HIGH-INTENSITY INTERVAL TRAINING AND MUSCLE OXIDATIVE CAPACITY

THE EFFECT OF HIGH-INTENSITY INTERVAL TRAINING ON SKELETAL MUSCLE OXIDATIVE CAPACITY IN MIDDLE-AGED SEDENTARY ADULTS

By

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ABSTRACT

There is growing appreciation of the potential for high intensity interval training (HIT) to rapidly stimulate metabolic adaptations that resemble traditional endurance training, despite a low total exercise volume. However, much of the work has been conducted on young active individuals and the results may not be applicable to older, less active populations. In addition, many studies have employed "all out", variable-load exercise interventions (e.g., repeated Wingate Tests) that may not be safe, practical or well tolerated by certain individuals. We determined the effect of a short program of low-volume, submaximal, constant-load HIT on skeletal muscle oxidative capacity and insulin sensitivity in sedentary middle-aged individuals who may be at higher risk for inactivity-related chronic diseases. Sedentary but otherwise healthy men (n=3) and women (n=4) with a mean (±SE) age, body mass index and peak oxygen uptake (VO_{2peak}) of 45±2 yr, 27±2 $kg \cdot m^{-2}$ and 30±1 $ml \cdot kg^{-1} \cdot min^{-1}$ were recruited. Subjects performed 6 training sessions over 2 wk, each consisting of 10 x 1 min cycling at 60% of peak power elicited during a ramp VO_2peak test (<90% of heart rate reserve) with 1 min recovery between intervals. Needle biopsy samples (v. lateralis) were obtained before training and <72 h after the final training session. Muscle oxidative capacity, as reflected by the maximal activity and protein content of citrate synthase, increased by ~20% after training, which is similar to changes previously reported after 2 wk of Wingate-based HIT in young active subjects. Insulin sensitivity, based on fasting glucose and insulin, improved by ~35% after training. These data support the notion that low-volume HIT may be a practical, time-efficient strategy to induce metabolic adaptations that reduce the risk for inactivity-related disorders in previously sedentary adults.

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Finally, I dedicate this thesis to my mother, my role model. Thank you for all you have done, for all you do and for the love you continue to give despite the challenges you are facing. You are truly an inspiration. I love you. ♥

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REVIEW OF LITERATURE

1.1. Introduction

The role of skeletal muscle in the maintenance of metabolic and overall health is multifaceted. Comprising approximately 30-40% of body mass (Janssen et al., 2000), skeletal muscle is sensitive to metabolic disruptions and consequently adapts (Coffey and Hawley, 2007). The intracellular adaptations to exercise training were initially investigated in rodents (Baar et al., 2002, Baldwin et al., 1972, 1973, Dudley et al., 1982, Holloszy, 1967) and subsequently in humans (Gollnick et al., 1973, Henriksson and Reitman, 1976, Holloszy and Coyle, 1984, Pilegaard et al., 2000, Saltin et al., 1976, Spina et al., 1996). Currently, aerobic exercise training is considered an effective method to elicit mitochondrial adaptations and to improve oxidative capacity within skeletal muscle (Coffey and Hawley, 2007, Hood, 2001, Menshikova et al., 2006, Pilegaard et al., 2003, Short et al., 2003, Spina et al., 1996, Taylor et al., 2005). The practical relevance of this scientific research is that exercise training may reverse the detrimental metabolic effects of a sedentary lifestyle on exercise capacity, vascular function, blood pressure and insulin sensitivity (Biolo et al., 2005, Hamburg et al., 2007). Despite the evidence for exercise prescription in reducing the risk of chronic diseases (Biolo et al., 2005, Blair & Brodney, 1999, Dowse et al., 1991, Hamburg et al., 2007, Warburton et al., 2006b, Wei et al., 2000), the minimal effective dose of exercise required for metabolic health has yet to be established. The purpose of this literature review is fourfold: i) to examine the role

of skeletal muscle in health and disease; ii) to explore the metabolic consequences of physical inactivity; iii) to discuss the physiological adaptations to traditional endurance exercise training; iv) and to consider high-intensity interval training (HIT) as an alternative, time-efficient strategy to improve musculoskeletal health and fitness.

1.2. Role of skeletal muscle in health and disease

Skeletal muscle and exercise are important factors in the development and preservation of oxidative potential (Coffey and Hawley, 2007, Holloszy and Coyle, 1984, Ross and Leveritt, 2001). Oxidative capacity is defined as the muscle's ability to utilize oxygen as reflected by the maximal activity of mitochondrial enzymes. The impact of habitual exercise training on metabolic health is evidenced by differences in the maximal activity of oxidative enzymes between athletes and sedentary individuals (Coyle et al., 1984, Daussin et al., 2008a, Gollnick et al., 1972, Weston et al., 1999). Both short (Baar et al., 2002, Burgomaster et al., 2005, Pilegaard et al., 2003, Spina et al., 1996, Talanian et al., 2007) and long-term (Short et al., 2003) exercise training have increased the maximal activity and protein content of skeletal muscle mitochondrial enzymes. Mitochondrial oxidative phosphorylation is responsible for up to 90% of cellular ATP production (Hawley and Lessard, 2007) and perturbations in ATP production may represent impaired oxidative metabolism manifested via diminished mitochondrial enzyme activity. This section will outline the role of skeletal muscle in health and disease by reviewing elementary concepts of mitochondrial metabolism, glucose regulation and by discussing the important link between the two; exercise.

1.2.1. Mitochondrial metabolism

Skeletal muscle oxidative capacity is dependent on the maximal activity and content of mitochondrial enzymes. During recovery from acute exercise, the transcriptional regulation of mitochondrial gene expression increases transiently within skeletal muscle (Russell et al., 2005). The short-term exercise-induced changes in gene expression peak rapidly and last for up to about 24 hours into recovery (Pilegaard et al., 2000, 2003). Acute exercise increases the mRNA content of mitochondrial transcription factor A (TFAM) and peroxisome proliferator-activated receptor α (PPARα) in untrained skeletal muscle (Pilegaard et al., 2003), providing a role for exercise in mitochondrial biogenesis. However in order to stimulate transcription of mitochondrial proteins, successive bouts of metabolically stressful exercise training are required. This is evidenced by greater post-exercise peroxisome proliferator activated receptor coactivator 1α (PGC-1α) transcription and mRNA content in trained versus untrained skeletal muscle (Pilegaard et al., 2003). Endurance training increases mitochondrial density (Zamora et al., 1995) and enzyme activity (Bassett and Howley, 2000, Billat, 2001, Coffey and Hawley, 2007, Holloszy and Coyle, 1984, Phillips et al., 1996), improving oxidative potential within skeletal muscle. The mitochondrial adaptations to habitual exercise can be induced quite rapidly (Spina et al., 1996) however training must be maintained to prevent the inactivity-related decline in enzyme activity (Coyle et al., 1984).

1.2.2. Glycemic regulation

The role of exercise in the regulation of blood glucose disposal is becoming increasingly accepted (Holloszy, 2005, Jessen and Goodyear, 2005, Rose and Richter, 2005, Yokoyama et al., 2008). Skeletal muscle glucose uptake is regulated in part by the translocation of glucose transporter protein 4 (GLUT 4) vesicles to the sarcolemmal membrane (Jessen and Goodyear, 2005). An increase in GLUT4 protein content has been shown after 7 days of exercise training (Houmard et al., 1995). Improved training-induced insulin sensitivity in rodents is associated with increased expression of GLUT4 (Ivy, 2004). Both insulin availability and skeletal muscle contraction initiate pathways leading to GLUT4 translocation (Figure 1; Krook et al., 2004).

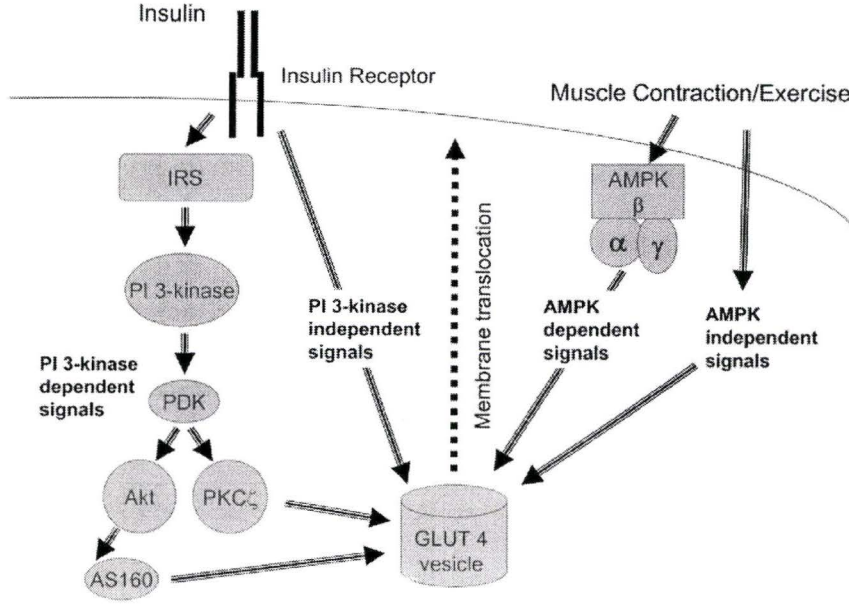

Figure 1. Pathways regulating glucose transport and GLUT4 translocation to the plasma membrane within skeletal muscle. (Krook et al., 2004)

Signalling mechanisms regulating contraction mediated glucose uptake are believed to involve activation of the purported "fuel sensing" enzyme 5' AMP-activated protein kinase (AMPK; Jørgensen et al., 2006, Kurth-Kraczek et al., 1999). Activation of AMPK depends on the energy state of skeletal muscle cells, specifically on decreased phosphocreatine (PCr) levels and on an increase in the AMP to ATP ratio. The activity of AMPK isoforms (α1 and α2) within skeletal muscle has been reported to stimulate glucose uptake independently of fitness levels (Nielsen et al., 2003). The findings of this study are promising for physically and metabolically unfit individuals in that they may acutely regulate blood glucose levels with exercise and muscular contraction.

1.3. Metabolic consequences of physical inactivity

The consequences of sedentary living on skeletal muscle metabolism are well documented (Biolo et al., 2005, Hamburg et al., 2007, Laufs et al., 2005, Zderic and Hamilton, 2006) and may be responsible for the development of modern chronic disease and premature death (Blair and Brodney, 1999, Booth et al., 2000, Katzmarzyk et al., 2003). Metabolic alterations associated with physical inactivity include dysfunctional mitochondria and impaired oxidative capacity, both strongly associated to insulin resistance (Bruce et al., 2003). Skeletal muscle insulin resistance is a common element in the development of cardiovascular disease (Reaven, 1995), type 2 diabetes and obesity (DeFronzo and Ferrannini, 1991). The purpose of this section is to review insulin resistance and mitochondrial dysfunction as detrimental effects of inactivity.

1.3.1. Insulin resistance

Insulin is a regulatory hormone implicated in metabolic processes such as glucose transport, protein synthesis and gene expression within skeletal muscle (Krook et al., 2004). Dysfunctional insulin action or insulin resistance is defined as the irresponsiveness of target tissues to normal circulating insulin concentrations (Goldstein, 2002). It has been suggested that the inactivity-related decline in insulin sensitivity amongst men and women (Short et al., 2003) may be related to oxidative capacity (Bruce et al., 2003). This is based on findings of reduced mitochondrial enzyme activity and protein levels within insulin resistant skeletal muscle (Heilbronn et al., 2007, Patti et al., 2003). The physiological manifestations of insulin resistance on the development of chronic disease have been extensively documented (DeFronzo and Ferrannini, 1991, Hamburg et al., 2007, Heilbronn et al., 2007, Reaven, 1993, 1995, White, 2003) however the pathophysiology of insulin resistance remains to be elucidated.

1.3.2. Mitochondrial dysfunction

The implication of mitochondrial dysfunction in the development of insulin resistance and type 2 diabetes mellitus is gaining support (Kelley et al., 2002, Schrauwen and Hesselink, 2004). Despite several attempts to explain the link between mitochondrial function and insulin sensitivity (Abdul-ghani et al., 2008, Patti et al., 2003, Petersen et al., 2004) no exact mechanism has been established. Patti et al. (Figure 2; 2003) have suggested that the interaction of a combination of factors may reduce gene transcription affecting oxidative metabolism. Perturbations in TCA cycle and electron transport chain

enzyme activity have been reported in mitochondria of type 2 diabetics (Kelley et al., 2002). In conjunction with impaired oxidative capacity, mitochondrial size, thickness and content are reduced in type 2 diabetic (Ritov et al., 2005) and obese (Kelley et al., 2002) individuals. The increase in oxidative enzyme activity after exercise training is also less in insulin resistant adults (Heilbronn et al., 2007). The metabolic consequences of insulin resistance on mitochondrial function have also been reported in the offspring of type 2 diabetics (Petersen et al, 2004), suggesting early manifestations of mitochondrial dysfunction.

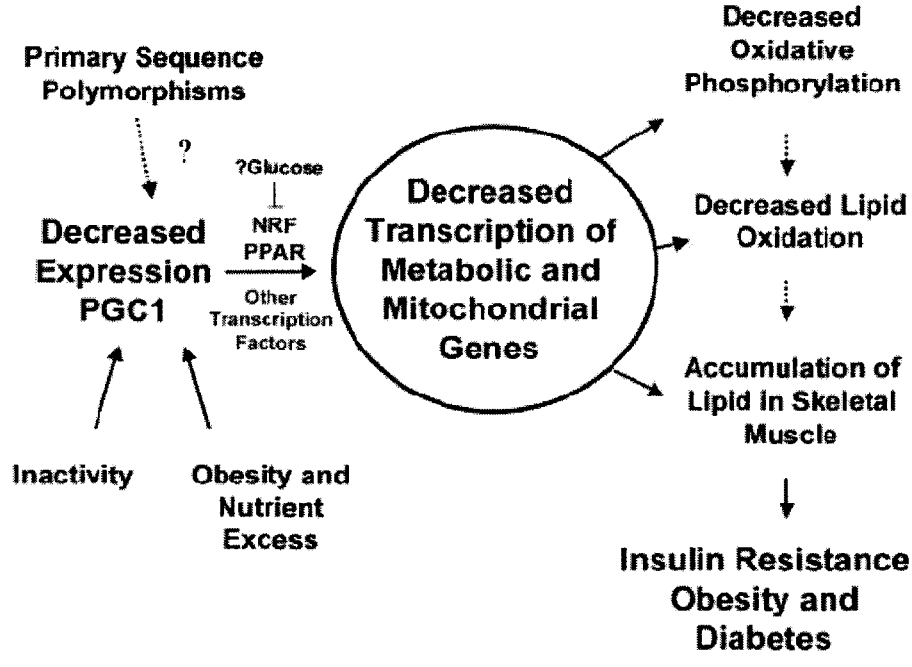

Figure 2. Proposed contribution of *PGC1* and *NRF-1* to expression and metabolic phenotype of insulin resistance and type 2 diabetes. (Patti et al., 2003)

The emergence of new technology such as quantitative reverse transcription-polymerase chain reaction (RT-PCR) has allowed researchers to quantify nuclear and

mitochondrially encoded gene expression in insulin-resistant and healthy individuals. PGC-1α/ β expression and PGC-1α mRNA are lower within insulin resistant skeletal muscle (Heilbronn et al., 2007, Patti et al., 2003) and this regulator or mitochondrial biogenesis may exhibit a reduced and delayed response to moderate exercise training (De Filippis et al., 2008). Obese individuals have been found to have normal PPARα, TFAM, PGC-1α and β content despite altered regulation of mitochondrial transcription factors (Holloway et al., 2008). These findings suggest that alterations in gene transcription and mitochondrial protein expression may appear well before the onset of insulin resistance.

1.4. Metabolic adaptations to exercise training

Exercise is a potent stimulus to prevent or reverse the onset of inactivity-related disorders such as type 2 diabetes (Holloszy, 2005, Laaksonen et al., 2005, Praet and van Loon, 2007, Yokoyama et al., 2004,), hypertension (Baker et al., 2007, Whelton et al., 2002), cardiovascular disease (Després and Lamarche, 1994, Dubach et al., 1997) and obesity (Warburton et al., 2006a, 2006b). Habitual exercise training promotes lean body mass leading to increased mitochondrial density and resulting in the regulation of proteins and enzymes that regulate muscle metabolism (Booth and Thomason, 1991, Hood, 2001). In fact, other than diet, physical activity is potentially the greatest intervention to simultaneously reduce the risk of all chronic diseases (Booth et al., 2000). The relationship between skeletal muscle oxidative capacity and the risk of chronic disease is demonstrated in Figure 3, where improvements in oxidative function with regular exercise reduce the risk of disease. However, discontinuation of training reverses

these adaptations in oxidative enzymes (Chi et al., 1983, Coyle et al., 1984) highlighting the importance of repeated exercise stress to maintain training-induced adaptations within organ systems, tissues and cells (Booth and Thomason, 1991).

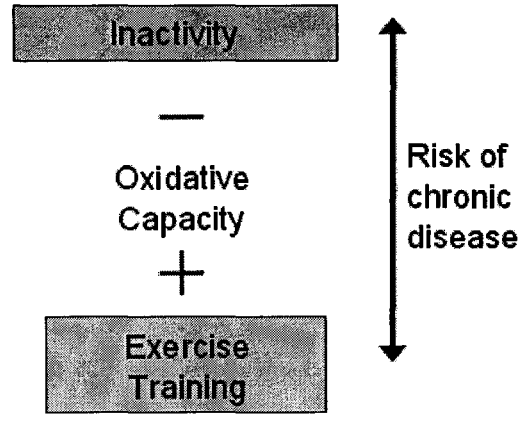

Figure 3. Relationship between oxidative capacity and the risk of chronic disease.

1.4.1. Traditional endurance training

Traditionally, aerobic training protocols have consisted of long bouts of light to moderate intensity exercise conducted several days per week (Table 1). The benefits of endurance training include improvements in fitness, mitochondrial content, oxidative capacity, glucose uptake and insulin sensitivity (Coffey and Hawley, 2007, Hawley, 2004, Holloszy and Coyle, 1984, Saltin et al., 1976, Short et al., 2003, Warburton et al., 2006b). Exercise has also been reported to attenuate the age-related decline in oxidative capacity (Proctor et al., 1995). The American College of Sports Medicine (ACSM) recommends moderate (30 min/d, 5d/wk) or high-intensity (20 min/d, 3d/wk)

cardiovascular exercise in addition to 2 days of strength training per week (ACSM, 2008).

Table 1. Examples of traditional endurance training protocols.

Reference	Intensity	Duration	Frequency
Gollnick et al., 1973	75% VO_{2peak}	1 hr	4 d/wk for 5 months
Saltin et al., 1976	70% VO_{2peak}	1 hr	4-5 d/wk for 4 weeks
Tabata et al., 1996	70% VO_{2peak}	1 hr	5 d/wk for 6 weeks
Phillips et al., 1996	59% VO_{2peak}	2 hr	5-6 d/wk consecutively
Spina et al., 1996	60-70% VO_{2peak}	2 hr	7 or 10 consecutively
Dubouchaud et al., 2000	75% VO_{2peak}	1 hr	6 d/wk for 9 weeks
Short et al., 2003	70-80% VO_{2peak}	20-40 min	3-4 d/wk for 16 weeks

1.4.2. Oxidative capacity

Endurance exercise training was first demonstrated to increase oxidative capacity over forty years ago (Holloszy, 1967). The maximal activity of skeletal muscle mitochondrial enzymes has since been used to quantify exercise-induced adaptations in oxidative capacity (Phillips et al., 1996, Spina et al., 1996, Tarnopolsky et al., 2007). Endurance training has been shown to increase the maximal activities of skeletal muscle citrate synthase (CS; LeBlanc et al., 2004a, LeBlanc et al., 2004b), cytochrome oxidase (COX; Leblanc et al., 2004a, Fernström et al., 2003), succinate dehydrogenase (SDH; Phillips et al., 1996) and β- hydroxyacyl CoA dehydrogenase β-HAD (Burgomaster et al., 2008). Pyruvate dehydrogenase (PDH) and pyruvate dehydrogenase kinase (PDK) have

also been used to quantify oxidative capacity through the entry of carbohydrate-derived acetyl units into the TCA cycle. The protein expression of PDH subunit $-E_1\alpha$ and the maximal activity of PDK2 have been shown to increase with several weeks of endurance training (LeBlanc et al., 2004b). Greater maximal activities of total COX, COX-II and –IV after endurance training (LeBlanc et al., 2004b, Short et al., 2003) reflect an increased efficiency of the ETC to produce ATP. Adaptations in mitochondrial enzyme activity can occur quite rapidly. The maximal activities of CS, β-HAD and carnitine acetyltransferase (CAT) have been documented to increase after only 7-10 days of endurance exercise training (Spina et al., 1996). Consequently to detraining, CS and SDH activities decrease quickly, with a half time of approximately 12 days (Coyle et al., 1984) and SDH returning to baseline within 6 weeks (Henriksson and Reitman, 1976).

1.4.2.1. Fibre type distribution

Endurance training improves VO_{2peak}, mitochondrial density and oxidative capacity (Holloszy and Coyle, 1984, Jones and Carter, 2000, Coffey and Hawley, 2007) corresponding to adaptations within skeletal muscle fibres (Chi et al., 1983, Howald et al., 1985). Continuous endurance training reduces type II (fast-twitch) muscle fibre density while increasing the percentage and cross sectional area of type I (slow-twitch oxidative) muscle fibres (Howald et al., 1985). Despite these adaptations, trained individuals manifest similar mitochondrial enzyme levels between type I and type II fibres (Chi et al., 1983). Oxidative capacity is associated with a greater proportion of type I oxidative muscle fibres (Proctor et al., 1995) evidenced by enhanced type I-SDH activity in trained individuals (Henriksson and Reitman, 1976). Accordingly, the

maximal activities of malate dehydrogenase (MDH), CS and β-HAD are higher in type I compared to type II fibres (Chi et al., 1983). Skeletal muscles rich in type I fibres also show a greater level of PPARδ and PGC-1α compared to type IIa (fast-twitch oxidative) and IIb (fast-twitch glycolytic)-rich muscles (Puigserver and Spiegelman, 2003, Wang et al., 2004). Regrettably, type I muscle fibres are unable to maintain mitochondrial enzyme activity as efficiently as type II fibres with detraining (Chi et al., 1983). However exercise at high intensity increases subsarcolemmal mitochondrial density in all three types of skeletal muscle fibres with the greatest increase occuring in type IIa (Howald et al., 1985). These findings suggest that high-intensity interval training can potentially elicit lasting adaptations within skeletal muscle.

1.4.2.2. Mitochondrial Biogenesis

The generation of mitochondria, referred to as mitochondrial biogenesis, is primarily regulated by PGC-1α (Puigserver and Spiegelman, 2003). Although the exact mechanisms regulating PGC-1α transcription are currently unknown, both short and long-term exercise training have been reported to increase expression of PGC-1α in rodent (Calvo et al., 2008, Taylor et al., 2005) and human (Burgomaster et al., 2008, Pilegaard et al., 2003, Short et al., 2003) skeletal muscle. Following an acute bout of exercise, the transcription of metabolic genes is transiently increased (Pilegaard et al., 2000, 2003). This transcription may occur via nuclear respiratory factors 1 and 2 (NRF-1, 2), activators of genes encoding for mitochondrial enzymes and mitochondrial biogenesis (Baar et al., 2002). Repeated sessions of endurance training may be required for an additive effect of the transient increases in transcription to lead to an accumulation of

mRNA. This theory proposes a mechanism for the up-regulation of metabolic protein expression and stresses the importance of frequent exercise sessions in the maintenance of functional and oxidative capacity.

1.4.3. Insulin sensitivity

Exercise prescription has emerged as an effective therapeutic intervention for the prevention and treatment of insulin resistance (Hawley, 2004). The effects of endurance training on glucose uptake are believed to result from increased mitochondrial (Taylor et al., 2005) and GLUT 4 content (Kristiansen et al., 2000). Both PGC-1α and GLUT4 (Short et al., 2003) mRNA are up-regulated in response to endurance exercise. Emerging reports have also provided evidence for the improvement of glucose transport activity via contraction mediated AMPK-dependent pathways (Holloszy, 2005, Krook et al., 2004).

Aerobic exercise training improves insulin sensitivity in type 2 diabetic (O'Gorman et al., 2006) and in sedentary insulin resistant individuals (Bruce et al., 2006, Houmard et al., 2004, Perseghin et al., 1996, Solomon et al., 2008). It has been shown that as little as a few weeks of low-intensity cycling can improve insulin action in type 2 diabetics (Yokoyama et al., 2004). The sensitivity of human skeletal muscle to insulin can be improved through different exercise programs (Wojtaszewski, 2000, Yokoyama et al., 2004, 2008). It is the repeated metabolic stress from regular physical activity that improves insulin sensitivity, preventing or reversing the insulin-resistant state preceding type 2 diabetes (Colberg, 2006, Hawley, 2004, Solomon et al., 2008).

1.5. High-intensity interval training: an alternate approach

Endurance training has traditionally been considered a safe and effective means of improving oxidative capacity (Baar et al., 2002, Gollnick et al., 1973, Holloszy and Coyle, 1984, Pilegaard et al., 2000, Spina et al., 1996) and is practiced by athletes and recreationally active individuals alike. Despite the metabolic and health benefits of endurance training, its time requirement poses a barrier to exercise for sedentary individuals (Booth et al., 1997). In view of the role of habitual exercise training in the prevention and treatment of chronic disease (Booth et al., 2000, Warburton et al., 2006a, 2006b), an alternative and time-efficient training solution is warranted.

Competitive athletes have recognized high-intensity interval training as a potent performance enhancing strategy for nearly a century (Billat et al., 2001). While is has also long been appreciated that intense intermittent exercise can stimulate oxidative changes in muscle (Jacobs et al., 1987, Linossier et al., 1993, Simoneau et al., 1985, 1987), over the last decade there has been renewed interest the physiological adaptations to HIT (Daussin et al., 2008a, 2008b, Gibala et al., 2006, Gibala and McGee, 2008, Harmer et al., 2008, Rodas et al., 2000, Talanian et al., 2007). Interval training consists of brief bouts of high-intensity activity interspersed by long or short, active or passive recovery periods (Ross and Leveritt, 2001). Compared to endurance training, HIT can improve exercise capacity and other metabolic markers despite a lower volume of work (Burgomaster et al., 2006, 2008, Gibala et al., 2006). The metabolic adaptations to HIT may improve oxidative capacity to a level sufficient to improve insulin sensitivity and to prevent the onset of chronic disease.

1.5.1. High-intensity training programs

The most common HIT protocols used to investigate skeletal muscle metabolism involve repeated Wingate Tests, or repeated 30 second bouts of "all out" exercise interspersed by ~3-4 minutes of recovery (Burgomaster et al., 2005, 2006, 2007, 2008, Gibala et al., 2006, Harmer et al., 2006, 2008, Iaia et al., 2007, McKenna et al., 1993). Considering the difficulty and impracticality of Wingate training, several other HIT programs have been evaluated in terms of performance, cardiovascular and metabolic adaptations (Daussin et al., 2008a, Esfarjani and Laursen, 2007, Helgerud et al., 2001, 2007, Laursen et al., 2002, Praet et al., 2008, Rodas et al., 2000, Talanian et al., 2007). Interval durations have ranged from 5-6 sec (Liniossier et al., 1997, Mohr et al., 2007) to 4 minutes (Helgerud et al., 2001, Talanian et al., 2007) and several intensities, rest periods and work to rest ratios have also been tried, evaluated and criticized (Esfarjani and Laursen, 2007, Helgerud et al., 2001, 2007, Laursen et al., 2002). Irrespective of the disagreement on the optimal training protocol, the consensus is that high-intensity interval training is a potent stimulus with the potential to improve metabolic and overall health.

1.5.2. Metabolic adaptations

1.5.2.1. Oxidative Capacity

High-intensity interval training rapidly induces improvements in oxidative capacity (Daussin et al., 2008a, Gibala et al., 2006, Gibala and McGee, 2008, Harmer et al., 2008, Rodas et al., 2000, Talanian et al., 2007) and is believed to be a time-efficient

training strategy (Coyle, 2005). Citrate synthase, the most commonly cited marker of oxidative metabolism, increases after Wingate-based training (Barnett et al., 2004, Burgomaster et al., 2005, 2006, 2008, Harmer et al., 2008). β-HAD on the other hand, is not responsive to short-term Wingate programs (Burgomaster et al., 2006) and may require long-term training (Burgomaster et al., 2008) or greater exercise volumes (Talanian et al., 2007). The response of mitochondrial enzymes to HIT is equivalent to a much larger volume of endurance training (Burgomaster et al., 2008, Gibala and McGee, 2008). Maximal activities of CS, β-HAD and COX, as well as the protein content of COX II, COX IV and PGC-1α have been shown to increase following both endurance and HIT (Burgomaster et al., 2008, Gibala et al., 2006). These enzymatic adaptations occurred to the same degree despite the ~90% smaller training volume in HIT.

1.5.2.2. Anaerobic metabolism

Glycolytic adaptations to high-intensity interval training (Ross and Leveritt, 2001) are seemingly more robust to detraining compared to oxidative enzymes (Linossier et al., 1997, Simoneau et al., 1987). Several studies have demonstrated HIT-induced increases in the maximal activities of hexokinase (HK; Harmer et al., 2008, Linossier et al., 1997, MacDougall et al., 1998), phosphofructokinase (PFK; Linossier et al., 1997, MacDougall et al., 1998, Rodas et al., 2000, Simoneau et al., 1987), lactate dehydrogenase (LDH; Linossier et al., 1997, Rodas et al., 2000, Ross and Leveritt, 2001, Simoneau et al., 1987) and in the active fraction of PDH (Burgomaster et al., 2006). During repeated bouts of high-intensity exercise, flux through PDHa is an important determinant of ATP production (Putman et al., 1995). However others have reported no training adaptations

to HIT in PFK (Barnett et al., 2004) and PDHa (Harmer et al., 2008) activities. Some glycolytic enzyme adaptations have been observed after HIT but not after continuous training (Harmer et al., 2008).

Resting glycogen content has been reported to be unaffected by training consisting of long interval durations (Talanian et al., 2007). However low-volume work has revealed significant increases in resting intramuscular glycogen (Barnett et al., 2004, Burgomaster et al., 2005, 2006, Gibala et al., 2006, Rodas et al., 2000,). The effects of high-intensity interval exercise on glycogen phosphorylase (PHOS) have been variable (Linossier et al., 1997, MacDougall et al., 1998). As occurs after endurance training (Green et al., 1992), net muscle glycogenolysis decreased during post-training exercise (Burgomaster et al., 2006, 2008, Harmer et al., 2008, Talanian et al., 2007) suggesting a glycogen sparing role for HIT.

1.5.2.3. Muscle Fibres

The literature evaluating the effect of HIT on skeletal muscle fibre type distribution is inconclusive. Wingate-type HIT has been reported to have no effect on the distribution of skeletal muscle fibre types (Barnett et al., 2004, Mohr et al., 2007). However a sprint training protocol including very short intervals (< 10 sec) increased the percentage and area of type II muscle fibres (Dawson et al., 1998). In the previous study, the percentage of type II fibres was highly correlated to short-sprint time and VO_{2max}. Early work demonstrated increases in the maximal activity of SDH in type II fibres with HIT (Henriksson and Reitman, 1976) though the distribution of skeletal muscle fibre types was not investigated. Higher-volume work including continuous and interval

training resulted in an increased proportion and area of type I fibres and area of type IIb fibres, and reduced proportion of type IIb fibres (Simoneau et al., 1985). Despite no training-induced adaptations, IMCL, glycogen and SDH content have been found greater in type I than type IIa and IIb muscle fibres before and after training (Praet et al., 2008). Further research is required to examine the effects of varying intensity and interval duration on the distribution and oxidative capacity of skeletal muscle fibres.

1.5.2.4. Transport Proteins

The skeletal muscle content of GLUT4 and COX4 has been shown to increase with low-volume HIT (Burgomaster et al., 2007). Monocarboxylase transporter 1 and 4 (MCT1, 4) content have also been shown to increase with only 3 sessions of HIT (Burgomaster et al., 2007). These findings suggest an important role for HIT in the regulation of glucose and lactate transport. The influence of HIT on fatty acyl translocase (FAT/CD36) has not been shown to be significant (Burgomaster et al., 2007, Talanian et al., 2007). Our laboratory was unable to report training-induced increases in plasma membrane fatty acid binding protein ($FABP_{pm}$) content however these findings were not consistent across laboratories (Talanian et al., 2007). The longer interval duration in the latter study suggests a possible role for volume in fatty acid transport.

1.5.2.5. Glycemic Regulation

The intensity of exercise has been suggested more important than volume in the regulation of glycemia (Zoeller, 2007). Correspondingly our laboratory has provided evidence for increased resting muscle glycogen and GLUT4 content following high-intensity interval training (Burgomaster et al., 2007, Gibala et al., 2006). It has been said

that as glycogen levels decrease during exercise, AMPKα2 activity and GLUT4 mRNA expression increase (Steinberg et al., 2006). Taken together, these data suggest an important interaction between glycogen content, AMPK, GLUT4 and glucose disposal.

Considering the importance of glucose regulation on determinants of insulin sensitivity (Holloszy, 2005), the combination of exercise-induced upregulation of GLUT4 (Burgomaster et al., 2007) and oxidative enzymes (Daussin et al., 2008b, Gibala et al., 2006, Harmer et al., 2008, Talanian et al., 2007) suggest that HIT may improve insulin sensitivity to a greater degree than traditional endurance training. In fact, a recent study by Trapp et al. (2008) revealed a greater reduction in body fat percentage, central abdominal fat and insulin sensitivity with high-intensity interval compared to endurance training. The improvements in muscle metabolism resulting from high-intensity physical activity may provide a primary means of reducing the burden of insulin resistance (Holloszy, 2005, Houmard et al., 2004).

1.5.3 Performance

The performance improvements elicited by HIT require a relatively low volume of work (Barnett et al., 2004, Burgomaster et al., 2005, 2006, 2007, 2008, Gibala et al., 2006, Laursen et al., 2002, McKenna et al., 1993). Increased Wingate peak and/or mean power output has been shown after HIT (Barnett et al., 2004, Burgomaster et al., 2005, 2008, McKenna et al., 1993) and constant load experimental trials (Burgomaster et al., 2006, Gibala et al., 2006, Laursen et al., 2002). Performance on 50, 250 and 750 kJ time trials has also been observed to increase following HIT (Burgomaster et al., 2006, Gibala

et al., 2006, Laursen et al., 2002,). These adaptations were maintained even after 6 weeks of detraining (Burgomaster et al., 2007). Increases in endurance time to fatigue during constant-load exercise (Burgomaster et al., 2005, Warburton et al., 2005) highlight the functional applications of HIT. Decreased time to exhaustion and increased aerobic capacity have been more prominent during HIT than moderate intensity exercise (Daussin et al., 2008a, Rognmo et al., 2004, Warburton et al., 2005).

1.5.4. Practicality

High-intensity interval training gained popularity in the 1980s and has since been regularly practiced by elite athletes as a vital component of their training programs. Though the metabolic and cardiovascular benefits of HIT are well known, this training strategy was previously believed to be unsafe for less fit individuals. However in the last decade, HIT has been researched as a theraputic intervention in highly functional coronary artery disease patients (Rognmo et al., 2004, Warburton et al., 2005) and older individuals (Amir et al., 2007). The safety and efficacy of HIT for improving functional capacity and reducing the risk of morbidity and mortality in these populations have been established. These findings have practical relevance for sedentary individuals with respect to facilitating the adoption of regular aerobic exercise considering that the most commonly cited exercise barrier is "lack of time" (Booth et al., 1997). Longitudinal exercise studies have demonstrated that improved fitness and resulting weight loss in adults can prevent or delay the progression from pre-diabetes to the complete onset of type 2 diabetes (Katzmarzyk et al., 2003, Laaksonen et al., 2005). However, to date no

study has specifically investigated the effect of low-volume high-intensity interval training on skeletal muscle metabolism in sedentary adults. Given the potency of HIT (Coyle, 2005), low-volume interval training may provide a time and cost-efficient strategy to reduce and/or prevent the occurrence of inactivity-related disorders. However, the debate still remains as to the type and volume of exercise required to significantly improve oxidative capacity and insulin sensitivity.

1.6. Summary and purpose of the present investigation

The role of physical activity in the prevention and treatment of chronic disabling conditions has become increasingly evident. The rapid metabolic improvements elicited through interval training are promising for individuals at risk of insulin resistance and inactivity-related disorders. The majority of research investigating the metabolic adaptations to high-intensity interval training has been conducted on young active individuals. To our knowledge, no studies have examined the effect of low-volume HIT on mitochondrial enzymes and insulin sensitivity in middle-aged sedentary individuals, a population at risk of chronic disease. Given that HIT can elicit metabolic adaptations equivalent to a high volume of endurance training, it is anticipated that HIT will provide a practical and time-efficient exercise strategy for sedentary individuals.

1.7. References

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- Chapter 2 -

THE EFFECT OF HIGH-INTENSITY INTERVAL TRAINING ON SKELETAL MUSCLE OXIDATIVE CAPACITY IN MIDDLE-AGED SEDENTARY ADULTS

2.1. Introduction

Habitual exercise training improves skeletal muscle mitochondrial capacity (Coffey and Hawley, 2007, Holloszy, 1967, Holloszy and Coyle, 1984, Hood, 2001, Menshikova et al., 2006) and contributes to the prevention and treatment of several chronic diseases (Holloszy, 2005, Jessen and Goodyear, 2005, Pedersen and Saltin, 2006, Praet and van Loon, 2007, Venables and Jeukendrup, 2008, Warburton et al., 2006a). The physiological manifestations of inactivity and insulin resistance contribute to the development of lifestyle-related disorders (DeFronzo and Ferrannini, 1991, Hamburg et al., 2007, Heilbronn et al., 2007, Reaven, 1993, 1995, White, 2003) however the pathophysiology of insulin resistance remains to be elucidated. Exercise training has been shown to improve insulin sensitivity (Bruce et al., 2006, Holloszy, 2005, Houmard et al., 2004, Ivy, 2004, Jessen and Goodyear, 2005, Perseghin et al., 1996, Trapp et al., 2008, Wojtaszewski et al., 2000, Yokoyama et al., 2004, 2008) due in part to increased mitochondrial content and oxidative capacity (Hawley, 2004, Holloszy and Coyle, 1984, Short et al., 2003). These and other adaptations may prevent or reverse the effects of sedentary living on overall health and skeletal muscle metabolism (Biolo et al., 2005, Hamburg et al., 2007, Hawley, 2004, Laufs et al., 2005, Warburton et al., 2006b, Zderic

and Hamilton, 2006) which have been linked to the development of disease and premature death (Blair and Brodney, 1999, Katzmarzyk et al., 2003).

Despite the clear benefit of endurance training in the maintenance of aerobic fitness and metabolic health (Holloszy and Coyle, 1984, Martin et al., 1993, Meredith et al., 1989) the time requirement of training may present a barrier to exercise for many individuals (Booth et al., 1997, Owen and Bauman, 1992). High-intensity interval training may represent a time-efficient alternative training solution. Consisting of brief bouts of high intensity activity interspersed by long or short period of either active or passive recovery (Ross and Leveritt, 2001), HIT stimulates many metabolic adaptations that resemble traditional endurance training despite a reduction in the total exercise volume (Burgomaster et al., 2008, Gibala et al., 2006). A 6 week comparison of endurance and high intensity training reported similar increases in oxidative capacity through increased maximal activities of CS and β-HAD (Burgomaster et al., 2008). Other changes include an increased protein content of glucose transporter 4 (GLUT4), COX-IV, monocarboxylate transporter 1 and 4 (MCT1,4) after only one week (3 sessions) of training, suggesting that the adaptations of skeletal muscle to HIT occur relatively quickly (Burgomaster et al., 2007). A number of laboratories have evaluated the effect of various intensities, interval durations, rest periods and work to rest ratios on performance, cardiovascular and metabolic adaptations (Burgomaster et al., 2005, Laursen et al., 2002, Mohr et al., 2007, Rognmo et al., 2004, Talanian et al., 2007), however there is no consensus regarding the optimal HIT protocol. In addition, the majority of research investigating the metabolic effects of HIT has been conducted in young active individuals

(≤30 yr) and it is unclear whether the findings can be applied to older, sedentary individuals, a population most at risk of chronic disease.

The primary purpose of the present study was to investigate the effects of a short 2 wk HIT program on skeletal muscle oxidative capacity in previously sedentary middle-aged adults who may be at higher risk for inactivity-related disorders. A secondary purpose of the study was to examine the effect of training on fasting glucose and insulin concentration and substrate oxidation during matched-work exercise. We tested the hypothesis that 2 wk of HIT would increase muscle oxidative capacity as reflected by the maximal activity and content of citrate synthase. In addition, we hypothesized that fasting plasma glucose, insulin sensitivity and lipid oxidation during matched work exercise would improve as a result of the HIT protocol.

2.2. Methods

2.2.1. Subjects

Seven sedentary but otherwise healthy men and women volunteered to participate in the study (Table 2). Qualification for the study was confirmed by medical screening including the completion of a general health questionnaire and the following measurements: height, weight, waist circumference (WC), resting heart rate (RHR), blood pressure (BP), fasting plasma glucose (FPG) and an electrocardiogram (ECG). Preliminary screening determined that participants (a) were not at increased risk of cardiovascular, pulmonary or metabolic disease; (b) did not present any contraindications to beginning an exercise program; and (c) were sedentary, defined as not having

participated in a regular exercise program (i.e. ≤ 2 sessions per week and ≤ 30 min per session) for at least 1 year prior to the study. The procedures for each visit were explained to participants upon arrival to the laboratory. Participants were informed of the purpose and potential risks associated with the study prior to providing written, informed consent. The experiment was approved by the Hamilton Health Sciences/Faculty of Health Sciences, McMaster University Research Ethics Board.

Table 2. Participant characteristics and peak oxygen uptake prior to training.

Subject	Sex M/F	Age	Weight (kg)	Height (cm)	BMI ($kg \cdot m^{-2}$)	WC (cm)	VO_{2peak} ($ml \cdot kg^{-1} \cdot min^{-1}$)
1	F	39	59	163	22	73	35
2	F	52	66	167	24	80	26
3	M	45	110	188	31	111	31
4	F	36	72	160	28	88	27
5	F	44	60	160	23	71	28
6	M	47	111	178	35	110	30
7	M	50	72	165	26	97	32
Men	3	47 ± 2	98 ± 13*	176 ± 6	31 ± 3	106 ± 5*	31 ± 1
Women	4	43 ± 7	64 ± 3	162 ± 2	24 ± 1	78 ± 4	29 ± 2
Total	7	45 ± 2	78 ± 9	168 ± 4	27 ± 2	90 ± 6	30 ± 1

Values are means ± SE (n=7). BMI, body mass index; WC, waist circumference; VO_{2peak}, peak oxygen uptake. *Main effect for sex ($P < 0.05$), such that men > women.

2.2.2. Preliminary testing

Waist circumference was measured using a non elastic tape at the midpoint between the iliac crest and the bottom of the rib cage while participants stood in a relaxed position with arms at their side. A stadiometer and physician's scale were used to measure height and weight, and body mass index (BMI) was calculated. Standing height was measured to the nearest 0.5 cm while weight was measured to the nearest 0.5 kg.

Resting heart rate and blood pressure were measured by an automatic inflation monitor (Spot Vital Signs®, Welch Allyn, Mississauga, ON). Finally, resting and post-exercise 12-lead ECGs were conducted the same day as the maximal oxygen uptake (VO_{2peak}) test using an electrocardiograph apparatus (MAC, Marquette Electronics Inc., Milwaukee, WI).

2.2.3. Pre-experimental protocol

Participants performed an incremental exercise test to exhaustion on an electronically braked cycle ergometer (Lode Excalibur Sport V2.0, Groningen, the Netherlands) to determine VO_{2peak}. Following a 3 min cycling warm-up at 50 Watts (W), the workload was increased by 1 W every 2 sec until participants reached volitional exhaustion. The end of the test was specified as the point at which pedal cadence fell below 40 revolutions per minute (r.p.m.) according to manufacturer's specifications that power outputs below 40 r.p.m. are inconsistent. A metabolic cart with an on-line gas collection system (Moxus Modular VO_2 System, AEI Technologies, Pittsburgh, PA) was used to acquire data in order to quantify oxygen consumption (VO_2), carbon dioxide production (VCO_2) and substrate oxidation via respiratory exchange ratio (RER). VO_{2peak} and W_{max} corresponded to the highest oxygen consumption and peak power output values achieved over a 15 s period, respectively. The gas analyzers were calibrated using a custom O_2/CO_2 mixture (VitalAire, Mississauga, ON) and the turbine volume measurement system was calibrated using a 3 L syringe. Heart rate was monitored continuously throughout the test by telemetry with a Polar A3 (Polar, Lake Success, NY)

monitor. Subsequent to completion of the incremental exercise test, participants returned to the laboratory on two occasions in order to: (a) determine the workload eliciting 50% and 60% W_{max}; and (b) become familiarized with the high-intensity training protocol. A summary of the study design is depicted in Figure 4.

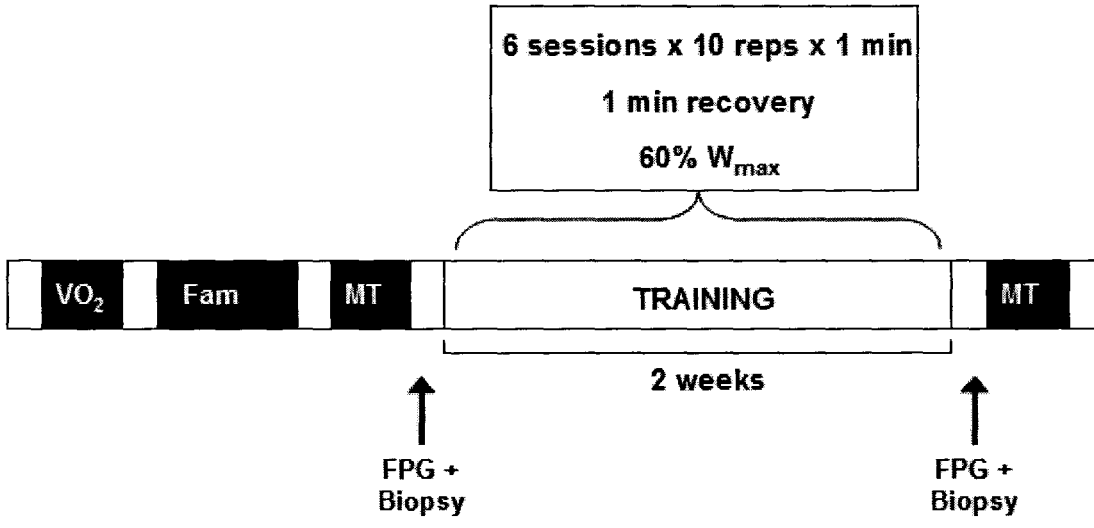

Figure 4. Overview of experimental protocol.
VO_2, Maximal oxygen uptake test; Fam, Familiarization session; MT: Metabolism trial; FPG: Fasting plasma glucose.

2.2.4. Fasting blood samples and resting muscle biopsies

Subjects reported to the laboratory (~8:00 am) following an overnight fast. A resting blood sample was obtained via venipuncture from an antecubital vein and treated according to manufacturer's instructions (Vacutainer®, BD, Mississauga, ON). The lateral portion of one thigh was prepared for the extraction of muscle biopsy samples

from the vastus lateralis (Bergström, 1975). The procedure was initiated by injection of a local anaesthetic (2% lidocaine) followed by a small incision in the skin and overlying tissues. The obtained biopsy samples were immediately frozen in liquid nitrogen and stored at -80°C until subsequent analysis. The resting blood and biopsy procedure was repeated after training.

2.2.5. Metabolism trials

All subjects in this study served as their own controls and completed two exercise trials before and after a 2 week period of HIT. Upon arrival to the laboratory at the start of each trial, a Teflon catheter was inserted into an antecubital vein and a resting blood sample was drawn. Participants then mounted an electrically-braked ergometer (Lode) and proceeded to warm-up for 3 min at 50W. Immediately following the warm-up, the workload was increased to elicit an intensity equivalent to ~50% of pre-training maximal workload (W_{max}) and subjects cycled for 30 min. Blood samples were obtained after 15 and 30 min of exercise and on each occasion the catheter was flushed with saline (0.9%, Baxter, Norfolk, UK) to prevent clotting. Heart rate was monitored continually (Polar A3, Lake Success, NY) and expired air samples were collected from 9-14 and 24-29 minutes of exercise (Moxus Modular VO_2 System). Breath samples were used for the determination of ventilation, VO_2, VCO_2, RER and to estimate rates of whole body fat and carbohydrate oxidation (Peronnet & Massicotte, 1991). The researchers provided verbal and physiological feedback throughout the duration of the test. The post-training exercise trial was conducted identically to the first (absolute workload ~50% of pre-

training VO_{2peak}) and was performed approximately 72 hours after the final exercise training session.

2.2.6. Training protocol

The training protocol was initiated several days following the pre-training muscle biopsy procedure. Participants completed 6 sessions of high-intensity interval cycling during a 2 week period, each session interspersed by 1-2 days of recovery (Monday, Wednesday, Friday). Each session was initiated by a 3 min warm-up at 50 W, followed by a series of 10 repeated high-intensity intervals (60 sec) and terminated with a 5 min cool-down at 50 W. The workload of each interval was set at sixty percent of the maximal workload (60% W_{max}) achieved during the VO_{2peak} test. The recovery interval between high-intensity cycling bouts was fixed at 1 min, where the ergometer resistance was set at 30 W and participants were asked to cycle at a low cadence (< 50 r.p.m.). Active recovery was utilized in order to minimize venous pooling in the lower extremities and to reduce any feelings of uneasiness related to high-intensity work. The exercise program was modified based on previous studies from our laboratory (Burgomaster et al., 2005, 2006, 2007, 2008, Gibala et al., 2006) and others (De Feyter et al., 2007, Rognmo et al., 2004, Talanian et al., 2007) and formulated to deliver a low-volume, constant-load interval training program. Heart rate was monitored carefully and non-invasively via telemetry during each exercise session. In order to ensure sufficient recovery, each training session was interspersed by 1-2 days of rest.

2.2.7. Physical activity and dietary controls

Participants were instructed to continue their normal daily activity practices and to refrain from strenuous physical activity throughout the experimental period. For 48 h before each experimental trial and biopsy, participants were asked to refrain from any type of exercise. Previous to the FPG test, participants were required to complete a 10-12 hour overnight fast. Otherwise, participants were instructed to maintain their habitual diet during the two week training period. Participants recorded their dietary intake for 24 h prior to the pre-training experimental procedures and replicated the diet using the same types and quantities of food before the post-training procedures. Subsequent dietary analyses (The Food ProcessorSQL 9.8, ESHA Research, Salem, OR) revealed no differences in the total energy content of diets prior to each experimental trial and biopsy (Table 3).

Table 3. Nutritional data.

	Biopsy		Metabolism Trial	
	Pre	Post	Pre	Post
Energy Intake (Kcal)	1454 ± 102	1659 ± 240	2066 ± 195	1954 ± 160
Carbohydrate (%)	64 ± 3	60 ± 2	62 ± 4	60 ± 3
Fat (%)	14 ± 2*	32 ± 1	17 ± 2	20 ± 1
Protein (%)	21 ± 3	22 ± 2	21 ± 2	23 ± 3
Protein (g·kg body wt^{-1})	0.9 ± 0.2	1.0 ± 0.2	1.1 ± 0.1	1.2 ± 0.2

Data collected for 24 h prior to each experimental trial. Values are means ± SE (n =7). %, Percent of total macronutrient intake; *Difference between experimental trials, $P < 0.05$.

Dietary analyses confirmed no differences in macronutrient intake prior to both experimental trials. Lipid intake between the first and second muscle biopsies was statistically different ($P < 0.05$), however there were no discrepancies in carbohydrate and protein intake. There were no differences in the amount of protein ingested per kg of body weight for both metabolism and biopsy trials.

2.2.8. Muscle analyses

Muscle biopsy samples were initially sectioned into several pieces under liquid nitrogen and stored for subsequent analysis. A small piece of wet muscle (10-15 mg) was homogenized to a 50 times dilution (Henriksson et al., 1986) and the resulting homogenate was analyzed spectrophotometrically (Ultrospec 3000 pro UV/Vis) for the maximal activity of citrate synthase (CS) using the methods of Carter et al. (2001). Total protein content of the homogenates was determined using a commercial assay kit (Pierce BCA Protein Assay Kit, Fisher Scientific, Ottawa, ON) and maximal enzyme activity was determined as moles per kilogram of protein per hour.

2.2.8.1 Western blotting

The total protein content of CS was quantified using Western blotting. A piece of frozen wet muscle was homogenized and 10 ug of protein was loaded and separated on 12.5% SDS-PAGE gels for 2 hours at 100V. Proteins were electro-transferred to nitrocellulose membranes at 100V for 1 hour and blocked overnight in 5% milk TBS-T. Membranes were incubated with primary antibodies against CS (1:5000; gift from Sandy Raha) for 2 hours at room temperature in 3% milk TBS-T.

Following 3 X 5-min washes in TBS-T, membranes were incubated in appropriate species specific secondary antibodies at 1:10000 dilution in 3% milk TBS-T for 1 hour at room temperature. Blots were developed using a chemilumiescent substrate (ECL Plus, Amersham/GE), exposed to X-ray film and quantified using Image J analysis software (NIH).

2.2.9. Blood analyses

Mixed venous blood samples were collected into tubes that contained sodium heparin or a clot activator (Vacutainer, BD, NJ). Collection tubes were immediately inverted 8 or 5 times as per the manufacturer's instructions and then placed on ice or left to clot at room temperature until the end of the trial. Blood samples were then centrifuged for 10 min at 1750 g. The resulting plasma was immediately analyzed for blood glucose and lactate (Ascensia Contour, Bayer, NY, and Accusport/Accutrend® Lactate, Sports Resource Group, US, respectively). Serum was stored at -80°C for subsequent analysis of insulin using a commercially available assay kit (Insulin EIA, Alpco Diagnostics, Salem NH). Hepatic insulin sensitivity was estimated using the ISI (HOMA) calculation method described by Matsuda and DeFronzo (1999, Calculation 1, where $k = 22.5 \times 18$).

$$\text{ISI (HOMA)} = \frac{k}{\text{FPG} \times \text{FPI}} \qquad (1)$$

2.2.10. Statistical Analyses

Subject descriptive characteristics were analyzed using paired sample t-tests and two-factor repeated measures ANOVA using trial (pre-training, post-training) and sex (men, women). Dietary information was analyzed via paired-samples t-tests (pre-training and post-training). Metabolism trial data were analyzed using two methods; a repeated-measures ANOVA with the factor trial (pre-training, post-training) for HR, RER, substrate oxidation, ventilation and VO_2; and a two-factor repeated measures ANOVA for blood measurements, with the factors trial (pre-training, post-training) and time (0, 15, 30 min). CS activity and CS content, glucose and insulin data were analyzed by paired sample t-tests for comparison between conditions (pre-training, post-training). The effect of gender on training-induced changes in CS, FPG and FPI levels were analyzed via a two-factor repeated measures ANOVA using trial (pre-training, post-training) and sex (men, women) as conditions. Bivariate and partial correlations amongst variables were evaluated using Pearson's correlation. Significance was set at $P<0.05$. Significant interactions and main effects were subsequently analyzed using Tukey's honestly significant difference post-hoc test.

2.3. Results

2.3.1. Fasting insulin and glucose

Fasting insulin concentration decreased from 8.1 ± 1.3 to 6.6 ± 1.1 $\mu IU \cdot ml^{-1}$ with training resulting in a 16% change (main effect for condition, $P < 0.01$, Figure 5). Insulin

sensitivity increased an average of 35% (16.6 ± 2.3 vs. 12.8 ± 2.3 arbitrary units) after 6 sessions of HIT (main effect for condition, $P < 0.05$, Figure 6).

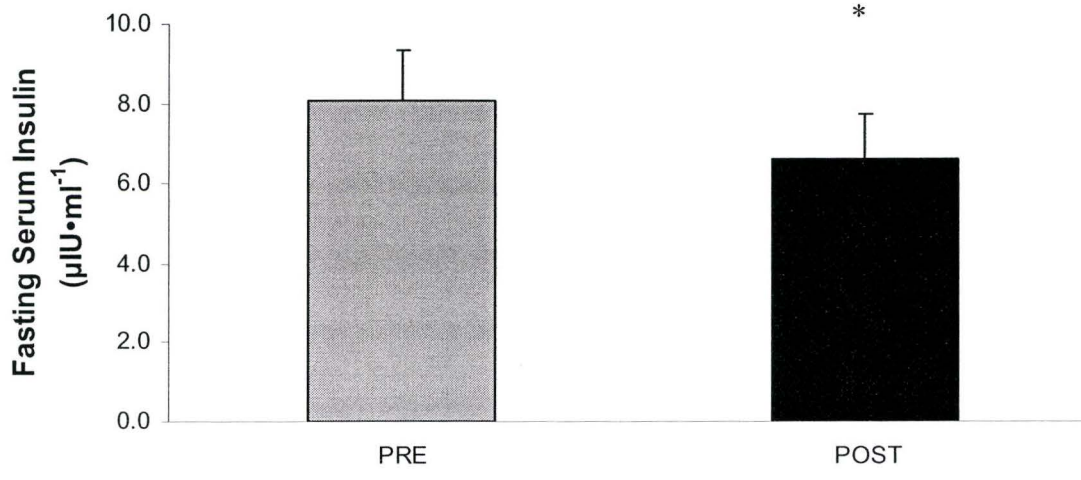

Figure 5. Fasting serum insulin measured before (PRE) and after (POST) 6 sessions of HIT.
Values are means ± SE (n=7); *Main effect for condition ($P < 0.01$), such that pre-training (PRE) > post-training (POST).

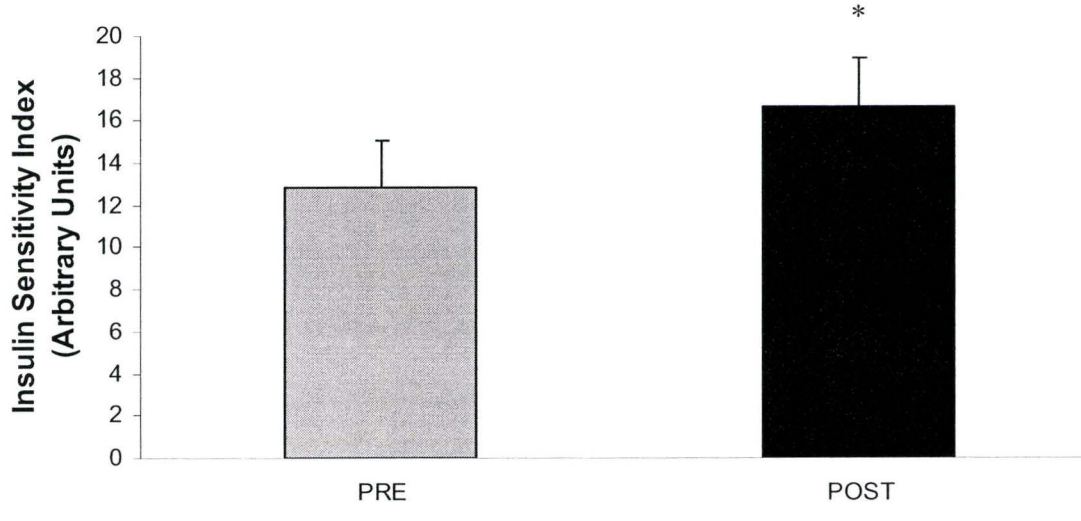

Figure 6. Insulin sensitivity calculated before and after 2 weeks of HIT.
Values are means ± SE (n=7); *Main effect for condition ($P < 0.05$), such that pre-training (PRE) < post-training (POST).

There was an interaction effect for fasting insulin between sex and training where fasting insulin levels decreased to a greater extent in men than women (26.8 ± 5.4 vs. 8.6 ± 4.1 %; $P < 0.05$, Figure 7).

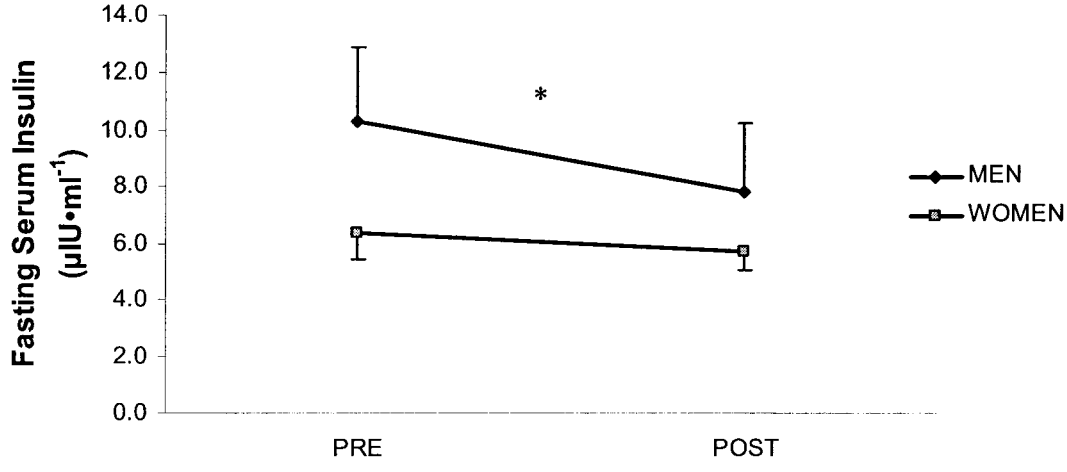

Figure 7. Fasting serum insulin of men and women measured before and after 6 sessions of HIT.
Values are means ± SE (n=7; men=3, women=4); *Interaction effect ($P < 0.05$), where the change in fasting serum insulin was greater in men than in women.

There were no differences in fasting glucose as a result of HIT however an overall decline in FPG was observed following training ($P = 0.091$). Fasting plasma glucose was lower in women compared to men prior to and following 6 sessions of HIT (4.4 ± 0.1 vs. 4.9 ± 0.1 mmol·L^{-1} and 4.1 ± 0.2 vs. 4.5 ± 0.3 mmol·L^{-1}; main effect for sex, $P < 0.05$; Figure 8).

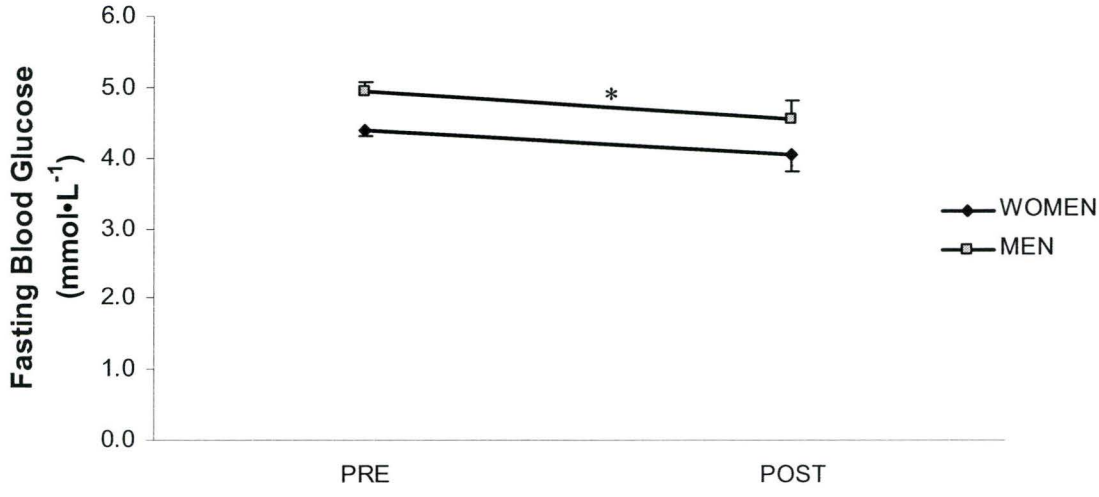

Figure 8. Fasting plasma glucose measured in fasting blood samples obtained before and after 6 sessions of HIT.
Values are means ± SE (n=7); *Main effect for sex ($P < 0.05$), such that women < men.

2.3.2. Mitochondrial enzyme activity and content

The maximal activity of CS increased by 23% as a result of training (12.9 ± 0.7 vs. 10.6 ± 0.6 mol·kg protein^{-1}·h^{-1} WW; main effect for condition, $P < 0.05$; Figure 9). There were no differences in the maximal activity of CS between men and women. Six sessions of HIT also increased the mitochondrial protein content of CS by 18% (main effect for condition, $P < 0.05$; Figure 10). Protein content of CS increased from 6105 ± 609 to 7199 ± 453 (arbitrary units) after 6 sessions of HIT.

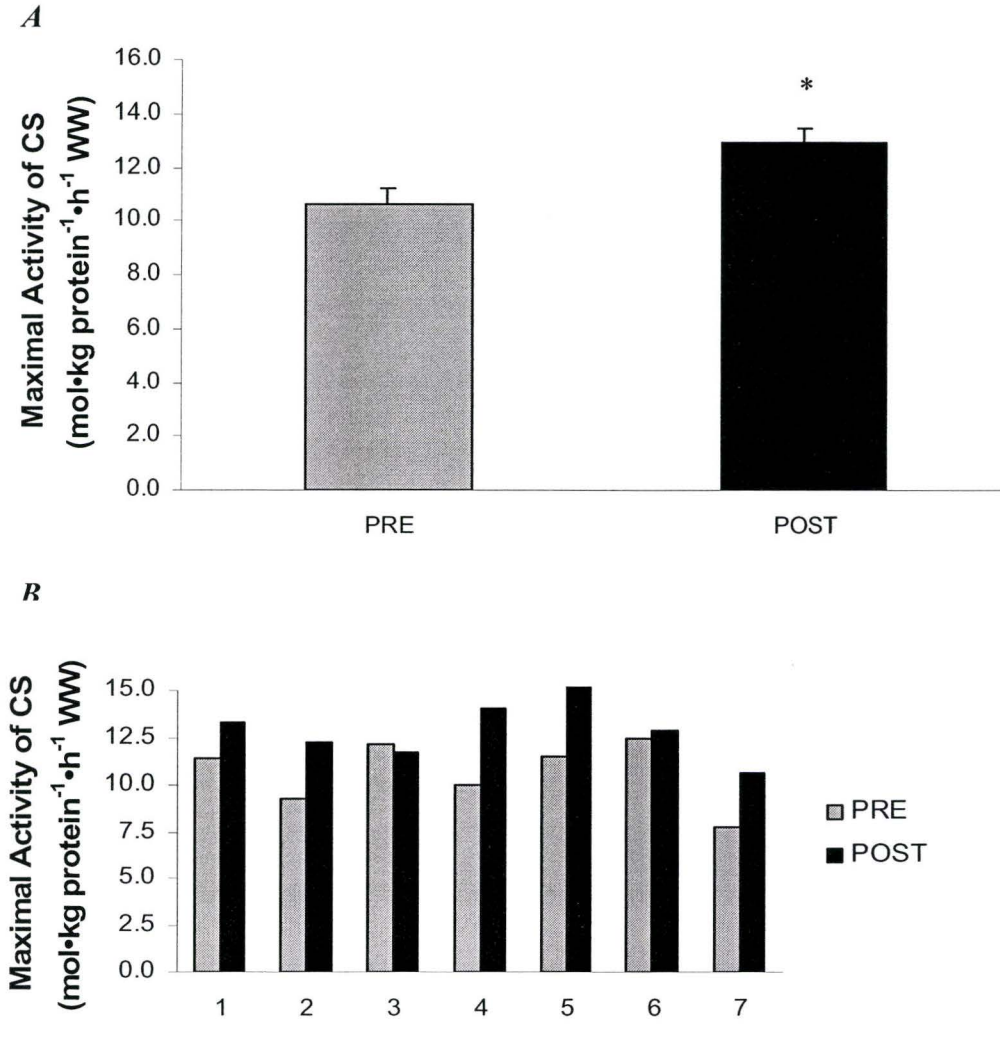

Figure 9. Maximal activity of mitochondrial enzyme citrate synthase (CS; *A*) and individual changes in CS (*B*), measured in biopsy samples obtained before and after 6 sessions of HIT.

Values are means ± SE (n=7); WW, wet weight. *Main effect for condition ($P < 0.05$), such that post-training (POST) > pretraining (PRE).

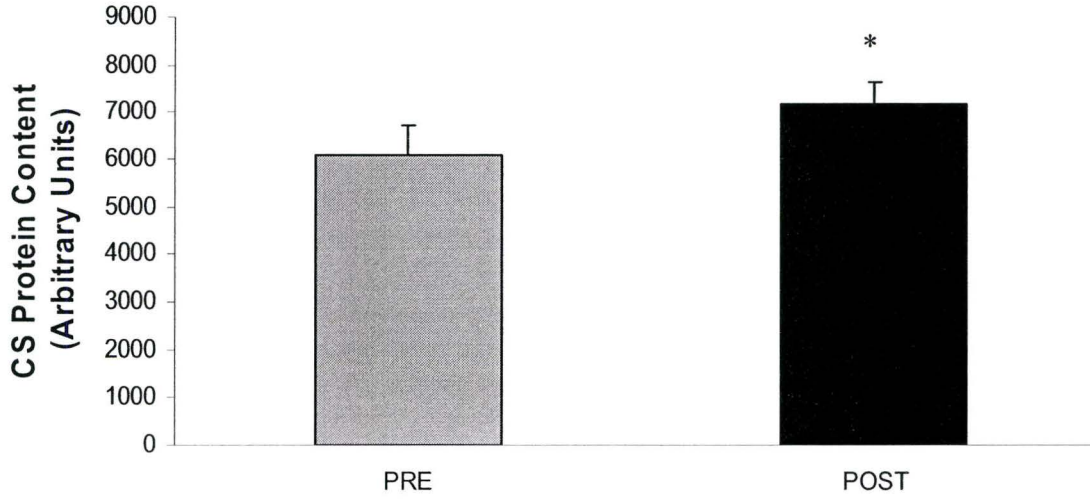

Figure 10. Total protein content of mitochondrial enzyme citrate synthase measured in biopsy samples obtained before and after 6 sessions of HIT.
Values are means ± SE (n=7); *Main effect for condition ($P < 0.05$), such that post-training (POST) > pretraining (PRE).

2.3.3 Metabolism trials

There were no differences in steady-state heart rate, RER, ventilation and oxygen uptake or calculated rates of substrate oxidation between trials (Table 4). There were no differences in blood glucose and blood lactate between experimental trials. Differences in plasma glucose and lactate levels between blood sampling times were observed (main effects for time, $P < 0.05$). Blood glucose and lactate were different than rest at 15 and 30 min of exercise ($P < 0.05$, Figures 11, 12).

Table 4. Steady-state cardiorespiratory data during cycling exercise at 50% W_{max} before and after 6 sessions of high-intensity interval training.

	Metabolism Trials	
	Pre	Post
Heart rate (beats·min^{-1})	147 ± 5	143 ± 4
RER	0.94 ± 0.01	0.93 ± 0.01
Ventilation (l·min^{-1})	50 ± 5	51 ± 6
VO_2 (ml·kg^{-1}·min^{-1})	22 ± 1	22 ± 1
CHO oxidation (g·min^{-1})	1.86 ± 0.23	1.86 ± 0.24
Fat oxidation (g·min^{-1})	0.16 ± 0.03	0.18 ± 0.02

Values are means ± SE (n =7). RER, respiratory exchange ratio; VO_2, oxygen uptake.

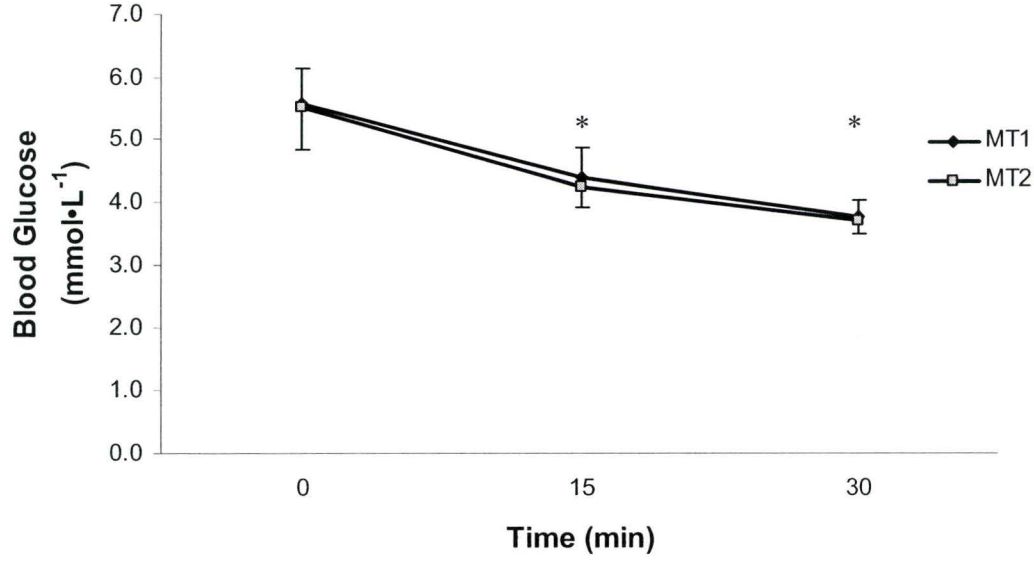

Figure 11. Blood glucose values during metabolism trials measured at 0, 15 and 30 min of exercise.
Values are means ± SE (n=7); *Significantly different from rest (Time = 0, $P < 0.05$).

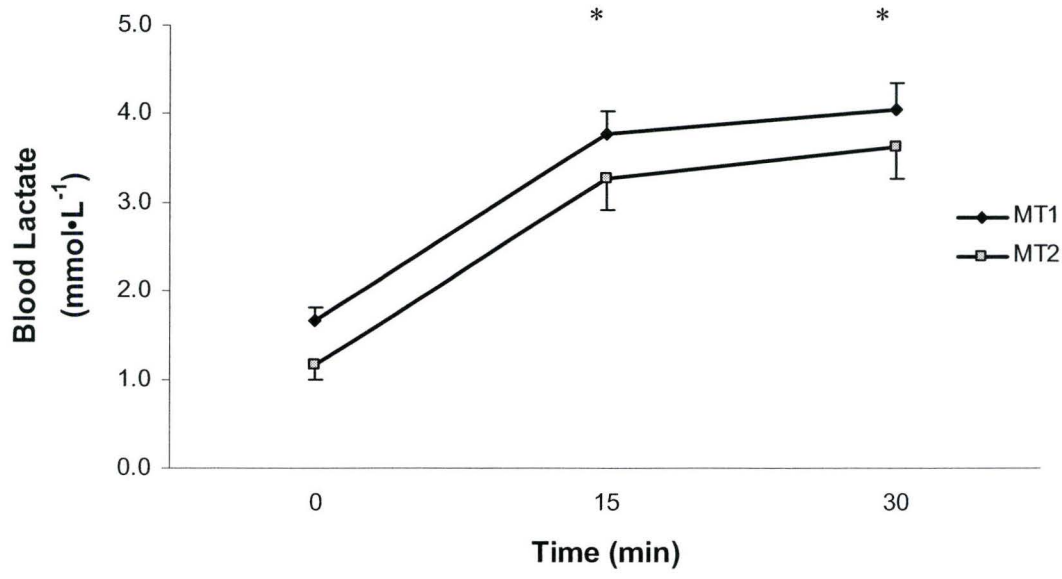

Figure 12. Blood lactate values during both metabolism trials measured at 0, 15 and 30 min of exercise.
Values are means ± SE (n=7); *Significantly different from rest (Time = 0, $P < 0.05$).

2.3.4. Correlations

The waist circumference and body weight of participants was correlated with sex ($r = 0.902, 0.799$; $P < 0.01, 0.05$). In turn, waist circumference was positively correlated with body weight and BMI ($r = 0.943, 0.926$, $P < 0.01$). The percentage of change in CS maximal activity was negatively correlated to the body weight of study participants ($r = -0.813$, $P < 0.05$; Figure 13) even when controlling for age ($r = -0.815$, $P < 0.05$).

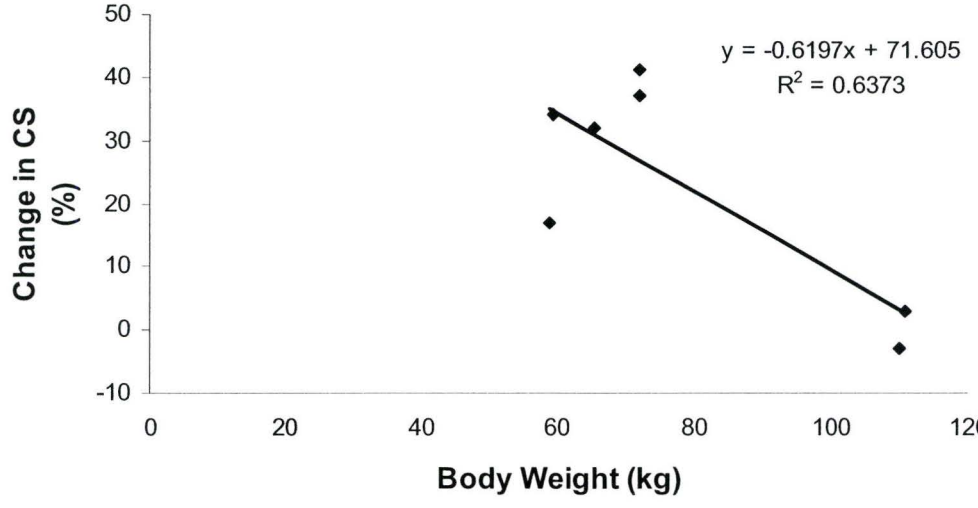

Figure 13. Correlation between the body weight of participants and training-induced changes in CS activity.
Values are means ± SE (n=7). Significant correlation (r = -0.813, $P < 0.05$).

Fasting plasma glucose values before and after training were also positively correlated with body weight (r = 0.786, 0.773, $P < 0.05$, Figure 14).

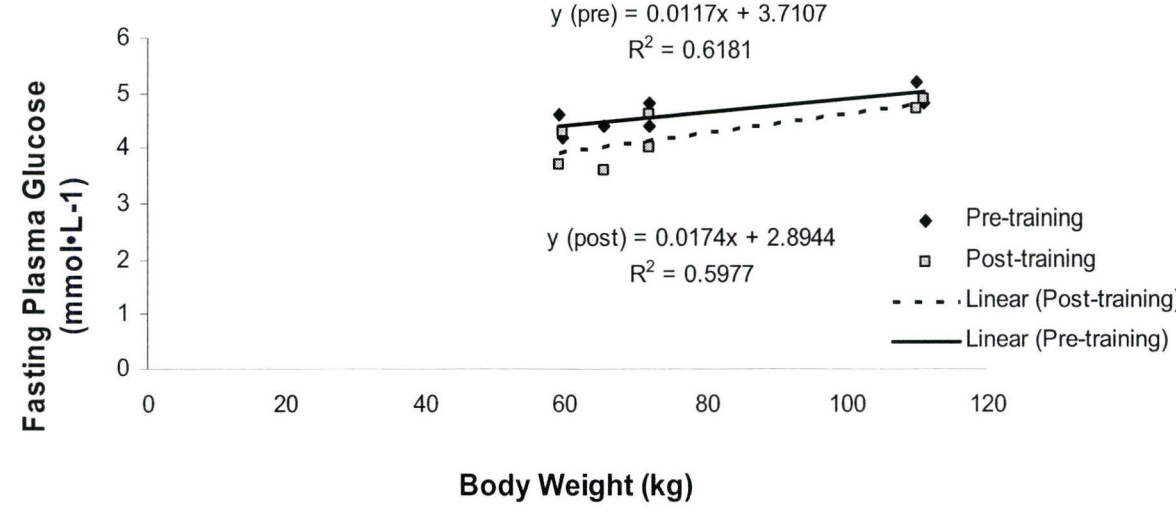

Figure 14. Correlation between body weight and fasting plasma glucose values before and after 6 sessions of HIT.
Values are means ± SE (n=7). Pre-training $R^2 = 0.618$ and post-training $R^2 = 0.598$. Significant correlation (r = 0.786, 0.773, $P < 0.05$).

However when sex was controlled, the correlation between FPG values and body weight was abolished. Controlling for age revealed a positive correlation between waist circumference and FPG values both prior to and following HIT ($r = 0.834, 0.814, P < .05$).

2.4. Discussion

The major novel finding from the present investigation was that 6 sessions of constant load HIT performed over 2 weeks increased skeletal muscle oxidative capacity and whole-body insulin sensitivity in sedentary middle-aged adults despite a low total exercise volume and training time commitment. These results extend previous findings from our laboratory that showed 6 bouts of "all out" HIT increased the maximal activity of oxidative enzymes in young active individuals (Burgomaster et al., 2005, Gibala et al., 2006). We also report the novel observation of a decline in fasting insulin concentration following 2 weeks of HIT. Although improvements in insulin sensitivity have been shown after HIT (Tjønna et al., 2008, Trapp et al., 2008), our results suggest that these adaptations occur rapidly and do not necessitate a high volume of work.

2.4.1. Skeletal muscle oxidative capacity

The maximal activity and content of citrate synthase measured in resting muscle biopsies increased by 23 and 18% after short duration HIT respectively, which is comparable to changes reported in young active individuals after Wingate-based HIT (Burgomaster et al., 2005, 2006, Rodas et al., 2000, Talanian et al., 2007) or traditional

high-volume endurance training (Spina et al., 1996). Citrate synthase is the most commonly used marker of muscle oxidative capacity (Baldwin et al., 1972, Barnett et al., 2004, Coyle et al., 1984, Harmer et al., 2008, MacDougall et al., 1998, Parra et al., 2000, Spina et al., 1996) and was the only mitochondrial enzyme measured in this study. Future studies could benefit from the measurement of additional marker enzymes for oxidative metabolism such as PDHa, β-HAD and COX to respectively provide specific information on carbohydrate, lipid oxidation and ETC function. However it must be noted that citrate synthase has previously been employed as the sole marker of oxidative metabolism (Barnett et al., 2004, Burgomaster et al., 2005, Harmer et al., 2008) and its use justified by its "constant proportion with other mitochondrial enzymes" (Green et al., 1999). Although mitochondrial enzyme activity reflects oxidative potential, the regulatory pathways leading to mitochondrial protein synthesis are not fully understood. During exercise, muscular contraction induces the phosphorylation and subsequent activation of PGC-1α by p38 MAPK (Wright et al., 2007), ultimately resulting in transient increases in PGC-1α transcription and mRNA content during recovery (Pilegaard et al., 2003). Once activated, PGC-1α may act in parallel with muscular contraction to increase mRNA levels of NRF-1 and NRF-2 during post-exercise recovery (Hood, 2001, Puigserver and Spiegelman, 2003). These transcription factors then turn on TFAM, which translocates to the mitochondria to regulate DNA replication and transcription (Puigserver and Spiegelman, 2003). We can only speculate that the improvements in CS activity observed in this study were the result of cumulative transient increases in citrate synthase mRNA

with repeated exercise sessions. Subsequent analyses may evaluate the effects of high-intensity interval training on resting NRF-1, NRF-2, TFAM and PGC-1α protein content.

2.4.2. Insulin sensitivity

A novel finding from the present study was that fasting insulin concentration decreased by 16% consequential to 6 sessions of HIT. High-intensity interval exercise has previously been shown to improve insulin action (Trapp et al., 2008) however to our knowledge this is the first study to report such rapid adaptations. The short (2 wk) high intensity training program employed in this study elicited changes in insulin sensitivity generally observed with high-volume endurance training (O'Donovan et al., 2005, Bruce et al., 2006) and lifestyle modification (Solomon et al., 2008). Improvements in glucose disposal have been attributed to greater expression of GLUT4 protein and translocation to the cell membrane (Ivy, 2004). Increases in the GLUT4 content have been reported with as little as 3 and 7 sessions of sprint interval (Burgomaster et al., 2007) and aerobic exercise (O'Gorman et al., 2006) respectively. Future investigations are warranted to evaluate high-intensity training adaptations in GLUT 4 content and in pathways leading to membrane translocation.

It has been suggested that duration is more important than intensity when prescribing exercise to improve insulin sensitivity (Houmard et al., 2004). However, the present study and previous work (Zoeller, 2007) suggest an important role for exercise intensity in glycemic regulation and insulin action. The mechanisms by which exercise training improves insulin sensitivity are not fully understood, however changes in muscle

oxidative capacity may be a factor (Bruce et al., 2006, Stump et al., 2003). The maximal activity and protein content of mitochondrial enzymes is often reduced in insulin resistant versus healthy skeletal muscle (Heilbronn et al., 2007, Østergärd et al., 2006, Patti et al., 2003) and exercise training is a well established method to improve mitochondrial enzyme activity and insulin sensitivity (O'Donovan et al, 2005, Østergärd et al., 2006, Segal et al., 1991). This has led some to suggest and explain a possible link between mitochondrial dysfunction and insulin resistance (Abdul-Ghani et al., 2008, De Filippis et al., 2008, Morino, et al., 2006) however no consensus has been reached. In contrast, recent work has demonstrated training-induced improvements in insulin sensitivity and aerobic fitness despite any changes in mitochondrial enzymes (Heilbronn et al., 2007). In these cases, contracted-mediated mechanisms including AMPK and GLUT 4 may explain the improvements in insulin action. Others have demonstrated reduced cardiorespiratory adaptations to training with increasing insulin resistance (Burns et al., 2007). Further research is required to elucidate the link between oxidative potential and insulin action.

2.4.3. Metabolism trials

Despite increases in oxidative capacity, substrate oxidation during constant-load exercise was unaffected by high-intensity interval training. This is consistent with previous work that failed to observe a change in RER or in the capacity for lipid oxidation after HIT (Venables and Jeukendrup, 2008). However our laboratory (Burgomaster et al., 2005) and others (Harmer et al., 2008) have reported reduced exercise RER after repeated Wingate training. Skeletal muscle lipid oxidation, as

reflected by the maximal activity of β-HAD, has also been reported to increase after a few sessions of high-volume HIT (Rodas et al., 2000, Talanian et al., 2007) and following several weeks of low-volume HIT (Burgomaster et al., 2008, Parra et al., 2000). These adaptations were similar to endurance training (Bruce et al., 2006, Spina et al., 1996) and taken together, suggest that the volume of training and the intensity of exercise intervals may be important determinants of subsequent adaptations in substrate oxidation. The volume of exercise and the length of the training program in this study may have been insufficient to elicit changes in fat oxidation capacity. Increases in fat oxidation and decreases in central abdominal fat after training have been associated with changes in insulin sensitivity during exercise (Trapp et al., 2008, Venables and Jeukendrup, 2008). Although exercise insulin and glucose area under the curve (AUC) did not change after training in the present study, resting insulin sensitivity was improved. Possible mechanisms for this adaptation include greater mitochondrial content, and increased GLUT4 protein content or translation to the sarcolemmal membrane.

Matched-work exercise trials were used to crudely assess cardiovascular (CV) function based on changes in HR and VO_2. Six sessions of HIT failed to elicit cardiovascular adaptations similarly to previous low-volume short-term training interventions (Burgotmaster et al., 2005, 2006) however several weeks of HIT induced adaptations in VO_{2peak} or exercise HR (Burgomaster et al., 2008, Rakobowchuk et al., 2008, Venables and Jeukendrup, 2008). Six weeks of low-volume HIT also improved arterial distensibility and endothelial function to the same degree as endurance training (Rakobowchuk et al., 2008). These findings suggest that the 6 sessions of HIT employed

in this study were not sufficient to increase markers of CV fitness and that adoption of this exercise program may result in changes in CV function over time.

2.4.4. Directions for future research

A limitation of the present study is that training-related changes in performance and exercise capacity were not measured. Future investigations should include timed or exhaustive exercise trials to provide quantitative information on the functional adaptations to high-intensity training. Similar experimental procedures employed to compare endurance and high-intensity training have demonstrated equal or greater performance and exercise capacity after high-intensity work (Burgomaster et al., 2008, Gibala et al., 2006, Helgerud et al., 2007, Rognmo et al., 2004, Warburton et al., 2005). The potency of low-volume HIT to elicit metabolic adaptations has been confirmed in young active individuals (Burgomaster et al., 2005, 2006, 2007, 2008, Gibala et al., 2006, Helgerud et al., 2001) and now in sedentary middle-aged adults. It is unclear if age, gender, activity level and insulin sensitivity influence metabolic adaptations to high-intensity interval training. Time-course studies in various populations would provide practical information on the physiological mechanisms underlying training-induced metabolic adaptations to high-intensity exercise. Although the training protocol employed in the current investigation elicited improvements in oxidative potential, subsequent work should consider the dose response relationship between exercise intensity and duration, and the magnitude of metabolic changes. In the future, investigators should also attempt to increase the study sample size in order to improve the power of statistical analyses.

2.5. Conclusion

In summary, the results of the present investigation demonstrate that low-volume HIT is a practical strategy to rapidly increase skeletal muscle oxidative capacity and insulin sensitivity in previously sedentary adults. Six sessions of high-intensity interval exercise increased the maximal activity of CS to a level consistent with endurance training (Phillips et al., 1996, Short et al., 2003, Spina et al., 1996) despite a lower volume of work. Reduced fasting blood insulin concentrations and a trend towards declining fasting blood glucose were also observed after just 6 bouts of interval exercise, proposing a role for HIT in the improvement and maintenance of insulin sensitivity. These findings provide novel information regarding the potency of low-volume HIT to improve insulin sensitivity, and to the same degree as traditional endurance training (Bruce et al., 2006, O'Donovan et al., 2005, Østergärd et al., 2006). Despite similar metabolic adaptations between this HIT and previous endurance training studies, the time requirement of the present protocol was relatively small (~20 min) and consequently participants completed 100% of the training sessions. Low-volume high-intensity interval training has resulted in strikingly superior exercise adherence compared to endurance training (Kanaley et al., 2001, O'Donovan et al., 2005, Rognmo et al., 2004,) proving HIT as a sensible exercise solution for sedentary individuals. Finally, given increasing evidence that HIT rapidly improves oxidative potential, clinical application of this training method may be a time and cost-efficient strategy to improve insulin sensitivity and to prevent the occurrence of type 2 diabetes.

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APPENDIX I

SUBJECT INFORMATION AND CONSENT FORMS

DESCRIPTION OF MEDICAL PROCEDURES

McMaster University
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Phone 905.525.9140
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http://www.mcmaster.ca/kinesiology/

PARTICIPANT INFORMATION SHEET

Title of Study:

The Effect of High-Intensity Interval Training on Skeletal Muscle Oxidative Capacity in Healthy Sedentary Adults

Locally Responsible Investigator and Principal Investigator:

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Sponsor:

Canadian Institutes of Health Research
Natural Sciences and Engineering Research Council

OVERVIEW

You are being invited to participate in the research study described above because you are a sedentary but otherwise healthy individual between the ages of 35 and 50 who may be at risk of developing an inactivity-related disorder. In order to decide whether or not you want to be part of this research study, you should understand what is involved and the potential risks and benefits. This form gives you detailed information about the research study, which will be discussed with you. Once you understand the study, you will be asked to sign this form if you wish to participate. Please take your time to make your decision. Feel free to discuss it with your friends and family or your family physician.

WHY IS THIS RESEARCH BEING DONE?

Physical inactivity or a sedentary lifestyle increases the risk of developing numerous chronic diseases including Type 2 Diabetes. It is well established that traditional endurance training, or prolonged periods of moderate-intensity exercise, reduces the risk of inactivity-related disorders. The most commonly cited barrier to exercise is "lack of time". Recent evidence demonstrates that improvements in fitness can be acquired relatively quickly with a surprisingly small amount of high-intensity interval exercise training. These findings are encouraging for those who struggle to adhere to a regular exercise program. However, most of this research has been conducted on young healthy people (aged 20-35 years). To date, no study has investigated the effect of this type of training in sedentary but otherwise healthy middle-aged persons.

WHAT IS THE PURPOSE OF THE STUDY?

The primary purpose of the study is to investigate the effects of low-volume, high intensity interval training (HIT) on skeletal muscle fuel utilization in healthy sedentary individuals. We believe that two weeks of HIT will increase markers of health and fitness including enzymes that regulate the use of carbohydrates and fats as fuel.

WHAT ARE MY RESPONSIBILITIES IF I PARTICIPATE IN THE STUDY?

Following routine medical screening, you will be required to visit the laboratory on 14 occasions during a 3-4 week testing period. The initial 6 visits will consist of baseline fitness and metabolism testing. The remaining visits will consist of high intensity low volume training and will be followed by two additional days of fitness and metabolism testing.

Visit 1 : Medical Screening, Fasting Plasma Glucose Test and Electrocardiogram

Medical screening will include the completion of a general health questionnaire and the following measurements: height, weight, waist circumference, resting heart rate and

blood pressure. For the fasting plasma glucose test, you will be asked to report to the laboratory in the morning following an overnight fast, or after 10-12 hours without food or drink ingestion (except for water). Upon arrival, a small sample of blood (1 ml) will be drawn from a forearm vein and immediately analyzed for plasma glucose (blood sugar). You will then undergo an electrocardiogram, where we will place 12 electrodes on your chest and monitor your heart. You will feel nothing during this procedure. You will then be asked to cycle at a low intensity to monitor your heart during exercise and once again after the exercise to monitor your recovery. This test is simply a precaution to identify any heart problems which may exclude you from the study.

Visit 2: VO_{2peak}

This test involves exercising on a cycle ergometer (stationary bicycle) at progressively increasing workloads to determine the amount of oxygen taken up by your body. During this test, you will wear a mouthpiece that will be connected to a gas analyzer. The exercise portion of the test will last approximately 10 to 15 min and heart rate will be monitored non-invasively throughout the test. The total time required for this visit will be approximately 30 min.

Visits 3-4: Exercise Training Familiarization

During these visits, you will be familiarized with all exercise equipment used in the study. This will help the investigators determine the workloads for subsequent testing and training. You will have the opportunity to practice the training protocol at a moderate pace and you will undergo a few trials cycling at the required intensity for training.

Visit 5: Metabolism Trial

You will be instructed to eat a light meal of your own chosing approximately 2-3 hours prior to the test. Upon arrival at the laboratory, a small catheter will be interested into an arm vein and a resting blood sample will be obtained. Following a 5 min warm-up, you will complete a 30 min cycling trial at an intensity corresponding to 60% $VO_{2peak.}$. Your heart rate will be monitored continuously throughout the task and breath samples will be obtained periodically. Blood samples also will be drawn at 0, 15 and 30 minutes to measure selected metabolites and hormones.

Visit 6: Muscle Biopsy

Please refer to the attached forms entitled "Description of Medical Procedures" for a detailed description of the procedures and their associated risks.

Visits 7-12: Exercise Training Sessions

Each exercise training sessions will consist of 10 x 60 second bouts of cycling at an intensity corresponding to 90% of VO_{2max}, with each interval separated by 60 seconds of rest or light cycling exercise. The workload will be similar to that performed during the Familiarization trial. Each training session will also consist of a 5 min warm-up and cool-down period and heart rate will be monitored non-invasively throughout training.

Visit 13-14: Fasting Plasma Glucose Test, Muscle Biopsy and Metabolism Trial

The Fasting Plasma Glucose Test and Muscle Biopsy will be performed on the same day whereas the metabolism trial will require another visit. The three tests will be identical in all respects to the tests describe above for visits 1, 5 and 6.

DESCRIPTION OF POTENTIAL RISKS AND DISCOMFORTS

Exercise Testing and Training

The potential risks and discomforts inherent to the exercise testing procedures in this study include fatigue, fainting, abnormal blood pressure, irregular heart rhythm, and in very rare cases, heart attack, stroke or death. These risks are similar to those associated to any form of strenuous physical activity. The investigators will make every effort to minimize these potential risks by evaluating your health history and by careful monitoring during testing.

Venous Blood Sampling and Muscle Biopsy

A complete description of the invasive medical procedures to be used in this study is attached. Please refer to this form for the potential risks and discomforts associated with these procedures.

HOW MANY PEOPLE WILL BE IN THIS STUDY?

For this study, we plan to recruit and test 12 subjects.

WHAT ARE THE POSSIBLE BENEFITS FOR ME AND/OR FOR SOCIETY?

As a result of this study, you will learn about your exercise capacity and receive information regarding exercise training to improve your metabolic profile and overall health.This protocol will benefit society by providing a time efficient strategy to increase physical activity and ultimately decrease the number of individuals who are sedentary. It is anticipated that with regular practice of this protocol, you could reduce your risk of inactivity-related disorders.

WHAT INFORMATION WILL BE KEPT PRIVATE?

All information and data obtained during this study will remain confidential. Appropriate measures consistent with Research Ethics Board guidelines will be taken to ensure privacy. The results from this study will be used for educational purposes and be shared with the scientific community. All personal information will be removed from the data and participants will be identified by a code number known only by the primary investigators. If the results of the study are published, your name will not be used and no information that discloses your identity will be released. Upon completion of the study, you will have access to your own data and the group data for your own interest.

CAN PARTICIPATION IN THE STUDY END EARLY?

Your participation in this study is entirely voluntary and you may choose to withdraw at any time. You also have the option of removing your data from the study. You have the freedom to refuse to answer any questions you do not feel comfortable with and still remain in the study. The investigators may also withdraw you from the study if circumstances arise which warrant doing so.

WILL I BE PAID TO PARTICIPATE IN THE STUDY?

If you agree to participate, you will be provided with an honorarium of $200.00. Should you not complete the study, you will receive a pro-rated amount based on the proportion of the study completed.

WILL THERE BE ANY COSTS?

Your participation will not involve any cost to you.

WHAT HAPPENS IF I HAVE A RESEARCH-RELATED INJURY?

If you are injured as a direct result of taking part in this study, all necessary medical treatment will be made available to you at no cost. Financial compensation for such things as lost wages, disability or discomfort due to this type of injury is not routinely available. By signing this consent form, you do not waive any legal rights under the law, nor are you releasing the investigator(s), institution(s) and/or sponsor(s) from their legal and professional responsibilities.

IF I HAVE ANY QUESTIONS OR PROBLEMS, WHOM CAN I CALL?

If you have any questions concerning this research project now or later, you may contact any one of the investigators listed on the first page of this document.

If you have questions concerning your rights as a research participant, you may contact the Office of the Hamilton Health Sciences/Faculty of Health Sciences, McMaster University Research Ethics Board Chair at 905-521-2100, ext. 42013.

The Hamilton Health Sciences/Faculty of Health Sciences, McMaster University Research Ethics Board Project Number for this study is 07-462.

CONSENT STATEMENT

SIGNATURE OF RESEARCH PARTICIPANT

I have read the preceding information thoroughly. I have had the opportunity to ask questions, and all of my questions have been answered to my satisfaction. I agree to participate in the study. I understand that I will receive a signed copy of this form.

_____
Name of Participant

_____ _____
Signature of Participant Date

Consent form administered and explained in person by:

_____
Name and title

_____ _____
Signature Date

SIGNATURE OF INVESTIGATOR

In my judgement, the participant is voluntarily and knowingly giving informed consent and possesses the legal capacity to give informed consent to participate in this study.

_____ _____
Signature of Investigator Date

DESCRIPTION OF MEDICAL PROCEDURES

Venous blood sampling

A small Teflon catheter will be inserted into a forearm vein with the assistance of a small needle, which is subsequently removed. The discomfort of this procedure is transient and very similar to having an injection by a needle, or when donating blood. Once the needle is removed there should be no sensation from the catheter. In any one experiment the total blood loss is typically less than 100 ml, which is approximately 1/6 of the blood removed during a donation to a blood bank. It is not enough blood to affect your physical performance in any way. After each blood sample has been taken, the catheter is "flushed" with a sterile saline solution in order to prevent blood from clotting in the catheter. This is a salt solution that is very similar in composition to your own blood and it will not affect you. Following removal of the catheter, pressure will be placed on the site in order to minimize bleeding and facilitate healing.

Muscle biopsy

This procedure involves the removal of a small piece of muscle tissue using a sterile hollow needle. The area over the muscle to be sampled will be cleaned and a small amount of local anaesthetic ("freezing") will be injected into and under the skin over the vastus lateralis (quadriceps) muscle. A small incision (approx. 4 mm) in the skin will then be made in order to create an opening through which to put the biopsy needle into the muscle. There is a small amount of bleeding from the incision, but this is minimal. During the biopsy, the biopsy needle will be inserted into your thigh through the incision. A small piece of muscle (approx. 50-100 mg; about the size of the eraser on the end of a pencil) will quickly be obtained and then the needle will be removed. During the time that the sample is being taken (5 sec), you may feel the sensation of deep pressure in the muscle and on some occasions this is moderately painful. However, the discomfort very quickly passes and you are quite capable of performing exercise. Occasionally, a biopsy sample yields little or no tissue sample and in this instance, it may be necessary to make a second attempt through the same incision site. Following the biopsy, the incision site will be closed with sterile bandage strips or a suture (stitch) and wrapped with a tensor bandage. You should refrain from excessive muscle use for the remainder of the day. Once the anaesthetic wears off, your leg may feel tight and often there is the sensation of a deep bruise or "Charlie Horse". You should not take any aspirin based medicine for 24 hours following the experiment as this can promote bleeding in the muscle. However, other analgesics such as Ibuprofen or Tylenol are acceptable alternatives. It is also beneficial to keep your limb elevated when you are sitting, and the periodic application of an ice pack will help reduce any swelling and residual soreness. The following day your muscle may feel uncomfortable upon movement, e.g. going down stairs. The tightness in the muscle usually disappears within 1-2 days, and the subjects routinely begin exercising normally within 2-3 days. In order to allow the incision to heal properly and minimize any risk of infection, you should avoid prolonged submersion in water for 2-3 days. Daily showers are acceptable, but baths, swimming, saunas, etc. should be avoided.

POSSIBLE RISKS AND DISCOMFORTS

Venous blood sampling

The insertion of a venous catheter for blood sampling is a common medical practice and involves minimal risk provided proper precautions are taken. The catheter is inserted under completely sterile conditions; however there is a theoretical risk of infection. There is also a chance of bleeding if adequate pressure is not maintained upon removal of the catheter. This may cause some minor discomfort and could result in bruising/skin discoloration that could last up to a few weeks. There is also the remote risk that trauma to the vessel wall could result in the formation of a small blood clot, which could travel through the blood stream and become lodged in a smaller vessel. However, we have never experienced such a complication in our laboratory after several thousand venous catheter placements.

Muscle biopsy

The biopsy technique is routinely used in physiological research, and complications are rare provided that proper precautions are taken. However, there is a risk of internal bleeding at the site of the biopsy, which can result in bruising and temporary discoloration of the skin. On occasion a small lump of fibrous tissue may form under the site of the incision, but this normally disappears within 2-3 months. As with any incision there is also a slight risk of infection, however this risk is virtually eliminated through proper cleansing of the area and daily changing of wound coverings. If the incision does not heal within a few days or you are in any way concerned about inflammation or infection, please contact us immediately. In very rare occasions there can be damage to a superficial sensory nerve that will result in temporary numbness in the area. There is also an extremely remote chance (1 in 1,000,000) that you will be allergic to the local anaesthetic. It is the collective experience of members in our laboratory that, in healthy young subjects, 1 in 2500 have experienced a local skin infection; 1 in 1000 have experienced a small lump at the site of the biopsy (in all cases this disappeared within approximately one week using gentle massage over the area of the lump); 1 in 2,000 have experienced a temporary loss of sensation in the skin at the site of incision (an area of numbness about the size of a quarter that lasted up to 4 months), and 1 in 100 have experienced mild bruising around the site of incision that lasted for 4-5 days. There is also a theoretical but extremely small risk of damage to a small motor nerve branch leading to the muscle that is being sampled but we have never experienced this complication.

APPENDIX II

SUBJECT RECRUITMENT POSTER

McMaster University

Are you sedentary?

Looking to get fit fast?

- Did you know?
- Inactivity increases your risk of chronic disease
- "Lack of time" is the most common excuse for not being active
- Short bursts of intense exercise can be a time efficient way to train

We are recruiting inactive but healthy individuals aged 35-50 y. Subjects will perform a short (2 week) interval exercise training program. This study requires blood sampling and two muscle biopsies. Individuals will receive remuneration for their participation. For more information or to participate, please contact:

Melanie: gardnems@mcmaster.ca

905-525-9140 ext. 26825

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APPENDIX III

RESEARCH ETHICS BOARD APPROVAL FORM

Hamilton Health Sciences

RESEARCH ETHICS BOARD

McMaster University

REB Office, 1057 Main St. W., Hamilton, ON L8S 1B7
Telephone: 905-521-2100, Ext. 42013
Fax: 905-577-8379

January 25, 2008

PROJECT NUMBER: **07-462**

PROJECT TITLE: The Effect of High-Intensity Interval Training on Skeletal Muscle Oxidative Capacity and Glycemic Regulation in Pre-Diabetics

PRINCIPAL INVESTIGATOR: **Dr. M. Gibala**

This will acknowledge receipt of your letter dated January 22, 2008 which enclosed the revised sections of the REB application, the revised Participant Information Sheet and Consent Form, the revised recruitment poster along with a response to the specific questions raised by the Research Ethics Board at their meeting held on December 18, 2007. Based on this additional information, we wish to advise your study has been given ***final*** approval from the full REB. The research protocol, including the revised Participant Information Sheet and Consent Form, version 2 dated January 23, 2008 and the recruitment poster was found to be acceptable on both ethical and scientific grounds. ***Please note*** attached you will find the Information Sheet with the REB approval affixed; all consent forms and recruitment materials used in this study must be copies of the attached materials.

We are pleased to issue final approval for the above-named study for a period of 12 months from the date of the REB meeting on December 18, 2007. Continuation beyond that date will require further review and renewal of REB approval. Any changes or amendments to the protocol or information sheet must be approved by the Research Ethics Board.

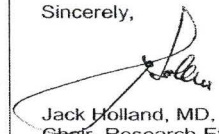
The Hamilton Health Sciences/McMaster Health Sciences Research Ethics Board operates in compliance with the ICH Guidelines Good Clinical Practice Guidelines, the Tri-Council Policy Statement: Ethical Conduct for Research Involving Humans and Division 5 Health Canada Food and Drug Regulations.

Investigators in the Project should be aware that they are responsible for ensuring that a complete consent form is inserted in the patient's health record. In the case of invasive or otherwise risky research, the investigator might consider the advisability of keeping personal copies.

A condition of approval is that the physician most responsible for the care of the patient is informed that the patient has agreed to enter the study.

PLEASE QUOTE THE ABOVE-REFERENCE PROJECT NUMBER ON ALL FUTURE CORRESTPONDENCE

Sincerely,



Jack Holland, MD, FRCP, FRCP (C)
Chair, Research Ethics Board

APPENDIX IV

SUBJECT CHARACTERISTICS

Subject Characteristics

Subject ID	Age	Sex	RHR	Height	Weight
			bpm	cm	kg
2	39	F	80	162.5	59.0
5	52	F	72	167.0	65.5
6	45	M	84	187.5	110.0
8	36	F	61	160.0	72.0
9	44	F	78	160.0	59.5
10	47	M	58	178.0	111.0
12	50	M	62	165.0	72.0
Average	**45**		**70.7**	**168.6**	**78.4**
SD	**6**		**10.4**	**10.4**	**22.5**
SEM	**2**		**3.9**	**3.9**	**8.5**

BMI	WC	BP	VO2peak	HRmax	RER-max
kg/m^2	cm	sys/dia	ml/kg/min		
22.3	73.0	111/72	34.80	175	1.50
23.5	79.5	112/72	25.70	169	1.42
31.3	111.0	150/98	31.30	179	1.32
28.1	88.0	120/72	27.30	188	1.38
23.2	71.0	132/91	28.00	173	1.36
35.0	110.0	126/82	30.00	171	1.34
26.4	96.5	127/74	31.80	169	1.39
Average	**27.1**		**29.8**	**175**	**1.4**
SD	**4.7**		**3.1**	**7**	**0.1**
SEM	**1.8**		**1.2**	**3**	**0.0**

APPENDIX V

FASTING INSULIN AND GLUCOSE

Fasting Plasma Glucose

Subject ID	FPG - Pre	FPG - Post	Change
	mmol/L	mmol/L	
2	4.6	3.7	-20%
5	4.4	3.6	-18%
6	5.2	4.7	-10%
8	4.4	4.6	5%
9	4.2	4.3	2%
10	4.8	4.9	2%
12	4.8	4.0	-17%
Average	**4.6**	**4.3**	**-8%**
SD	**0.3**	**0.5**	**11%**
SEM	**0.1**	**0.2**	**4%**

Fasting Plasma Insulin

Subject ID	FPI-pre	FPI-post	Change
	mmol/L	mmol/L	
2	7.1	6.9	-4%
5	7.5	6.6	-13%
6	9.1	6.7	-27%
8	3.7	3.7	0%
9	7.2	5.9	-18%
10	15.2	12.5	-18%
12	6.6	4.2	-36%
Average	**8.1**	**6.6**	**-16%**
SD	**3.5**	**2.9**	**13%**
SEM	**1.3**	**1.1**	**5%**

APPENDIX VI

METABOLISM TRIAL RESULTS

Metabolism Trial – Pre-training

Subject ID	HR-2	Vent-2	RER-2	VO2-2	VO2-2	VCO2-2
	bpm			ml/kg/min	ml/min	ml/min
2	145	42.55	0.98	24.43	1440.1	1414.0
5	153	39.93	0.95	19.46	1289.0	1217.8
6	159	67.20	0.96	20.59	2269.1	2174.6
8	159	41.13	0.93	20.73	1494.8	1388.8
9	144	41.57	0.92	21.65	1296.0	1189.9
10	142	71.06	0.96	21.79	2420.8	2314.3
12	128	46.36	0.89	23.28	1679.6	1500.8
Average	**147**	**50.0**	**0.9**	**21.7**	**1698.5**	**1600.0**
SD	**11**	**13.3**	**0.0**	**1.7**	**462.9**	**455.3**
SEM	**5**	**5.4**	**0.0**	**0.7**	**189.0**	**185.9**

Glucose			Lactate		
T0	**T1**	**T2**	**T0**	**T1**	**T2**
4.6	3.2	3	1.8	4.5	4.6
n/a	n/a	n/a	n/a	n/a	n/a
5.2	3.8	3.5	1.2	2.7	3.0
6.2	4.6	4.5	1.9	4.5	4.8
4.7	3.6	3.3	1.2	3.9	4.7
8.3	6.8	4.8	1.9	3.7	3.8
4.4	4.3	3.6	2.0	3.3	3.4
5.6	**4.4**	**3.8**	**1.7**	**3.8**	**4.1**
1.5	**1.3**	**0.7**	**0.4**	**0.7**	**0.8**
0.6	**0.3**	**0.2**	**0.1**	**0.2**	**0.2**

Metabolism Trial – Post-training

Subject ID	HR-2	Vent-2	RER-2	VO2-2	VO2-2	VCO2-2
	bpm			ml/kg/min	ml/min	ml/min
2	137	37.18	0.94	22.75	1342.0	1260.2
5	158	41.1	0.9	19.98	1304.9	1170.0
6	152	68.54	0.94	21.77	2399.5	2261.8
8	148	46.07	0.92	22.98	1657.8	1520.0
9	127	41.89	0.94	23.08	1371.6	1284.2
10	141	75.08	0.96	21.58	2398.5	2308.3
12	135	45.92	0.93	23.86	1754.9	1632.5
Average	**142.5**	**50.8**	**0.9**	**22.3**	**1747.0**	**1633.8**
SD	**10.6**	**14.8**	**0.0**	**1.3**	**475.9**	**472.4**
SEM	**4.3**	**6.0**	**0.0**	**0.5**	**194.3**	**192.9**

Glucose			Lactate		
T0	T1	T2	T0	T1	T2
4.6	3.8	3.4	1	3.9	3.9
n/a	n/a	n/a	n/a	n/a	n/a
5.2	4.0	4.1	1.0	3.0	3.0
5.0	4.6	4.1	1.0	2.4	3.5
4.6	3.2	3.0	1.0	4.6	4.5
9.2	5.6	4.3	2.1	3.7	4.7
4.5	4.2	3.3	0.9	2.1	2.2
5.5	**4.2**	**3.7**	**1.2**	**3.3**	**3.6**
1.8	**0.8**	**0.5**	**0.5**	**1.0**	**0.9**
0.7	**0.2**	**0.1**	**0.1**	**0.3**	**0.3**

APPENDIX VII

CITRATE SYNTHASE RESULTS

Citrate Synthase Activity

Subject	CSPre	CSPost	% Change
	mol/kg protein/h ww		
2	11.41	13.31	17%
5	9.22	12.19	32%
6	12.13	11.74	-3%
8	9.98	14.04	41%
9	11.44	15.38	34%
10	12.49	12.84	3%
12	7.72	10.62	37%
Average	**10.63**	**12.87**	**23%**
SD	**1.72**	**1.56**	**0.18**
SEM	**0.65**	**0.59**	**0.07**

Citrate Synthase Content

Subject	CSPre	CSPost	% Change
	arbitrary units		
2	7670	7706	47%
5	6171	8264	34%
6	8303	8987	8%
8	3856	5716	48%
9	4534	7066	55%
10	5484	5960	9%
12	6712	6690	0%
Average	**6104**	**7198**	**18%**
SD	**1610**	**1197**	**0.18**
SEM	**608**	**452**	**0.07**