletal muscle health and exercise capacity in a wide range of populations. Our studies examined alterations in skeletal muscle metabolism in response to extremely intense training in adults of average fitness, thus the findings of this work are limited to young healthy persons. Despite recent evidence that suggests that

interval training can stimulate improvements in health and fitness in persons with various disease conditions, a widespread concern regarding the safety of intense training exists particularly in regards to special populations. Additional research is required to determine the training frequency, intensity, and duration of exercise that can be performed as well as the recovery between bouts in a safe and practical manner. Moreover, studies should include functional outcome measures (e.g., the six-minute walk, tests of muscle strength and fatigueability, etc.) in order to determine the efficacy of interval training on activities of daily living in people with various disease conditions.

6.6 SUMMARY

The results from these studies demonstrated that SIT rapidly increased muscle oxidative potential and transport proteins associated with CHO metabolism, and induced metabolic adaptations during exercise that were comparable to traditional ET. This work adds novel insights to the growing body of evidence that shows the potency of SIT to elicit adaptations in human skeletal muscle, despite a marked reduction in training volume and time commitment. Additional research is warranted to resolve the precise nature and magnitude of adaptations that can be elicited and maintained over the long term in various populations, the intracellular events that mediate skeletal muscle remodelling in response to SIT, and the effectiveness of modified interval-training strategies on skeletal muscle metabolism and exercise performance in humans.

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