

**THE IMPACT OF INTERVAL INTENSITY IN
OVERWEIGHT YOUNG MEN**

by

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Lay Abstract

Considering the increasing global prevalence of overweight and obesity and their propensity for disease, this study was undertaken in an attempt to optimize exercise prescription for this at-risk group by determining if the benefits associated with interval training occur in an intensity dependent manner. 19 sedentary, overweight males (Age: 22.7 ± 3.9 yrs, BMI: 31.4 ± 2.6 kg/m², WC: 106.5 ± 6.6 cm) performed interval training for three weeks at either 70% or 100% of their peak work rate on a cycle ergometer. Aerobic capacity measurements, time to completion trials, muscle biopsies, and fasted blood samples were all performed pre and post training. Analyses of aerobic capacity and exercise performance demonstrate greater improvements made in the 100% compared to the 70% group, while measures of skeletal muscle oxidative capacity indicate equivalent changes between groups. Taking into account the similar increases in mitochondrial content in both groups and understanding the influence of both oxygen supply and demand in determining maximal oxygen consumption, the greater increases in aerobic capacity achieved by the 100% group may be the result of enhanced cardiovascular adaptations. These findings suggest that some of the health benefits associated with interval exercise may be intensity dependent. Therefore, there may be additional benefit to exercise at higher intensities.

Co-Authorship

This thesis presents the work of Colin Boyd in collaboration with Dr. Brendon Gurd.

The impact of interval intensity in overweight young men is presented according to the guidelines for the Journal of Physiology. Colin Boyd was responsible for reviewing relevant literature to identify the research question, conducting the training study, performing the data analyses, and drafting the manuscript. All aspects were a collaborative effort between Colin Boyd and Dr. Brendon Gurd. In addition, Dr. Craig Simpson provided medical supervision and contributed to study design. Finally, Dr. Mary Jung contributed to the drafting of the manuscript.

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List of Abbreviations

ADP – adenosine diphosphate

AICAR – analog 5-aminoimidazole-4-carboxamide ribonucleotide

AMP – adenosine monophosphate

AMPK – AMP kinase

ANOVA – analysis of variance

ATP – adenosine triphosphate

βHAD – β-hydroxyacyl-CoA dehydrogenase

BMI – body mass index

bpm – beats per minute

COX – cytochrome c oxidase

cm – centimetre

CS – citrate synthase

CV – confidence variable

DMM – Dual Mode Model

EDTA – ethylene diamine tetra-acetic acid

EGTA – ethylene glycol tetra-acetic acid

ET – endurance training

ETC – electron transport chain

FS – feeling scale

g – gram

GAPDH – glyceraldehyde 3-phosphate dehydrogenase

GLUT4 – glucose transporter 4

HCl – hydrogen chloride

HIT – high-intensity interval training

HR – heart rate

IL-6 – interleukin-6

kcal – kilocalorie

kg – kilogram

L – litre

m – metre

M – molar

min – minute

mL – millilitre

mM – millimolar

mRNA – messenger ribonucleic acid

NAD⁺ – nicotinamide adenine dinucleotide

NAMPT – nicotinamine phosphoribosyltransferase

nm – nanometre

O₂ – oxygen

pg – picogram

PGC-1 α – peroxisome proliferator-activated receptor gamma coactivator 1-alpha

P_i – inorganic phosphate

RPM – revolutions per minute

RSV – resveratrol

s - second

SD – standard deviation

SDS-PAGE – sodium dodecyl sulfate polyacrylamide gel electrophoresis

SIRT1 – silent mating type information regulation 2 homolog 1

SV – stroke volume

TNF α – tumor necrosis factor alpha

μg – microgram

μIU – micro international unit

μL – microlitre

μmol – micromole

VO $_2$ – volume of oxygen

W – watts

WC – waist circumference

yrs - years

Chapter 1

Introduction

1.1 General Introduction

Obesity has reached epidemic proportions in both developed and developing countries around the world, making it the most prevalent nutritional problem over the past 20 years (Lau *et al*, 2007). Overweight and obesity are precursors to a litany of diseases including cardiovascular disease, diabetes, hypertension, dyslipidemia, certain types of cancer, and premature mortality (Foster and Burton, 1985). In addition to these individual implications, the economic burden associated with physical inactivity and obesity was estimated at \$9.6 billion in 2001 (Katzmarzyk and Janssen, 2004). The most recent reports suggest that 36.8% of the Canadian adult population is overweight (Statistics Canada, 2011), including a substantial proportion (30.7%) of Canada's young adults between the ages of 20-34 (Statistics Canada, 2011). Srinivasan et al. (Srinivasan *et al*, 1996) have shown a tendency for 58% of overweight adolescents to remain overweight and obese as they became young adults. From the initial group of overweight and obese adolescents measured, those that remained overweight at follow up as adults (28 yrs. old) had significantly higher blood pressure, total cholesterol, triglycerides, resting glucose, and resting insulin as compared to the overweight and obese adolescents who had become lean adults (Srinivasan *et al*, 1996). Thus, the prevention of future disease begins with

interventions targeting younger overweight and obese individuals before they become overweight/obese adults (Guo *et al*, 2000).

1.2 Endurance Training: A Failed Cure

Endurance training (ET), defined as a bout of prolonged steady-state activity, is an effective strategy to reduce body weight (Ross *et al*, 2000, Bouchard *et al*, 1990), improve cardiovascular and metabolic health (Hawley, 2004, Holloszy and Coyle, 1984), increase insulin sensitivity (Bruce *et al*, 2006, Houmard *et al*, 2004), and reduce the risk of developing metabolic disorders such as type 2 diabetes (Sieverdes *et al*, 2010). However, despite the knowledge that 150 minutes of moderate to vigorous endurance exercise per week will improve health (Canadian Society for Exercise Physiology, 2011), adherence to endurance training programs is poor. This is perhaps due to the large time demand required by ET, as evidenced by individuals often citing lack of time as a major barrier to exercise (King *et al*, 2000, Trost *et al*, 2002). Due to lack of adherence to low-intensity ET, recent American College of Sports Medicine (ACSM) guidelines have placed greater emphasis on shorter-duration, higher intensity exercise (Haskell *et al*, 2007).

1.3 High Intensity Interval Training

High-intensity interval training (HIT) consists of repeated bouts of intense exercise, often near or above maximal aerobic capacity, interspersed by periods of recovery. HIT is emerging as a potential alternative to traditional endurance exercise (Gibala *et al*, 2006) due to its reduced overall exercise time (150 minutes per week of

moderate exercise vs. 60 minutes per week for the protocol utilized in the current thesis). Importantly, interval training has been shown to induce numerous physiological improvements such as increased VO_2peak , mitochondrial biogenesis, and metabolic adaptations to the same extent as endurance exercise (Burgomaster *et al*, 2005, Burgomaster *et al*, 2008, Gibala and McGee, 2008) as well as reducing various cardiovascular disease risk factors (Earnest, 2009).

However, despite the effectiveness of HIT, it is important to recognize that the increased efficiency comes at the cost of higher exercise intensity. Intensity and pleasure of exercise are inversely related and as such the requisite high intensity of HIT protocols may negatively impact adherence (Ekkekakis *et al*, 2008). Specifically, the exercise intensity of HIT protocols can surpass the ventilatory threshold and require supramaximal workloads in some cases (Gibala *et al*, 2006, Gibala and McGee, 2008, McKay *et al*, 2009). Accordingly, it would be beneficial to find a minimum threshold intensity for interval training at which subjects can experience improvements without excessive displeasure. To this point there has been very limited research directed towards determining the optimal/minimal dose of HIT required to improve fitness and health (Hazell *et al*, 2010, Dalleck *et al*, 2010). Given the proposed relationship between exercise intensity, pleasure, and adherence, finding the lowest intensity of HIT still associated with benefit could have significant implications for exercise adherence.

1.4 HIT for Overweight Young Adults

Research examining the application of HIT in populations with, or at risk for, cardiometabolic disorders is limited but recent work has attempted to accommodate such at-risk groups (Gibala *et al*, 2012). As little as six sessions of HIT over 2 weeks has been shown to improve aerobic capacity (Whyte *et al*, 2010) and insulin sensitivity (Whyte *et al*, 2010, Hood *et al*, 2011) in a sedentary, overweight population as well as improve glucose control in type 2 diabetics (Little *et al*, 2011). Further, HIT has stimulated increased muscle oxidative capacity and GLUT4 protein content in sedentary overweight (Hood *et al*, 2011) and type 2 diabetic populations (Little *et al*, 2011).

Unfortunately, there is an inverse relationship between body weight and enjoyment of exercise where obese individuals report significantly lower pleasure ratings during incremental exercise than normal weight and overweight individuals (Ekkekakis *et al*, 2010). Thus, the fact that more at-risk groups enjoy exercise less, especially at higher intensities (Ekkekakis *et al*, 2008) is a dangerous combination and requires the development of a HIT protocol that will be effective and promote adherence in this special population. As such, there is a need for HIT to evolve from the extreme demands of maximal and supramaximal intensities to a more practical level. At present there is little information available regarding what the optimal intensity of interval exercise is, making informed and effective prescription of HIT impossible.

1.5 Study Purpose and Objectives

The purpose of this research was to examine the dose-response relationship of the intensity of interval exercise to its cardiometabolic benefits, particularly in this at-risk group. Knowledge of such a relationship will facilitate prescription of effective and time-efficient HIT protocols for sedentary, overweight populations. This relationship was explored through the original research presented in this thesis involving a HIT exercise protocol performed on a stationary cycle ergometer. Sedentary, overweight young men were separated into two groups performing 9 sessions of a HIT protocol over a duration of three weeks at either 70% or 100%. Their improvements were measured in terms of aerobic capacity, exercise performance, skeletal muscle oxidative capacity, maximal cardiovascular function, and inflammation status.

1.6 Thesis Organization

This manuscript-style thesis conforms to the regulations as outlined in the Queen's School of Graduate Studies and Research "General Forms of Theses". Chapter 2 is a review of the literature regarding interval exercise and the underlying mechanisms that mediate its effects on health. Chapter 3 contains the manuscript detailing the study of the impact of interval intensity of HIT in a sedentary, overweight population and its health benefits. Chapter 4 then provides a discussion of the implications of this research as well as the ongoing search for the optimal exercise dose. Finally, several appendices are attached to the end of this document to provide more complete examples of items referred to throughout the thesis.

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Chapter 2

Literature Review

2.1 Overview

Research on interval training as an alternative mode of exercise has increased substantially over the past ten years as it emerges as a viable alternative to more traditional forms of exercise while maintaining the important physiological benefits. This chapter will begin with an exploration of the development of interval training to this point, followed by an examination of the underlying cardiovascular and metabolic adaptations that mitigate its response. The purpose of this chapter is to provide the reader with an understanding of the current body of literature surrounding the physiology of interval training.

2.2 Key Definitions

Moving forward, an understanding of some key terms will aid in the appreciation of this review. The ultimate goal of this research is to benefit groups at risk for future disease, specifically young overweight (waist circumference greater than 94 cm) and obese (waist circumference greater than 102 cm) adults (Lean *et al*, 1995). The mode of exercise most thoroughly described throughout this thesis will be high intensity interval training (HIT), which is characterized by relatively brief, intermittent periods of muscle contraction against maximal or near maximal resistance separated by up to a few minutes of rest or low-intensity exercise for recovery (Gibala, 2009). This could include periods of

running interspersed between periods of walking, but will most often refer to intervals of cycling against a work load designed to elicit a certain percentage of peak oxygen (O_2) uptake followed by an interlude of cycling against no resistance.

The important benefits associated with exercise will also be thoroughly discussed throughout this thesis. Aerobic capacity will be the primary outcome of the research and will be measured in terms of VO_{2peak} , the peak rate of O_2 consumed by an individual during incremental exercise (Krahenbuhl *et al*, 1985). This gives an indication of the body's gas transport system as well as its pulmonary, cardiovascular, and muscular capacities (Krahenbuhl *et al*, 1985), and is one of the most important predictors of all-cause and cardiovascular-specific mortality (Keteyian *et al*, 2008). We will also be examining possible mechanisms that may contribute to determining adaptations to VO_{2peak} with training. Among these mechanisms are increases in skeletal muscle mitochondrial content, the products of mitochondrial biogenesis. As the name suggests, mitochondrial biogenesis refers to the creation of new mitochondria within tissue, specifically following exercise (Little *et al*, 2011b). Although exercise can induce mitochondrial biogenesis in multiple tissues such as adipose tissue (Laye *et al*, 2009) liver (Boveris and Navarro, 2008), brain (Navarro *et al*, 2004), and kidney (Navarro *et al*, 2004), this thesis will be focusing on mitochondrial biogenesis within skeletal muscle.

2.3 Prevalence of obesity

Obesity has reached epidemic proportions around the world, making it the most prevalent nutritional problem over the past 20 years (Lau *et al*, 2007). In 2005, an

estimated 23.2% of the world's adult population was overweight, and 9.8% was obese (Kelly *et al*, 2008). If current trends were to continue, 57.8% of the world's adult population, a total of 3.3 billion people, could be overweight or obese by the year 2030 (Kelly *et al*, 2008). Others project 80% of all American adults will be overweight or obese if current trends continue for the next 15 years (Wang *et al*, 2008). These same calculations suggest that, by the year 2030, the costs attributable to overweight and obesity could range from \$860.7 to 956.9 billion US dollars, making up approximately 16% of healthcare expenditure (Wang *et al*, 2008).

The most recent data for the Canadian population describes 19% of males and 21% of females aged 20 to 39 years as obese, based on body-mass index (BMI) measurements (Shields *et al*, 2010). Similarly, 21% of males and 31% of females aged 20 to 39 were considered to be at high risk for health problems based on their waist circumference (Shields *et al*, 2010). Consider as well that 37% of males and 23% of females were considered overweight based on BMI, totalling 56% of males and 44% of females as overweight or obese (Shields *et al*, 2010). This is problematic as longitudinal studies have indicated that maximum BMI in young adulthood is a strong predictor of adulthood BMI, total body fat, and percent body fat (Guo *et al*, 2000). Thus, the fitness and health of this at-risk population (young overweight/obese adults) must be improved prior to adulthood as a BMI of $>25 \text{ kg/m}^2$ at age 18 is associated with significantly increased mortality after 20 years as compared to those with a BMI below 25 (Hoffmans

et al, 1988). Exercise is a possible preventative intervention for this condition, but the optimal exercise modality for this population is unknown.

2.4 Traditional Endurance Training

Known to stimulate important health benefits, endurance training (ET), defined as a bout of prolonged steady-state activity, has traditionally been prescribed according to national physical activity guidelines (Haskell *et al*, 2007, Canadian Society for Exercise Physiology, 2011). ET can facilitate improvements in exercise capacity through physiological adaptations that allow an individual to sustain a submaximal workload for longer (Coyle, 1995) or achieve a higher power output over a fixed distance or time (Hawley, 2002). In fact, as little as 3 days of ET elevates submaximal exercise tolerance and improves endurance capacity (Green *et al*, 1995). Additionally, ET is useful to reduce body weight (Ross *et al*, 2000), improve cardiovascular and metabolic health (Hawley, 2004, Holloszy and Coyle, 1984), increase insulin sensitivity (Bruce *et al*, 2006, Houmard *et al*, 2004), and reduce the risk of developing metabolic disorders such as type 2 diabetes (Sieverdes *et al*, 2010). However, national physical activity guideline recommendations of 150 minutes of exercise per week (Canadian Society for Exercise Physiology, 2011) make ET highly time consuming, an important factor considering individuals often cite lack of time as a major barrier to exercise (Stutts, 2002, Trost *et al*, 2002). Further, lack of enjoyment has been implicated as a reason why individuals are reluctant to engage in traditional endurance exercise (Bartlett *et al*, 2011, Wisloff *et al*, 2007, Leslie *et al*, 1999).

2.5 HIT – All grown up

HIT is an effective alternative to traditional ET, inducing similar or even superior changes in a range of physiological and health-related markers in both healthy individuals and diseased populations when compared on a matched-work basis (Wisloff *et al*, 2007, Tjonna *et al*, 2009, Hwang *et al*, 2011). HIT induces equivalent or superior improvements, compared to ET, in BMI (Tjonna *et al*, 2009, Buchan *et al*, 2011), body weight (Tjonna *et al*, 2008), mean arterial pressure (Buchan *et al*, 2011, Tjonna *et al*, 2008), endothelial function (Tjonna *et al*, 2009, Tjonna *et al*, 2008), capillary density (Daussin *et al*, 2008), VO₂peak (Tjonna *et al*, 2009, Daussin *et al*, 2008, McKay *et al*, 2009, Macpherson *et al*, 2011, Helgerud *et al*, 2007), VO₂ kinetics (Daussin *et al*, 2008, McKay *et al*, 2009), exercise performance (Buchan *et al*, 2011, Macpherson *et al*, 2011, Gibala *et al*, 2006), β -oxidation (Tremblay *et al*, 1994), and muscle oxidative capacity (Daussin *et al*, 2008, Burgomaster *et al*, 2005, Burgomaster *et al*, 2008). Further, many of the benefits of HIT, as compared to ET, can occur in as little as two (Gibala *et al*, 2006, Burgomaster *et al*, 2005) to three (McKay *et al*, 2009) weeks of training as well as requiring less than 25% of the time and less than 10% of the energy of ET (Gibala *et al*, 2006, Burgomaster *et al*, 2005). This reduced time commitment associated with HIT is important considering lack of time remains one of the most commonly cited barriers to regular exercise participation (Stutts, 2002, Trost *et al*, 2002). The innate active recovery phases associated with HIT also help to remove lactic acid from working muscles (Bangsbo *et al*, 1994), likely extending tolerable exercise time (Hwang *et al*, 2011).

Additionally, HIT has been shown to be more enjoyable than moderate-intensity continuous exercise through higher ratings of perceived enjoyment despite concomitant higher ratings of perceived exertion (Bartlett *et al*, 2011).

2.5.1 Optimizing HIT

Despite reports that low-volume HIT may induce similar physiological remodelling comparable with moderate-intensity ET (Burgomaster *et al*, 2008, Gibala and McGee, 2008), supra-maximal HIT such as Wingate-based training may not be safe, tolerable, or appealing for some individuals (Gibala *et al*, 2012, Gayda *et al*, 2012, Gaesser and Angadi, 2011). Further, exercise that exceeds the ventilatory threshold has shown significant and relatively homogenous reductions in pleasure as compared to lower intensities, an effect that could negatively impact adherence to higher intensities of exercise (Ekkekakis *et al*, 2008). This negative relationship could be further exacerbated in obese populations who report lower pleasure ratings during incremental exercise than their normal weight and overweight peers (Ekkekakis *et al*, 2010). Accordingly, more practical models of HIT that demand less intensity but remain time efficient and can be applied to populations such as those at risk for chronic metabolic disease are currently being developed (Gayda *et al*, 2012). Such HIT protocols continue to be effective at inducing rapid skeletal muscle remodeling towards a more oxidative phenotype despite a reduction in intensity (Little *et al*, 2010b).

2.5.2 Dose-response relationship

A major limitation to the prescription of HIT is our lack of knowledge regarding whether physiological adaptations associated with HIT are dose-dependent. Only a handful of studies have manipulated HIT protocols in terms of training frequency (Dalleck *et al*, 2010) or interval duration (Helgerud *et al*, 2007, Hazell *et al*, 2010). Similar to any pharmacologic or health-based intervention, the precise dose of exercise to be prescribed must be carefully calculated prior to dissemination throughout a population. This notion takes on particular relevance considering the potential negative impact of high intensity exercise on adherence due to possible nausea, light-headedness (Richards *et al*, 2010) and decreases in the pleasure associated with exercise (affective response; (Ekkekakis *et al*, 2008), especially in obese populations (Ekkekakis *et al*, 2010). At this point we are not aware of any research that has compared the impact of identical HIT training protocols conducted at different intensities. This information is crucial for prescription of effective HIT protocols that will maximize benefit to the individual while remaining time efficient. Therefore, the purpose of this original research was to begin to understand the optimization of HIT protocols in terms of maximizing benefit while minimizing exercise intensity with a focus on the underlying mechanisms related to improved health.

2.6 VO₂peak – The gold standard measurement of aerobic fitness

Owing to its non-invasive nature, low relative cost, and breadth of relevant information, the VO₂peak test is an important tool in the assessment of aerobic capacity

and health (Myers *et al*, 2002). The importance of aerobic capacity as an indicator of health has been reported in various longitudinal studies in healthy (Ekelund *et al*, 1988, Blair *et al*, 1989) and diseased (Keteyian *et al*, 2008, Kavanagh *et al*, 2002) populations. Of all established risk factors, low aerobic capacity appears to be the strongest predictor of all-cause and cardiovascular specific mortality (Keteyian *et al*, 2008, Myers *et al*, 2002). The physical fitness level for optimal health in men has been estimated at 35 mL/kg/min among healthy men and women, with 7.9% reductions in mortality for every increase of 3.5 mL/kg/min (Blair *et al*, 1989). Similarly, a 1 mL/kg/min increase in VO_2 peak is associated with between a 3.4% and 15% decrease in risk for all-cause and cardiovascular disease-specific mortality among cardiovascular-diseased populations (Keteyian *et al*, 2008, Myers *et al*, 2002, Kavanagh *et al*, 2002). Accordingly, increases in VO_2 peak are paramount to improved health and are an important outcome of aerobic exercise training. The current thesis will work towards optimizing HIT for improving VO_2 peak in overweight and obese young adults, a pre-clinical population.

2.6.1 A Closer Look at the Components of VO_2

The Fick equation describes VO_2 as the product of cardiac output (Q) and the arterio-venous O_2 difference (a- vO_2 diff) (Astrand, P.O. & Rohald, K., 1986). Cardiac output refers to the product of heart rate (HR) and stroke volume (SV) ($Q = HR \cdot SV$) (Crisafulli *et al*, 2007), where stroke volume is defined as the amount of blood pumped per contraction of the heart. The arterio-venous O_2 difference accounts for the differing content of O_2 between arterial and venous blood (a- vO_2 diff = $[O_2]$ arterial blood – $[O_2]$

venous blood) (Bhambhani *et al*, 1994). The propensity of O₂ to leave the arterial blood and be transported into skeletal muscle, thereby increasing the (a-vO₂diff), is determined by both conductance into and consumption by the skeletal muscle. Conductance in this case is dependent on capillarization (Hepple *et al*, 1997) and consumption is a function of mitochondrial content (Robinson *et al*, 1994). The resulting equation is:

$$(1) \quad \text{VO}_2 = Q \cdot \text{a-vO}_2\text{diff} \quad (\text{Astrand \& Rohald, 1986})$$

2.6.2 Supply or Demand – Which is limiting during maximal exercise?

The dynamic interaction between skeletal muscle and the cardiovascular system during exercise make it difficult to determine which of the two are limiting during maximal exercise (Figure 1, Richardson *et al*, 2000). With the existence of evidence for both mechanisms available, determining which of either skeletal muscle or the cardiovascular system is limiting VO_{2peak} becomes a complex process dependent on a variety of factors. Acute increases in O₂ supply during exercise have shown increases in VO_{2peak} through elevated O₂ delivery to skeletal muscle (Knight *et al*, 1993, Richardson *et al*, 1999), thereby pointing to VO_{2peak} as being limited by O₂ supply. This limit of O₂ delivery has thus been defined as the upper limit of muscle VO_{2peak} (Hepple, 2000). However, studies increasing the relative fraction of inspired O₂ (from 12 to 21 to 100% O₂) have reported diminishing returns in increased intracellular PO₂ at the greatest content of inspired O₂ (Richardson *et al*, 1999). This would indicate that the elevated O₂

availability is in excess of the capacity of skeletal muscle to consume O₂, determined by both mitochondrial content (Robinson *et al*, 1994, Mcallister and Terjung, 1990) and capillarization (Hepple *et al*, 1997, Hughson *et al*, 1996). Therefore, it is important to consider alternative determinants of O₂ consumption in addition to bulk O₂ delivery and increases in SV that may facilitate improvements in VO₂peak.

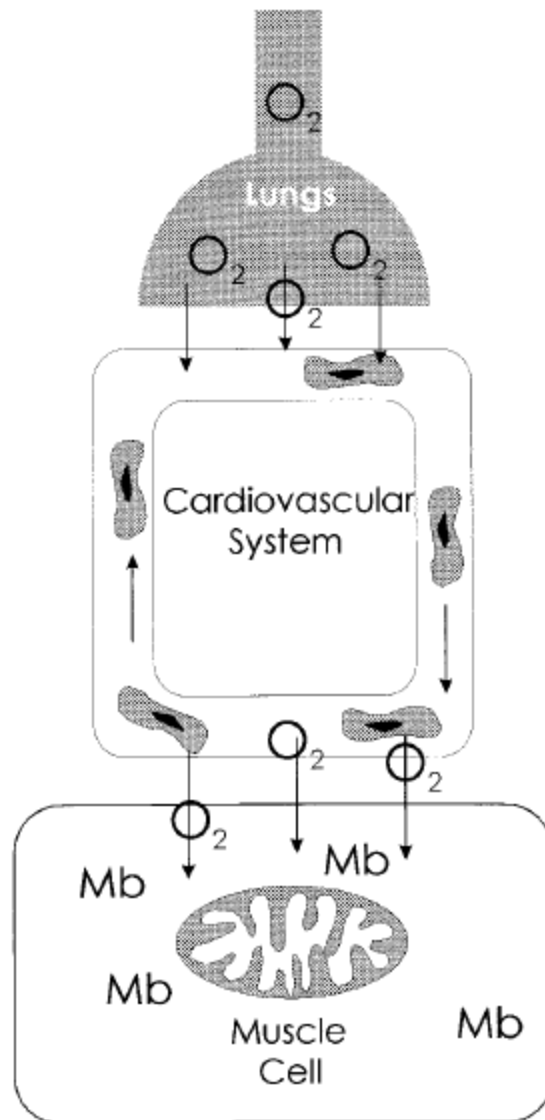


Figure 1. Oxygen (O₂) delivery by the cardiorespiratory system must match the demand of working skeletal muscle. Figure from Richardson et al. (2000). Mb; myoglobin.

2.6.3 VO₂peak improvement through capillarization and mitochondrial biogenesis

Support for the theory that skeletal muscle can independently influence VO₂peak is confirmed by the highly adaptable nature of skeletal muscle in response to altering metabolic demand (Hepple, 2000). The a-vO₂diff term of the Fick equation, a marker of the maximal extraction of O₂ from blood, is a function of both conductance and utilization of O₂ from the arterial system into skeletal muscle. The presence of an O₂ gradient between the arterial system and skeletal muscle indicates a resistance to O₂ diffusion, which is known to be dependent on number of capillaries (Mathieu-Costello *et al*, 1996, Poole *et al*, 1989) as well as the size of the capillary-to-fiber interface (Mathieu-Costello, 1993, Poole and Mathieu-Costello, 1996). The concept that the capillary-to-fiber interface is a major site of resistance for O₂ flux is also consistent with evidence supporting a diffusion limitation to VO₂peak (Wagner, 1995, Wagner, 1988). This is easily understood with the Fick equation as a reduced opportunity for O₂ diffusion would lead to a decrease in the a-vO₂diff, thereby limiting VO₂.

Just as capillaries determine O₂ conductance into skeletal muscle, mitochondria set the O₂ demand of a muscle. Of importance when considering the ability of muscle to utilize O₂ is the understanding that mitochondrial oxidative capacity is limiting (McCallister and Terjung, 1990). Accordingly, an increase in mitochondrial electron transport chain (ETC) activity, as can occur with exercise training, is required to increase VO₂peak (Robinson *et al*, 1994). Therefore, independent of O₂ availability, the tight

respiratory control obtained by an elevated mitochondrial content after training may be a possible mechanism by which mitochondria contribute to an increased VO_2 peak (Robinson *et al*, 1994). Interestingly, a proportional relationship exists with the complementary adaptation of capillaries and mitochondria, demonstrated by a constant ratio of the size of the capillary to fiber interface and mitochondrial volume/fiber length (Mathieu-Costello, 1993, Poole and Mathieu-Costello, 1996). This relationship is thought to be an important factor in determining blood-tissue O_2 exchange kinetics (Mathieu-Costello, 1993).

2.6.4 O_2 pulse: An indirect measurement of stroke volume and cardiovascular function

O_2 pulse is simultaneously the product of the SV and the arterio-venous O_2 difference as well as the ratio of absolute O_2 consumption (VO_2 , L/min) to HR (VO_2/HR) (Astrand & Rohald, 1986). O_2 pulse has been suggested as a direct result of alterations in SV with exercise (Kasch *et al*, 1973, Mahler *et al*, 1985), which is one of the most important markers of the functional state of the heart and relative fitness (Crisafulli *et al*, 2007, Whipp *et al*, 1996). Due to the invasive measurement techniques required to directly measure SV, indirect measurement by calculation from the Fick equation is considered an acceptable substitute (Crisafulli *et al*, 2007). Accordingly, a separation of the cardiac output into its components with isolation for $\text{SV} \cdot \text{a-vO}_2\text{diff}$ and the resulting O_2 pulse can be performed as follows:

$$(2) \quad \text{VO}_2 = (\text{HR} \cdot \text{SV}) \cdot \text{a-vO}_2\text{diff}$$

$$(2.1) \quad \text{VO}_2/\text{HR} = \text{SV} \cdot \text{a-vO}_2\text{diff}$$

$$(2.2) \quad \text{O}_2 \text{ pulse} = \text{VO}_2/\text{HR} \quad (\text{Bhambhani } et al, 1994)$$

In the current thesis we will be measuring VO_2peak and HR as well as mitochondrial content. Based on the theory that mitochondrial content and capillary density change in concert (Mathieu-Costello, 1993), we can assume that oxidative capacity represents an index of a-v O_2diff . Therefore, having measured VO_2 and HR, we will be able to estimate the a-v O_2diff using equation 2.1 (based on changes in skeletal muscle oxidative capacity) and will therefore be able to solve for SV.

2.6.5 Ability of HIT to improve VO_2peak

Cardiorespiratory endurance has long been recognized as one of the fundamental components of physical fitness (Astrand, P.O. & Rohald, K., 1986). While the intensity, duration, and frequency of training are directly related to improvements in VO_2peak (Pollock, 1977), traditional ET programs have typically required a minimum intensity of ~55-65% HR_{peak} to stimulate improvement in VO_2peak (Astrand, P.O. & Rohald, K., 1986). Based on the importance of VO_2peak as an independent predictor of all-cause mortality and cardiovascular-specific mortality (Ekelund *et al*, 1988, Blair *et al*, 1989), the ability of HIT to improve VO_2peak on par with ET is of utmost importance (Gibala *et al*, 2006). HIT is proven to improve VO_2peak (Helgerud *et al*, 2007, Burgomaster *et al*, 2008, McKay *et al*, 2009) and can further be used to improve VO_2 kinetics and optimize aerobic performance (McKay *et al*, 2009, Bailey *et al*, 2009).

However, the minimal intensity of HIT required to improve VO_2peak is currently unknown. The ability to train at higher intensities and with greater stimulus for adaptation is a powerful advantage of HIT by allowing heightened cellular and cardiovascular stress than would otherwise be possible. Such stress to the vascular system may induce greater improvements in both the pumping capacity of the heart (SV) as well as conductance at the tissue level ($a\text{-vO}_2\text{diff}$) above more moderate intensity exercise. As well, research in HIT conducted at various levels of interval intensity to establish a dose-response relationship is extremely limited. While interval duration has been examined in cardiac patients (Meyer *et al*, 2012) and both interval duration (Hazell *et al*, 2010) and training frequency (Dalleck *et al*, 2010) have been studied in young healthy adults, the impact of interval intensity is unknown. Further, no study has attempted to optimize HIT for sedentary, overweight/obese populations. These are major deficiencies in our knowledge of HIT and are necessary components in our ability to prescribe effective and efficient HIT programs.

2.6.6 Hypothesis 1

Taking into account the various factors associated with improving aerobic capacity, I formulated the hypothesis using the components of the Fick equation. Based on the greater stimulus for adaptation with higher intensity exercise, I hypothesized that both SV and capillarization would improve to a greater extent in the group exercising at 100% of their peak work rate (100% group). Due to practical limitations in measurement of both SV and capillarization, changes in cardiovascular function (primarily SV, see

discussion above) will be estimated based on the quotient of absolute O_2 consumption and HR (O_2 pulse). Accordingly, I hypothesized that VO_{2peak} would increase in both the 70% and 100% groups with a greater improvement in the 100% group due to improved central cardiovascular function and peripheral adaptation. It is also important to be cognizant of the contribution of increased mitochondrial content to greater a- vO_2 diff in determining VO_{2peak} .

2.7 Exercise mediated mitochondrial biogenesis

Molecular physiologists have been trying to understand the underlying mechanisms of exercise adaptations since the first descriptions of elevated mitochondrial capacity following endurance training (Holloszy, 1967). Mitochondrial biogenesis is now known as an important adaptation to exercise training and refers to the synthesis of new mitochondria based on the coordinated expression of activated mitochondrial and nuclear genes with the interaction of various transcription factors and coactivators (Ojuka, 2004). The increases in number of mitochondria and mitochondrial content, such as components of the electron transport chain (ex. cytochrome oxidase), that result from mitochondrial biogenesis facilitate greater efficiency and capacity of skeletal muscle.

2.7.1 Mitochondrial content in overweight/obesity and disease

Decreased mitochondrial volume and a reduced capacity for substrate oxidation have been linked to obesity (Holloway *et al*, 2008), insulin resistance (Petersen *et al*, 2004), type 2 diabetes (Schrauwen-Hinderling *et al*, 2007), and cardiovascular disease (Nisoli *et al*, 2007). Consequently, the peripheral adaptations that take place in the muscle

following exercise are considered some of the more important responses for enhanced endurance performance, resistance to fatigue, and improved cardiovascular and metabolic health. Little et al. (Little *et al*, 2011b) posit that the effect of HIT to improve health and reduce all-cause mortality (Booth *et al*, 2002, Warburton *et al*, 2006) may be related to exercise induced mitochondrial biogenesis at a systemic level.

2.7.2 PGC-1 α : The master regulator of mitochondrial biogenesis

A major breakthrough in the understanding of mitochondrial biogenesis came with the discovery of the transcriptional coactivator peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PGC-1 α) (Wu *et al*, 1999). PGC-1 α is involved in activating both nuclear and mitochondrial transcription, resulting in mitochondrial biogenesis and upregulation of genes involved in lipid metabolism and oxidative phosphorylation (Wu *et al*, 1999, Lin *et al*, 2002). Overexpression of PGC-1 α in skeletal muscle and myotubes results in large increases in functional mitochondria (Wu *et al*, 1999, Lin *et al*, 2002). PGC-1 α is also established as a key determinant of the adaptive response following acute exercise and exercise training (Burgomaster *et al*, 2008, Little *et al*, 2011c, Gurd *et al*, 2010, Gurd *et al*, 2011b).

Importantly, activation of PGC-1 α by post-translational modification appears to be responsible for the initial step in increasing transcription of mitochondrial genes following exercise (Canto and Auwerx, 2009). Wright et al. (2007) have demonstrated that most of the PGC-1 α in skeletal muscle resides in the cytosol at rest, but exercise

induces translocation of PGC-1 α to the nucleus where it facilitates mitochondrial biogenesis. Various researchers have shown increases in nuclear abundance of PGC-1 α after high-intensity interval training (HIT) (Little *et al*, 2010b, Gurd *et al*, 2011b), as well as after acute bouts of endurance exercise (Little *et al*, 2010a) and high intensity intervals (Little *et al*, 2011c), indicating increased activation. This increase in the nuclear content of PGC-1 α is associated with PGC-1 α transcriptional activity and has been shown with (Little *et al*, 2011c, Gurd *et al*, 2011a) and without an increase in corresponding whole muscle PGC-1 α (Little *et al*, 2010a, Little *et al*, 2010).

2.7.3 AMPK – *The energy monitor of the cell*

Healthy cells require that high ratios of ATP:ADP and ATP:AMP are maintained. When the activity of ATPases exceed the capacity of ATP synthases during cellular stress, the ATP:ADP ratio will decrease, stimulating the adenylate kinase reaction to produce AMP (Hardie and Hawley, 2001). Because the AMP:ATP ratio varies as the square of the ADP:ATP ratio, the cellular concentrations of AMP will change at a much greater rate than those of ATP or ADP (i.e. If the ADP:ATP ratio rises 10-fold, the AMP:ATP ratio rises 100-fold) (Krebs, 1964). Therefore, AMP is an important indicator of cell energy status and is monitored by AMP-activated protein kinase (AMPK) (Hardie *et al*, 1998, Hardie and Carling, 1997). Processes that increase ATP-utilization, such as muscle contraction, as well as those that inhibit ATP-production are stimuli for AMPK activation (Hardie *et al*, 2006, Fryer and Carling, 2005).

Stimulation of AMPK has been shown *in vivo* and *in vitro* both with exercise and pharmacological treatment. Exercise-induced stimulation of AMPK has been shown in mice (Jaeger *et al*, 2007) and human (Gibala *et al*, 2009) models and AMPK is known to be an upstream modulator of PGC-1 α transcriptional activity in skeletal muscle by directly phosphorylating PGC-1 α at residues Thr177 and Ser538 (Jaeger *et al*, 2007). Use of the AMP analog 5-aminoimidazole-4-carboxamide ribonucleotide (AICAR) to activate AMPK in rodent models have shown increases in transcription and activity of mitochondrial enzymes in skeletal muscle (Putman *et al*, 2003, Suwa *et al*, 2006). In particular, studies of gene knockout models have demonstrated the importance of AMPK activity in the expression of mitochondrial proteins (Jorgensen *et al*, 2006, Nilsson *et al*, 2006). As the activation of AMPK in exercising muscle seems to depend mainly on local mechanisms (Hayashi *et al*, 1998), activity and content of AMPK in skeletal muscle may be controlled by intensity and time of exercise (Egan *et al*, 2010, Jorgensen *et al*, 2007).

2.7.4 SIRT1 – A controversial regulator of PGC-1 α

Sirtuins are a family of NAD⁺ dependent deacetylases involved in a variety of biological processes that deacetylate lysine residues, allowing them to exert regulatory effects in the cell. SIRT1, one of seven mammalian homologs of Sir2, catalyzes NAD⁺ dependent protein deacetylation, yielding a nicotinamide product (Blander and Guarente, 2004) which is a noncompetitive inhibitor of sirtuins (Bitterman *et al*, 2002). An emerging theory is that SIRT1 activity can therefore be modulated by nicotinamide levels in the cell as well as by NAD⁺. SIRT1 has been shown to physically interact with and

deacetylate PGC-1 α at multiple lysine sites, consequently increasing PGC-1 α activity in vitro (Gerhart-Hines *et al*, 2007, Rodgers *et al*, 2005). However, the effect of SIRT1 on PGC-1 α with respect to transcriptional control in vivo is less clear (Gurd *et al*, 2012). Conflicting reports demonstrate an increasing (Little *et al*, 2010b), decreasing (Gurd *et al*, 2009), or unchanging (Chabi *et al*, 2009) relationship of SIRT1 protein to mitochondrial biogenesis and oxidative capacity following exercise training/chronic contractile activity.

Resveratrol (RSV), a natural polyphenolic compound mainly found in the skin of grapes (Baur and Sinclair, 2006), has been shown to significantly increase SIRT1 activity resulting in the deacetylation of PGC-1 α (Lagouge *et al*, 2006). Importantly, these effects have improved resistance to obesity and insulin resistance among RSV-treated mice (Lagouge *et al*, 2006). RSV is proposed to act through an allosteric interaction mechanism that causes an increase in SIRT1 affinity for both NAD⁺ and the acetylated substrate (Howitz *et al*, 2003), but this view has been challenged (Pacholec *et al*, 2010). Alternative theories argue that RSV may target AMPK in addition to (Baur *et al*, 2006) or independent of (Centeno-Baez *et al*, 2011) SIRT1. These findings suggest that the mitochondrial biogenesis and PGC-1 α activation that occur with RSV treatment may be caused by activation of AMPK rather than, or in addition to SIRT1 (Gurd *et al*, 2012).

2.7.5 Exercise-induced mitochondrial adaptations

Increases in mitochondrial content are well-established adaptations within the exercised muscle, however, the molecular mechanisms underlying these changes are just beginning to be clarified (Hood, 2009). The process appears to begin with exercise-

induced activation of signaling reactions leading to the subsequent activation of coactivator proteins and transcription factors (Hood, 2009). At the onset of exercise, numerous chemical changes occur such as calcium cycling, ATP turnover, and O₂ consumption stimulating signaling pathways involved in mitochondrial biogenesis. These signaling pathways lead to downstream activation of kinases, phosphatases, and deacetylases such as the previously mentioned AMPK and SIRT1, producing the posttranslational modification of proteins (Figure 2, Sakamoto and Goodyear, 2002). When activated, these factors phosphorylate nuclear transcription factors and coactivators depending on the intensity and duration of the contractile activity as well as the fiber type composition of the muscle (Ljubcic and Hood, 2008).

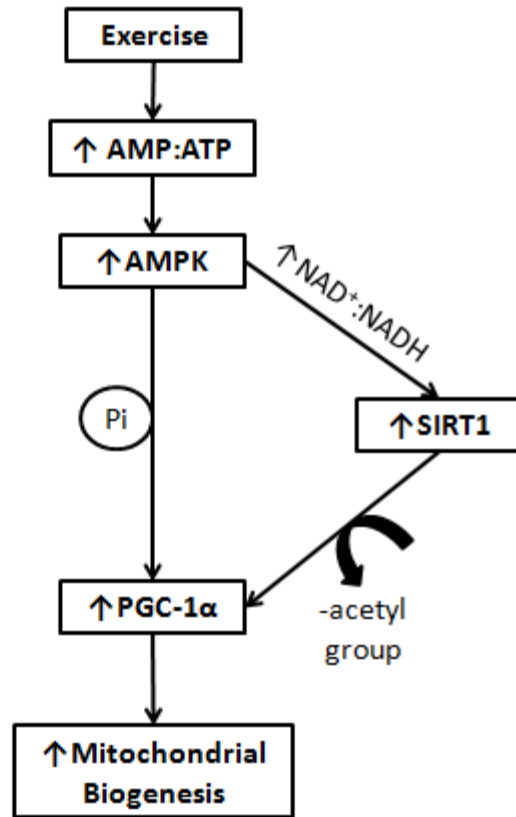


Figure 2. Summary of exercise induced mitochondrial biogenesis

2.7.6 HIT specific mitochondrial biogenesis

There is a growing appreciation of the ability of HIT to stimulate the skeletal muscle remodeling normally associated with traditional endurance training (Gibala and McGee, 2008). Despite the reduced volume of exercise associated with HIT, brief interval exercise has been shown to increase phosphorylation of AMPK, a key component in the signaling pathway that activates both PGC-1 α and SIRT1 (Egan *et al*, 2010, Gibala *et al*, 2009). As a result, HIT has been shown to increase markers of oxidative capacity such as the maximal activity of mitochondrial enzymes (Gibala *et al*, 2006, Gurd *et al*, 2010, Burgomaster *et al*, 2005, Little *et al*, 2011a) and COX IV protein content (Gibala *et al*, 2006, Gurd *et al*, 2010, Gurd *et al*, 2011a, Little *et al*, 2011a, Hood *et al*, 2011). Further, HIT has been shown to increase the relative proportion of PGC-1 α in the nucleus, indicating that the enzyme is being translocated both acutely (Little *et al*, 2011c) and over the course of the training period (Little *et al*, 2010b, Gurd *et al*, 2011a). The result is that HIT is a potent stimulus to induce mitochondrial biogenesis and produce healthy functioning cells. However, there is much to be gained from a better understanding of how mitochondrial content responds to interval training at various intensities.

2.7.7 The HIT-intensity hypothesis

In order to meet the increased intensity demand of intervals at 100% as compared to 70%, more ATP must be utilized to power the skeletal muscle contractions, resulting in a greater production of AMP. This leads to an increase in the AMP:ATP ratio; an effect that does not necessarily occur with low intensity exercise (Howlett *et al*, 1998). This

differential AMP:ATP ratio regulates the extent to which AMPK is activated (Egan *et al*, 2010) and consistent with this, increases in PGC-1 α transcriptional activity occur in an intensity dependent manner (Egan *et al*, 2010, Nordsborg *et al*, 2010). As well, the influence of the AMPK-Nampt axis on the NAD⁺ salvage pathway plays an important role in determining intramuscular NAD⁺ and the resulting SIRT1 activity (Canto *et al*, 2009, Fulco *et al*, 2008), whose deacetylatory action on PGC-1 α will further increase mitochondrial biogenesis. Therefore, exercise at higher intensities should stimulate mitochondrial biogenesis to a greater extent via increased AMPK activation than exercise at lower intensities.

2.7.8 Hypothesis 2

Based on this literature, I hypothesized that both exercise groups would significantly increase markers of mitochondrial content and oxidative capacity, with greater increases occurring in the 100% group. I suspected these enhanced improvements would be due to increased content and activation of PGC-1 α by exercise mediated mechanisms such as phosphorylation by AMPK and deacetylation by SIRT1.

2.8 Systemic inflammation – A systemic problem

While acute inflammation is a natural part of the body's defence mechanism against injury and infection, low-grade inflammation may contribute to a number of diseases (Mathur and Pedersen, 2008). Specifically, chronic low-grade inflammation is a hallmark of obesity, type 2 diabetes, and metabolic syndrome (Dandona *et al*, 2004, Alexandraki *et al*, 2006, Haffner, 2006), and refers to a condition in which systemic

concentrations of proinflammatory factors are elevated in circulation (Wilund, 2007, Troseid *et al*, 2010). Sustained levels of circulating proinflammatory factors are known to influence the pathogenesis of atherosclerosis due to plaque formation in the endothelium (Libby, 2002), insulin resistance induced via dysregulation of skeletal muscle metabolism (Dandona *et al*, 2004), and dysfunction of immune cells (Romeo *et al*, 2010).

2.8.1 Exercise training reduces inflammation

The ability of acute exercise to reduce circulating levels of proinflammatory factors (Balducci *et al*, 2010, Goldhammer *et al*, 2005) and increase anti-inflammatory factors is established (Croft *et al*, 2009, Petersen and Pedersen, 2005). Various cross-sectional reports describe inverse correlations between self-reported physical activity and systemic inflammation (Pitsavos *et al*, 2005, King *et al*, 2003, Dufaux *et al*, 1984), while studies of exercise interventions have recorded reduced systemic inflammation among healthy young adults (Mattusch *et al*, 2000) as well as individuals with coronary heart disease (Goldhammer *et al*, 2005) and chronic heart failure (Gielen *et al*, 2003). Taken together, the available evidence suggests an ability of exercise to reduce systemic inflammation, however, the relationship between exercise and inflammation appears quite complex (Bruunsgaard, 2005).

The type, intensity, and duration of exercise may all affect the ability of exercise to reduce inflammatory markers (King *et al*, 2003, Bruunsgaard, 2005). However, the effect of different types of exercise training (such as HIT) on low-grade inflammation is complex, and relatively poorly understood. For example, while correlational data obtained

from marathon runners has suggested that the IL-6 response to exercise may be dependent on the intensity of the exercise and on the amount of muscle mass being activated (Ostrowski *et al*, 2000), other, more recent reports have demonstrated no change in IL-6 following HIT (Croft *et al*, 2009, Stensvold *et al*, 2012). Thus, the impact of HIT on systemic inflammation remains unclear, as does the impact of altered HIT intensity. Further, we are unaware of any study that has examined changes in pro- and anti-inflammatory factors following HIT in young overweight/obese adults.

2.8.2 Partners in crime – TNF α and IL-6

For the purposes of this thesis we will be considering the proinflammatory cytokines tumor necrosis factor alpha (TNF α) and interleukin-6 (IL-6). Obesity is strongly correlated with increased levels of circulating TNF α as it is produced mainly in adipose tissue (Krogh-Madsen *et al*, 2006, Lee and Pratley, 2005). TNF α is thought to play a direct role in the metabolic syndrome (Petersen and Pedersen, 2005) as high levels of plasma and skeletal muscle TNF α , have a direct effect on glucose metabolism causing insulin resistance (Plomgaard *et al*, 2007, Plomgaard *et al*, 2005) as well as increasing whole-body lipolysis (Plomgaard *et al*, 2007, Plomgaard *et al*, 2008). Changes in TNF α usually only occur as a result of highly strenuous, prolonged exercise (e.g. marathon running) and may not accompany the increases in cytokines seen elsewhere following training (Pedersen and Febbraio, 2008). TNF α has been reported to decline following exercise in patients with chronic heart failure (Gielen *et al*, 2003) as well as the elderly (Greiwe *et al*, 2001), but similar reductions did not occur in older obese adults following

moderate cycling training (Ferrier *et al*, 2004). Interestingly, TNF α increased after 12 weeks of resistance training among individuals with metabolic syndrome (Stensvold *et al*, 2012) raising the possibility that higher intensities of HIT may actually promote systemic inflammation.

Despite considerable controversy surrounding the role of IL-6 following exercise (Mathur and Pedersen, 2008, Pedersen and Febbraio, 2007), it is now thought that one role of IL-6 may be to enhance glucose uptake via increased GLUT4 translocation (Mathur and Pedersen, 2008, Febbraio and Pedersen, 2002, Glund *et al*, 2007). Further, despite its consideration as a proinflammatory cytokine, some research has suggested that IL-6 is anti-inflammatory (Pedersen, 2006) and may exert an inhibitory effect on TNF α production (Starkie *et al*, 2003). Balducci *et al*. (Balducci *et al*, 2010) show a decrease in IL-6 after 1 year of high-intensity aerobic exercise in individuals with type 2 diabetes and metabolic syndrome, whereas 12 weeks of interval training in a similar population was unable to reproduce this result (Stensvold *et al*, 2012). Currently, the effect of different types of exercise such as HIT and resistance training on low-grade inflammation among those with metabolic syndrome is poorly understood (Stensvold *et al*, 2012) and we are unaware of any study that has examined the impact of HIT intensity on changes in either TNF α or IL-6 in overweight/obese young adults.

2.8.3 Adiponectin

Adipose tissue can operate as an endocrine organ, performing metabolic functions and secreting proteins, such as adiponectin (Ahima and Flier, 2000) which is induced

during adipogenesis. Typically, adiponectin levels are decreased in obesity and in diseases such as type 2 diabetes (Arita *et al*, 1999, Stepan and Lazar, 2002). Hypoadiponectemia is also linked to the development of insulin resistance (Hotta *et al*, 2001). Importantly, elevated adiponectin is linked with increased insulin sensitivity and improvement in metabolic syndrome in adults (Tjonna *et al*, 2009, Tjonna *et al*, 2008). Conflicting reports of exercise training increasing (Christiansen *et al*, 2010a), decreasing (Moran *et al*, 2011), or having no effect (Christiansen *et al*, 2010b) on plasma adiponectin levels make the influence of exercise on adiponectin unclear, again possibly due to differences in exercise intensity, duration, modality, and metabolic profile of the individuals (Richards *et al*, 2010). However, despite no change in healthy adults (Richards *et al*, 2010), HIT has been shown to increase fasting adiponectin levels in those with metabolic syndrome (Tjonna *et al*, 2008, Oberbach *et al*, 2006). Taking into account our poor understanding of the effect of interval intensity on adiponectin in overweight and obese young adults, further research is required.

2.8.4 Hypothesis 3

Despite the difficulty in formulating a hypothesis considering the controversial literature surrounding the systemic inflammation accompanying overweight and obese individuals and the impact of exercise training, I hypothesized that there would be decreases in plasma concentrations of IL-6 and TNF α and increased adiponectin following HIT. Similar to our other hypotheses, we expected the differences in the 100% group to be greater than the differences in the 70% group.

2.9 Summary

With the increasing prevalence of overweight and obesity and its related health problems, there is considerable interest in understanding the physiology surrounding these conditions. Further, exercise modalities designed to efficiently counteract the growing presence of overweight and obesity are of particular interest but much work remains to be done before such exercise can be effectively prescribed. The optimal dose of exercise both in terms of efficacy as well as practicality needs to be elucidated. The original research presented here seeks to help resolve these questions through a three week interval training intervention with groups exercising at either 70% or 100% of their VO_2peak . Pre- and post-measures of aerobic capacity, exercise performance, oxidative capacity, mitochondrial content, and markers of systemic inflammation compared between groups will help determine if there is any impact of interval intensity in overweight and obese young adults.

2.10 References

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Chapter 3

The impact of interval intensity in overweight young men

Abstract

Purpose: The purpose of this research was to determine if the adaptations to high intensity interval training (HIT) occur in an intensity dependent manner in overweight young adults.

Methods: 19 sedentary males (Age: 22.7 ± 3.9 yrs, BMI: 31.4 ± 2.6 kg/m², WC: 106.5 ± 6.6 cm) performed 9 sessions of interval training over three weeks at either 70% or 100% peak work rate.

Results: VO₂peak increased by 10.4% and 27.9% in the 70% and 100% groups respectively with significant effects of both training and interaction. Exercise performance improved by 8.6% in the 70% group and 14.1% in the 100% group with a significant effect of training and a significant difference in the improvement between groups. COX I protein content, COX IV protein content, and citrate synthase maximal activity all demonstrated equivalent increases between groups with a significant effect of training for each. β HAD maximal activity tended to increasing with training but did not reach statistical significance ($p = 0.07$). PGC-1 α and SIRT1 protein contents also increased significantly with training, while AMPK protein content decreased following the intervention. Plasma adiponectin concentrations decreased by 12.9% and 19.4% in the

70% and 100% groups respectively with a significant effect of training while no changes were detected in plasma concentrations of either IL-6 or TNF α following training.

Conclusion: These findings suggest that improvements in aerobic capacity and exercise performance may occur in an intensity dependent manner following HIT.

Keywords: overweight, interval intensity, VO₂peak, skeletal muscle oxidative capacity

Introduction

High intensity interval training (HIT), a training modality that alternates between brief repeated bursts of intense exercise and periods of active rest, has established its ability to improve clinically relevant outcomes. Specifically, HIT conducted in a various populations and with a number of different protocols, is known to improve VO_2 peak (Wisloff *et al*, 2007, Warburton *et al*, 2005, Whyte *et al*, 2010), functional exercise measures such as time to completion trials (Gibala *et al*, 2006), time to exhaustion trials (Warburton *et al*, 2005, Burgomaster *et al*, 2005), cardiovascular function (Wisloff *et al*, 2007, Tjonna *et al*, 2009, Helgerud *et al*, 2007), and markers of oxidative capacity (Hood *et al*, 2011, Little *et al*, 2011a) and mitochondrial content (Hood *et al*, 2011, Burgomaster *et al*, 2008) in skeletal muscle. Additionally, HIT reduces systemic inflammation among post percutaneous coronary intervention patients (Munk *et al*, 2011) and individuals with metabolic syndrome (Stensvold *et al*, 2012).

These physiological benefits are achieved in significantly less time and require less total exercise energy expenditure than traditional endurance training (ET) (Gibala *et al*, 2006, McKay *et al*, 2009). However, while emerging evidence suggests that HIT may be enjoyable (Little *et al*, 2011a), perhaps to a greater extent than ET (Bartlett *et al*, 2011, Tjonna *et al*, 2008), the near maximal or supramaximal intensities typically associated with HIT represent a potential threat to the adoption of HIT by the general population (Wisloff *et al*, 2007, Whyte *et al*, 2010, Little *et al*, 2011a). Exercise at such demanding intensities is typically associated with negative feelings and poor exercise adherence

(Ekkekakis *et al*, 2008), a relationship that may be exacerbated in obese and/or diseased populations (Ekkekakis *et al*, 2010). The safety of higher intensity protocols (i.e. supramaximal intervals) for populations with cardiovascular risk or disease has also been questioned (Gaesser and Angadi, 2011, Gayda *et al*, 2012).

Accordingly, there is a need for research examining the minimal intensity and volume of HIT required to induce beneficial adaptation (Gaesser and Angadi, 2011, Gibala *et al*, 2012). Specifically, there is a need for HIT to be optimized such that the associated adaptations (improved aerobic capacity, skeletal muscle oxidative capacity, inflammatory response, etc.) are maintained while the negative feelings and safety concerns associated with high intensity exercise are minimized. While interval duration has been examined in cardiac patients (Meyer *et al*, 2012) and both interval duration (Hazell *et al*, 2010) and training frequency (Dalleck *et al*, 2010) have been studied in young healthy adults, the impact of interval intensity is unknown. Further, no study has attempted to optimize HIT for sedentary, overweight/obese populations.

Therefore, the purpose of this study was to compare the impact of 3 weeks of interval training using a 1-minute on 1-minute off protocol (adapted from (Hood *et al*, 2011, Little *et al*, 2011a) at two different intensities: 70% and 100% peak work rate. We have examined this effect on measures of aerobic capacity, exercise performance, maximal cardiovascular function, skeletal muscle oxidative capacity, changes in mediators of mitochondrial biogenesis, and inflammation status. We hypothesized that the adaptations to exercise would occur in an intensity dependent manner and therefore there

would be greater improvement in these measures with higher exercise intensity following the training intervention.

Methods

Participants

Nineteen overweight, sedentary males volunteered to participate in this study (participant characteristics are presented in Table 1). All participants reported participating in less than 1 hour per week of aerobic exercise (jogging, cycling, etc.) at enrollment and overweight was defined as a waist circumference greater than 94 cm (Lean *et al*, 1995). Participants were matched on pre-test waist circumference and VO₂peak before being separated into two groups, completing exercise training utilizing repeated intervals at either 70% or 100% of their maximal aerobic capacity. The study was approved by the Health Sciences Human Research Ethics Board at Queen's University and all participants provided written informed consent (Appendix A).

Experimental Approach

The experimental protocol consisted of (i) baseline testing; (ii) a 3-week training intervention, and (iii) post-testing.

Baseline testing

Participants arrived for the first laboratory visit in the morning following an overnight fast (≥ 8 h). Resting blood samples were collected by venipuncture from an antecubital vein in sterile tubes (BD Vacutainer, Franklin Lakes, NJ) with and without ethylenediaminetetraacetic acid (EDTA) as an anticoagulant. Plasma was separated by centrifugation at 3500 RPM for 10 minutes at 4°C while serum was separated by centrifugation at 3500 RPM for 15 minutes at 4°C. Samples were stored at -80°C until analysis. Participants were then fed a standardized breakfast and allowed to rest for 1 hour

before a resting muscle biopsy sample was taken using the Bergstrom needle biopsy technique (Bergstrom, 1975). Biopsies were performed under sterile conditions with local anesthesia (2% lidocaine) using a custom modified Bergstrom biopsy needle and manually applied suction. Muscle tissue was immediately blotted, snap-frozen in liquid nitrogen, and stored at -80°C until analysis.

Forty-eight hours following the muscle biopsy, participants returned to the lab for a $\text{VO}_{2\text{peak}}$ incremental ramp test to exhaustion on a Monark Ergonomic 874E stationary ergometer (Vansbro, Sweden). The $\text{VO}_{2\text{peak}}$ ramp protocol consists of a five minute loadless warm-up followed by a step increase to 70 W for one minute and subsequent increases in work rate of $21 \text{ W}\cdot\text{min}^{-1}$ to volitional exhaustion (determined by the inability of the participant to maintain a cadence of 60 RPM). Gas exchange and heart rate were measured with a metabolic cart (Moxus AEI Technologies, Pittsburgh, PA). Relative $\text{VO}_{2\text{peak}}$, absolute $\text{VO}_{2\text{peak}}$ and HR_{peak} were calculated as the average of their respective values measured in the final 30s of the protocol. Peak O_2 pulse was calculated as absolute $\text{VO}_{2\text{peak}}$ divided by HR_{peak} from the incremental ramp protocol.

Twenty-four hours following the incremental ramp test, participants completed a 500 kcal time to completion trial at a self-selected cadence as quickly as possible against a load expected to elicit 50% $\text{VO}_{2\text{peak}}$ at 60 RPM. Participants were given no temporal, verbal, or physiological feedback and were only aware of how many calories they had expended, which was calculated and displayed on the ergometer. Exercise duration and average power were recorded upon completion of each test.

Training protocol

Training was performed three times per week for three weeks with a progression of 8 intervals per session during Week 1, 9 intervals per session during Week 2, and 10 intervals per sessions during Week 3. After a five minute loadless warm-up, participants completed 60 s intervals at either 70% or 100% of the work rate expected to elicit VO_2peak while maintaining a cadence of 80 RPM. Active rest periods, involving 60 s of loadless cycling, were interspersed between all intervals. Each training session ended with a five minute loadless cool down period. All training sessions were completed on the same Monark ergometer.

RPM and HR were recorded in 1-second intervals using the Polar Team² Pro System (Polar, Lachine, QC) during the 1st training session for all participants. The affective dimension was also assessed in each participant's first training sessions using a single-item, 11-point Likert bipolar Feeling Scale (FS) (Hardy and Rejeski, 1989; Appendix D). The FS ranges from -5 (Very Bad) to +5 (Very Good) and explores the affective dimension (acute affect) of pleasure-displeasure.

Post-training measures

Post-training tests were conducted in an identical manner as the baseline measures. Fasted blood and a resting muscle biopsy were sampled 72 h following the final training session. 48 h after the muscle biopsy, participants performed an incremental VO_2peak ramp protocol, then a 500 kcal time to completion trial 24 h later. Participants were also asked to complete a survey after completing the training intervention regarding

their perceived enjoyment and self-efficacy of exercise (Appendix C). Perceived enjoyment of interval exercise was assessed by the question “How enjoyable would it be for you to do high intensity interval training 3 days per week?” Responses were recorded on a 1 (not enjoyable at all) to 7 (extremely enjoyable) scale. Scheduling self-efficacy was reported using the single item measure of confidence “How confident are you that you could schedule interval training sessions three times per week?” and task self-efficacy was assessed using the single item measure “How confident are you that you would complete interval training sessions three times per week?”. Both self-efficacy questions utilized a 10-point likert scale ranging from 1 (not confident at all) to 10 (completely confident).

Fasting glucose, insulin, and HOMA

Fasted blood glucose was determined via a hexokinase reaction assay performed at the Kingston General Hospital (Kingston, Ontario). Fasted insulin levels were determined with a commercially available (enzyme-linked immunoabsorbent assay (ELISA) kit, Alpco Diagnostics, Salem, NH). All samples were run in duplicate, with the CV being <10% for all values. Insulin sensitivity was estimated using homeostatic model assessment – insulin resistance (HOMA-IR) with the equation:

$$\text{HOMA-IR} = (\text{fasting insulin } (\mu\text{IU/mL}) \times \text{fasting blood glucose (mmol/L)}) / 22.5$$

(Matthews *et al*, 1985)

Cytokines

Fasted blood samples were assayed for plasma interleukin-6 (IL-6), tumor necrosis factor alpha (TNF α), and adiponectin with commercially available high sensitivity ELISA kits (R & D Systems, Minneapolis, MN). All samples from individual participants were tested in duplicate on the same assay plate. Repeat analysis was performed on duplicates that varied by more than 15% and the average of the duplicates was used in the analyses (Chard, 1990). The absorbance of the fluorescent conjugate-antibody complex in the wells was obtained at 490, 490, and 540 nm and corrected at 650, 650, and 540 nm with a Biotek (Winooski, VT) multiplate reader (Synergy Mx) for IL-6, TNF α , and adiponectin respectively. The concentration was calculated from the fluorescence of the conjugate-antibody complex by calculating the value on the linear-log scale obtained with the standards provided. Values are reported in pg/mL (IL-6, TNF α) and ng/mL (adiponectin).

Western Blot Analysis

30-50 μ g segments of frozen muscle tissue were separated from the whole muscle sample in liquid nitrogen. The segments were then placed in a pre-chilled tube containing 2 mL of lysis buffer (210 mM sucrose, 2 mM EGTA, 40 mM NaCl, 30 mM Hepes, 20 mM EDTA) before being homogenized at 20000 RPM for 3 s. Protein concentrations were determined for whole muscle lysates isolated from tissues using a commercial protein assay (Pierce, Rockford, IL). Samples were diluted to the same concentration with a mixture of 4x Laemmli sample buffer and water and then denatured by heating at 95°C

for 5 minutes. Proteins were separated by SDS-PAGE using an 8.0% (PGC-1 α , AMPK), 10.0% (COX I, COX IV), or 12.0% (SIRT1) polyacrylamide gel and were subsequently transferred to a polyvinylidene difluoride membrane. Commercially available antibodies were used for the detection of PGC-1 α (Calbiochem, San Diego, CA), AMPK α , GAPDH (Millipore, Temecula, CA), COX I, COX IV (Cell Signalling, Danvers, MA), and SIRT1 (Abcam, Cambridge, MA). Proteins were visualized by chemiluminescence detection according to the manufacturer's instructions (Millipore, Billerica, MA). Blots were imaged using the FluorChem Cell Biosciences imaging system and quantified using AlphaView technology. Equal protein loading for all Western blots were confirmed using GAPDH.

Citrate Synthase and β -Hydroxyacyl-CoA Dehydrogenase Activity

A portion of the muscle sample (~20 mg) was homogenized by hand for 30 s in glass hand homogenizers on ice and used to determine maximal citrate synthase (CS) and β -hydroxyacyl-CoA dehydrogenase (β HAD) activity. Total CS activity was measured in Tris·HCl buffer (100 mM Tris HCl, 1mM DTNB, 3mM acetyl CoA, pH = 7.3) and 10% Triton-X. The reaction was started with the addition of 10 mM oxaloacetate and activity was measured spectrophotometrically at 37°C by measuring the release of CoASH to the colorimetric agent DTNB at 412 nm (Colowick and Kaplan, 1957). The β HAD assay was performed in Tris·HCl buffer (1 M Tris·HCl, 200 mM EDTA, 5 mM NADH, pH = 7.0) and 10% Triton-X. The reaction began with the addition of 5 mM acetoacetyl-CoA and

the absorbance was measured for 3 min at 340 nm at 37°C to detect the disappearance of NADH (Bergmeyer and Grassl, 1986).

Statistics

Two-way, repeated measures ANOVAs were used to compare the effects of interval intensity and time. Post hoc tests were conducted using the Bonferroni test. Additionally, unpaired t-tests of change scores were used to compare changes between groups to isolate the strength of the change. Linear regressions were conducted to evaluate the strength of the relationship between the changes in VO_2peak and various markers of oxidative capacity. Data analysis was completed with GraphPad Prism v 5.01 (GraphPad Software, Inc., La Jolla, CA). Statistical significance was accepted at $p < 0.05$ unless otherwise noted.

Results

VO₂peak and Submaximal Exercise Performance

VO₂peak increased by 10.4% and 27.9% in the 70% and 100% groups respectively. Both a significant ($p < 0.01$) effect of training and a significant ($p < 0.05$) interaction effect were observed (Table 1). Post hoc analyses demonstrate a significant ($p < 0.05$) difference from pre-test in the 100% group, but not in the 70% group ($p > 0.05$). Analysis by unpaired t-test revealed that the increase in the 100% group was significantly ($p < 0.05$) greater than that observed in the 70% group (Figure 3A). A significant ($p < 0.001$) effect of training was also demonstrated for the time to complete 500 kcal test with decreases of 8.6% in the 70% group and 14.1% in the 100% group observed (Table 1). Post hoc analyses revealed that these changes were significantly ($p < 0.001$) different from pre-test for both groups, while an unpaired t-test of change scores determined that the improvement made by the 100% group was significantly ($p < 0.05$) greater than that made by the 70% group (Figure 3B). Peak power achieved on the incremental ramp protocol showed a significant ($p < 0.001$) effect of training and significant ($p < 0.01$) difference from pre-test with the 70% and 100% groups showing increases of 6.9% and 8.9% respectively (Table 1).

Muscle Oxidative Capacity

Whole muscle COX I increased by 8.2% and 19.1% in the 70% and 100% groups respectively, resulting in a significant ($p < 0.01$) effect of training (Figure 4A). Post hoc analyses revealed a significant ($p < 0.05$) increase above pre-test for the 100% group, but

not the 70% group ($p > 0.05$). Quantification of COX IV content also demonstrated a significant effect of training ($p < 0.01$) with increases of 17.1% in the 70% group and 17.8% in the 100% group (Figure 4A). These changes were found to be significantly different from pre-test with post hoc tests ($p < 0.05$).

Maximal activity of CS increased by 7.8% (Pre-test: 43.8 ± 4.7 $\mu\text{mol}/\text{min}/\text{g}$, Post-test: 47.2 ± 5.1 $\mu\text{mol}/\text{min}/\text{g}$) in the 70% group and 14.4% (Pre-test: 43.6 ± 4.5 $\mu\text{mol}/\text{min}/\text{g}$, Post-test: 49.9 ± 8.8 $\mu\text{mol}/\text{min}/\text{g}$) in the 100% group resulting in a significant ($p < 0.05$) effect of training for both groups (Figure 4C). Maximal activity of βHAD tended to be higher post-training with both the 70% (Pre-test: 2.3 ± 1.5 $\mu\text{mol}/\text{min}/\text{g}$, Post-test: 2.7 ± 1.9 $\mu\text{mol}/\text{min}/\text{g}$) and 100% (Pre-test: 2.7 ± 0.7 $\mu\text{mol}/\text{min}/\text{g}$, Post-test: 3.1 ± 0.4 $\mu\text{mol}/\text{min}/\text{g}$) groups demonstrating increases of 17.4% and 14.8% respectively. However, this effect did not reach statistical significance ($p = 0.07$, Figure 4C).

There were no significant ($p > 0.05$) correlations between the change in VO_2peak and the change in any of COX I ($r^2 = 0.04$), COX IV ($r^2 = 0.08$), or maximal CS activity ($r^2 = 0.05$; data not shown).

Regulators of Mitochondrial Biogenesis

PGC-1 α whole muscle protein content increased by 24.8% and 21.7% in the 70% and 100% groups respectively, resulting in a significant ($p < 0.05$) effect of training (Figure 5A). Post hoc analyses revealed no significant ($p > 0.05$) differences from pre-test for either group. Decreases in AMPK protein of 6.1% in the 70% group and by 11.8% in

the 100% group resulted in a significant ($p < 0.05$) effect of time (Figure 5A). These changes were not significantly ($p > 0.05$) different from pre-test values as determined by post hoc analyses. SIRT1 protein content increased by 10.0% and 43.2% in the 70% and 100% groups respectively, resulting in a significant ($p < 0.05$) effect of training (Figure 5A). Post hoc analyses demonstrate a significant ($p < 0.01$) difference from pre-test in the 100% group, but not in the 70% group ($p > 0.05$).

Cardiovascular O₂ delivery capacity

Significant ($p < 0.05$) effects of training and an interaction effect occurred in measures of peak O₂ pulse. Increases of 8.8% in the 70% group and 28.3% in the 100% group were observed (Figure 6) with post hoc analyses demonstrating that only the increase in the 100% group was significantly different ($p < 0.05$) from pre-test.

Inflammatory markers

Plasma adiponectin concentrations decreased by 12.9% in the 70% group and 19.4% in the 100% group with a significant main effect of time ($p < 0.05$, Table 2). No significant effects or changes were detected in plasma concentrations of either IL-6 or TNF α following training.

Affect scores

Subjects in the 100% group reported significantly ($p < 0.001$) lower acute affect scores during the 2nd, 4th, 6th, and 8th intervals as well as the 4th, 6th, and 8th rest periods of their first training sessions as compared to subjects in the 70% group (Figure 7). There was a trend for affect to be higher during the recovery periods following each interval, however this effect was not significant when analyzed using a 2-way repeated measures

ANOVA. There were no significant ($p > 0.05$) differences in the reported values of perceived enjoyment, scheduling self-efficacy, or task self-efficacy between groups following the training intervention (Table 3).

Table 1. Subject Characteristics

	70 %		100 %	
	Pre	Post	Pre	Post
N	10	-	9	-
Age (yrs.)	22.7 ± 4.3	-	22.7 ± 3.8	-
Height (cm)	184 ± 8	-	180 ± 7	-
Body mass (kg)	105 ± 14	103 ± 15	102 ± 12	102 ± 11
BMI (kg/m²)	30.7 ± 3.0	30.2 ± 3.0	32.3 ± 2.1	32.2 ± 1.9
Waist circumference (cm)	105 ± 8	103 ± 8	108 ± 5	107 ± 6
Abs. VO₂peak (mL/min)	3610 ± 968	3856 ± 732†	3605 ± 587	4453 ± 515*†§
Rel. VO₂peak (mL/kg/min)	35.8 ± 8.1	38.6 ± 6.4†	35.4 ± 5.4	44.7 ± 5.0*†§
Peak power (W)	293 ± 39	313 ± 47*†	308 ± 49	336 ± 49*†
HRpeak (bpm)	189 ± 11	185 ± 7†	196 ± 8	190 ± 10*†
Peak O₂ pulse (mL/min/bpm)	19.2 ± 5.4	20.9 ± 4.2†	18.4 ± 3.1	23.6 ± 3.6*†§
Time to 500 kcal (s)	2481 ± 560	2277 ± 588*†	2365 ± 599	2034 ± 533*†
Glucose (mmol/L)	4.7 ± 0.2	4.7 ± 0.2	4.8 ± 0.4	4.7 ± 0.4
Insulin (µIU)	10.0 ± 2.5	10.5 ± 3.2	12.1 ± 5.5	10.9 ± 4.2
HOMA-IR	2.1 ± 0.6	2.1 ± 0.6	2.5 ± 1.1	2.3 ± 0.9
Training HR (bpm)	141 ± 17	-	166 ± 12‡	-
Interval WR (W)	206 ± 27	-	308 ± 48‡	-

Values are mean ± SD. yrs, years; cm, centimetres; kg, kilograms; BMI, body mass index; m, metres; mmol/L, millimoles per litre; µIU, micro international units; HOMA-IR, homeostatic model assessment of insulin resistance; W, watts; s, seconds; HRpeak, maximal heart rate from VO₂peak test; bpm, beats per minute; Training HR, average heart rate from first training session.

*Significantly different (p < 0.05) from Pre-test within group.

†Significant (p < 0.05) effect of training.

‡Significantly different (p < 0.05) from 70%.

§Significant (p < 0.05) interaction between groups.

Table 2. Effect of training on plasma pro- and anti-inflammatory markers

	70 %		100 %	
	Pre	Post	Pre	Post
Adiponectin (ng/mol)	81.60 ± 42.32	71.06 ± 28.24 [†]	68.40 ± 25.62	55.14 ± 15.94 [†]
IL-6 (pg/mol)	1.74 ± 1.31	1.69 ± 1.46	1.79 ± 0.89	2.27 ± 1.00
TNFα (pg/mol)	2.22 ± 1.61	2.07 ± 1.55	1.83 ± 1.03	1.84 ± 0.76

Values are mean ± SD. IL-6, interleukin-6; TNF α , tumor necrosis factor alpha; ng/mol, nanograms per mole; pg/mol, picograms per mole.

[†]Significant (p < 0.05) effect of training.

Table 3. Post-intervention Survey Results

	70%	100%
Perceived Enjoyment	6.20 ± 0.92	6.06 ± 0.81
Scheduling Self-efficacy	8.10 ± 1.97	7.89 ± 1.36
Task Self-efficacy	8.80 ± 1.48	8.44 ± 2.30

Values are mean ± SD.

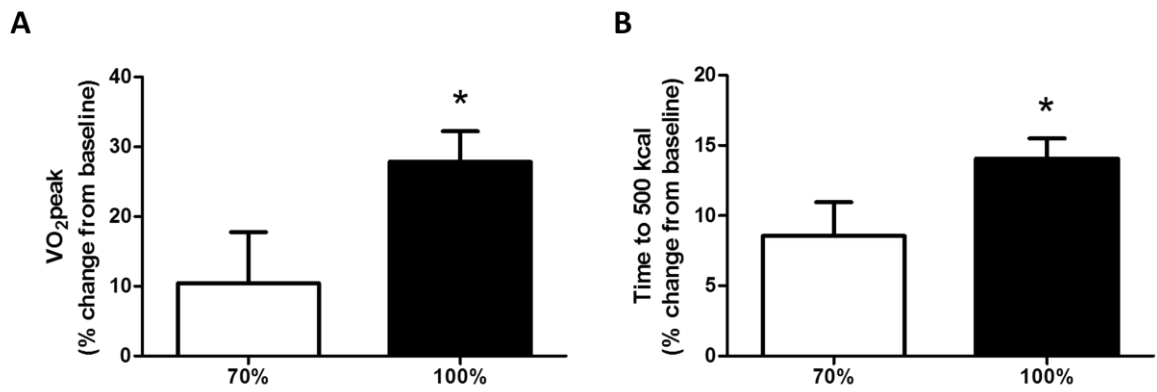


Figure 3. Higher intensity exercise led to greater improvements in measures of aerobic capacity and exercise performance. Both VO₂peak and time to 500 kcal increased to a greater extent in the 100% group .

*Significantly different from 70% (p < 0.05).

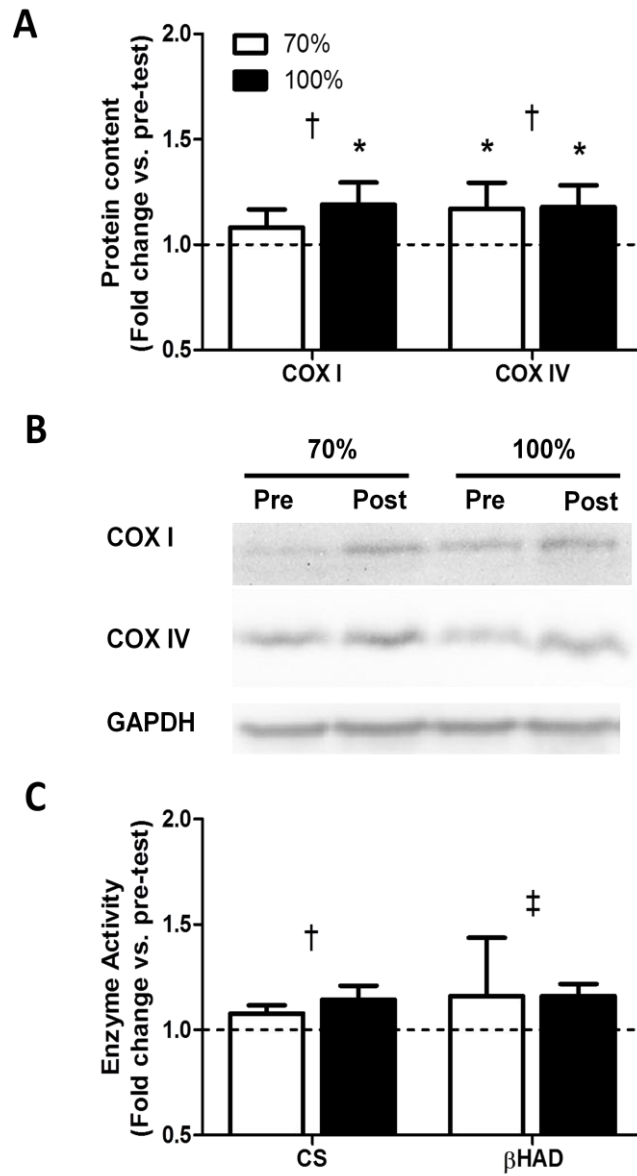


Figure 4. Increases in markers of skeletal muscle oxidative capacity were equivalent between groups. A) Protein content of COX I and COX IV and C) maximal enzyme activity of citrate synthase (CS) increased with training. There was a trend for maximal activity of β -hydroxyacyl-CoA dehydrogenase (β HAD) to increase with training (C, $p = 0.07$).

*Significantly different ($p < 0.05$) from pre-test.

†Significant ($p < 0.05$) effect of training.

‡Effect of training ($p = 0.07$).

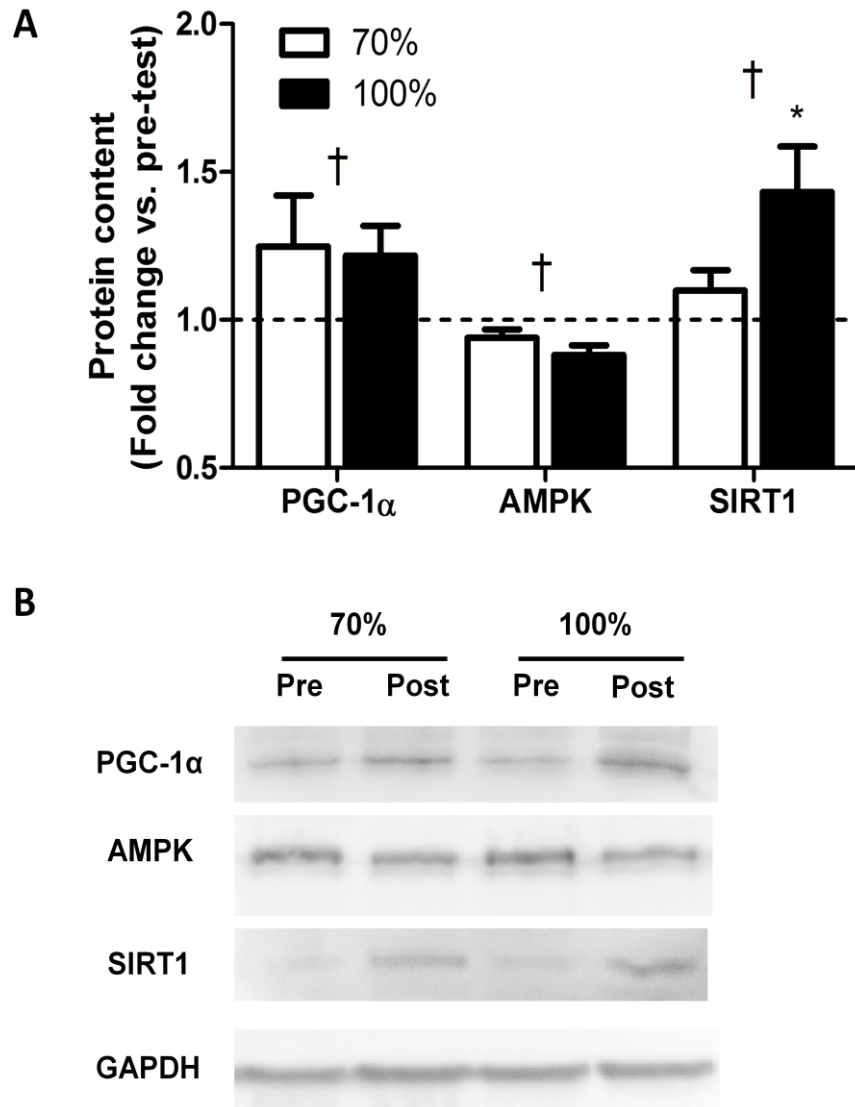


Figure 5. Effects of exercise training on PGC-1 α , AMPK, and SIRT1 protein content.

A) PGC-1 α and SIRT1 protein content increased with training in both groups. AMPK protein content decreased in both groups following exercise training (A).

*Significantly different ($p < 0.05$) from pre-test.

†Significant ($p < 0.05$) effect of training.

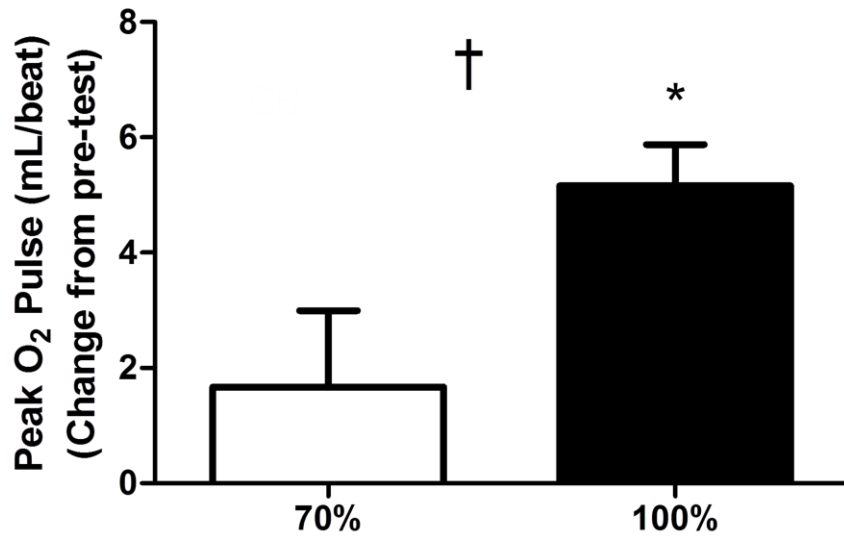


Figure 6. Peak O₂ pulse increases in an intensity dependent manner. The 100% group demonstrated greater increases in peak O₂ pulse over the 70% group.

*Significantly different ($p < 0.05$) from pre-test.

†Significant ($p < 0.05$) effect of training.

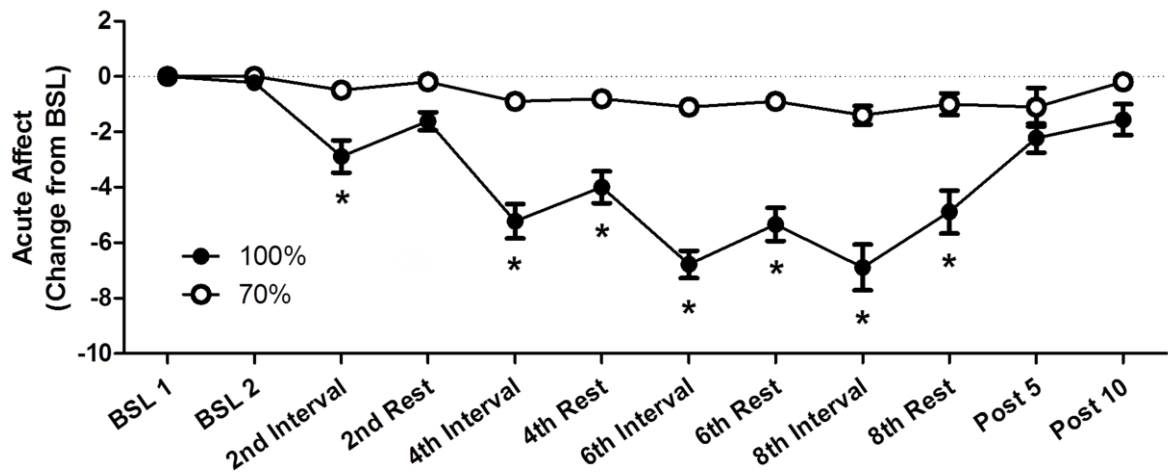


Figure 7. 100% group reported greater decreases in acute affect during training.

Acute affect decreases were greater among the 100% group during intervals and some rest periods of the first training session.

*Significant different ($p < 0.05$) from the 70% group.

Discussion

This study sought to determine the impact of HIT dose, specifically the effect of interval intensity, by examining changes in aerobic fitness, submaximal exercise performance, mitochondrial content, and markers of inflammation following a 3-week training intervention in overweight and obese young men. The major findings of this study were: 1) aerobic capacity and exercise performance were improved in both the 70% and 100% groups with improvements occurring in an intensity dependent fashion, 2) increases in skeletal muscle oxidative capacity were present in both groups and were independent of interval intensity, 3) the greater increase in peak O₂ pulse observed in the 100% group, combined with the equivocal changes in skeletal muscle oxidative capacity, suggest that cardiovascular adaptation induced by HIT are intensity dependant, 4) markers of systemic inflammation were largely unchanged by either intensity of HIT, and 5) despite a lower affective response during intervals at 100%, no differences were observed between groups in post training measures of exercise enjoyment, or exercise self-efficacy.

VO₂peak and submaximal exercise performance

As HIT becomes increasingly accepted as an effective means of improving health and fitness there is a need for studies attempting to determine the optimal dose of HIT. This study represents one of the first attempts to examine the impact of interval intensity on aerobic fitness and submaximal exercise performance. Importantly, while intervention with intervals at both 70% and 100% of VO₂peak improved aerobic capacity and exercise

performance, these improvements were intensity dependent (Figure 3). These results agree with previous reports demonstrating that improvements in VO_2peak following steady-state ET occur in an intensity dependant fashion (Wenger and Bell, 1986). Further, the intensity dependent increase in VO_2peak observed in the present study may help explain why improvements in VO_2peak following HIT are equivalent (McKay *et al*, 2009, Macpherson *et al*, 2011, Daussin *et al*, 2008) or superior (Tjonna *et al*, 2009, Helgerud *et al*, 2007) to those observed following ET despite the significantly lower exercise volume typically associated with HIT. Given the clinical relevance of VO_2peak (Myers *et al*, 2002, Keteyian *et al*, 2008), and despite the possible psychological (Ekkekakis *et al*, 2008) and safety (Guiraud *et al*, 2011) concerns associated with high intensity exercise, these results highlight the importance of continuing to promote intensity as we move towards the optimization of HIT.

Muscle oxidative capacity and mitochondrial content

Based on previous reports of greater increases in PGC-1 α mRNA following higher intensity exercise (Egan *et al*, 2010, Nordsborg *et al*, 2010), we hypothesized that mitochondrial content would increase to a greater extent following higher intensities of HIT. Specifically, our hypothesis was that intensity dependant increases in AMP (Howlett *et al*, 1998), would activate AMPK in an intensity dependant fashion (Egan *et al*, 2010). Subsequently, AMPK would activate PGC-1 α transcriptional activity directly by phosphorylation (Jaeger *et al*, 2007) and indirectly by altered [NAD⁺] and SIRT1 activity (Canto *et al*, 2009, Canto *et al*, 2010). Contrary to this hypothesis, there was no effect of

interval intensity on either protein content of COX I or COX IV (Figure 4A) or the maximal activities of CS or β HAD (Figure 4C).

The existence of an intensity effect on mitochondrial adaptation has been demonstrated in murine muscle (Dudley *et al*, 1982, Harms and Hickson, 1983, Terjung, 1976). However, the present results, combined with the typically equivalent adaptations observed between high intensity HIT and lower intensity ET (Gibala *et al*, 2006, Burgomaster *et al*, 2008, Tjonna *et al*, 2008) question whether this relationship extends to humans. While comparisons between HIT and ET are complicated by differences in both exercise volume (duration and energy expenditure; (Gibala and McGee, 2008) and potential differences in fibre type recruitment (Coyle, 2005), there is currently little evidence available supporting a dose-dependent effect of intensity on mitochondrial adaptations following HIT, or following exercise training in general. Importantly, when intensity was isolated (and training duration was held constant) in the current study, interval intensity did not impact the observed increases in muscle oxidative capacity induced by HIT.

As mentioned above, we hypothesized that high intensity of exercise would alter the cellular activities of AMPK, SIRT1 and PGC-1 α to a greater extent than low intensity exercise. While our study design makes it impossible to comment on whether the activity of this signalling pathway was acutely altered in an intensity dependant fashion, our findings of similar increases in PGC-1 α (Figure 5A) between groups suggest that chronic activation of this pathway by HIT is intensity dependant. Increases in whole muscle PGC-

1 α following HIT are consistent with previous reports (Gurd *et al*, 2010, Gurd *et al*, 2011, Perry *et al*, 2010), as is the apparent relationship between changes in PGC-1 α and changes in oxidative capacity (Gurd *et al*, 2010, Gurd *et al*, 2011, Little *et al*, 2011b). The observed increase in whole muscle SIRT1 protein content, which appears to be intensity dependent (70%, +9%; 100%, +43%), (Figure 5A), adds to the discrepant findings surrounding changes in SIRT1 following exercise training (Gurd *et al*, 2010, Gurd *et al*, 2010, Gurd *et al*, 2011, Gurd *et al*, 2009, Little *et al*, 2010, Ljubicic and Hood, 2009). While this issue has been discussed in detail elsewhere (Gurd, 2011, Gurd *et al*, 2012) the present results highlight the need for future work examining the implications of altered whole muscle SIRT1 in humans, and the role of SIRT1 protein content in determining skeletal muscle mitochondrial content *in vivo*.

Cardiovascular adaptation to HIT

Changes in O₂ pulse (VO₂/HR) can be used in combination with the Fick equation ($VO_2 = Q [HR \times SV] \times a-vO_2\text{diff}$) to provide information regarding the capacity of the cardiovascular system to deliver (SV) and extract (a-vO₂diff) oxygen (O₂ pulse [VO₂/HR] = SV x a-vO₂diff). Based on the observed changes in muscle oxidative capacity (i.e. no difference between groups) and the assumed relationship between changes in oxidative capacity and capillary density (Mathieu-Costello, 1993, Mathieu-Costello *et al*, 1996), it would be expected that the peak a-vO₂diff would have increased to the same degree in both groups. Therefore, we believe that the greater change in O₂ pulse (Figure 6) and VO₂peak in the 100% group reflect greater adaptation in SV; a conclusion that is

supported by previous research demonstrating a strong correlation between O₂ pulse and SV (Bhambhani *et al*, 1994, Whipp *et al*, 1996). This interpretation agrees with the classical view that SV limits maximal aerobic capacity (Richardson *et al*, 2000) and with previous results demonstrating coincident increases in SV and VO₂peak (Daussin *et al*, 2007, Murias *et al*, 2010). Thus, our results, combined with reports that increases in SV are intensity dependent (Helgerud *et al*, 2007) provided a minimal stimulus is met (Macpherson *et al*, 2011), suggest that changes in SV with HIT are linked to interval intensity. Further studies examining the mechanism(s) underlying this effect, and the minimal intensity required to increase cardiovascular function are needed.

Systemic Inflammation

Chronic low-grade inflammation is a hallmark of obesity and may contribute to a number of diseases such as type 2 diabetes and metabolic syndrome (Mathur and Pedersen, 2008, Dandona *et al*, 2004). While previous exercise interventions have shown reductions in markers of systemic inflammation (Balducci *et al*, 2010, Goldhammer *et al*, 2005), we did not detect any decreases in the proinflammatory markers IL-6 or TNF α following HIT (Table 2). The lack of change observed in these two inflammatory factors may indicate that improvements in inflammation require a longer period of regular exercise than 3 weeks (Stensvold *et al*, 2012) or that the population examined in the present study (young overweight/obese adults) did not exhibit low-grade inflammation prior to our intervention. The observed decrease in adiponectin following HIT in both groups (Table 2) is in agreement with previous work (Moran *et al*, 2011). However, at

present the impacts of intensity, duration and modality of exercise training on adiponectin levels, along with the implications associated with altered resting adiponectin levels require further study (Richards *et al*, 2010).

Interval intensity and tolerability of HIT

To examine the overall tolerability of these two interval intensities, affect, perceived enjoyment, and self-efficacy were explored. These psychological variables are important predictors of future exercise behaviour, and provide information on the likelihood that participants would consider engaging in this type of exercise again. In accordance with Ekkekakis' Dual Mode Model (DMM) (Ekkekakis, 2005), the affective response to the intervals was significantly lower in those that performed intervals at 100%. DMM proposes that affect – how pleasurable exercise is perceived to be – declines considerably as the intensity of exercise increases. Given that affect assessed during an exercise bout predicts engagement in that exercise behaviour up to 12 months afterwards (Williams *et al*, 2008), these findings suggest that intervals performed at 100% may not be adhered to.

In contrast to the affect data, participants reported equally high ratings of enjoyment in each exercise intensity group, and high confidence to a) successfully complete high-intensity intervals and b) schedule high-intensity interval exercise into their weekly routine. These findings support preliminary reports of enjoyment of high-intensity interval exercise (Bartlett *et al*, 2011). Self-efficacy is often touted as the most influential predictor of future volitional exercise behaviour (Bandura, 2004). The fact that

self-efficacy was equally high in both conditions suggests that, although perceived as less pleasurable in the more intense exercise condition, participants perceived HIT as manageable and were confident that they could schedule such activity into their lives on a regular basis. Future research examining adherence to HIT is needed.

Practical implications and future directions

Our results suggest that highly demanding or supramaximal intensities of HIT are not required to achieve important health benefits associated with exercise. However, the greater improvement in aerobic capacity and exercise performance observed in the 100% group indicate that training at higher intensities augments the training response. Interestingly, the equivalent responses on measures of exercise enjoyment and scheduling and task self-efficacy indicate that training at higher intensities may not preclude adherence (Table 3, Figure 7). Despite the similar mitochondrial adaptations observed, our results suggest that intensity should continue to be stressed as HIT is optimized and should health practitioners choose to prescribe HIT to overweight/obese/diseased populations.

Summary

In summary, we have examined interval training in overweight and obese young adults at two different intensities to determine if the responses occur in an intensity dependent manner. Our results indicate that improvements in aerobic capacity and exercise performance are intensity dependent but that changes in markers of skeletal muscle oxidative capacity are not. These results, combined with a greater change in O₂

pulse in the 100% group suggest that the additional improvements in aerobic capacity are a results of a greater cardiovascular adaptation, specifically, enhanced stroke volume. Further research concerning the impact of interval intensity on mechanisms underlying the adaptations to HIT would be beneficial for optimization of HIT protocols and exercise prescription.

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Chapter 4

General Discussion

4.1 Summary of Key Findings

Our results indicate that improvements in aerobic capacity and exercise performance occur in an intensity dependent manner. Considering the equivalent improvements made in measures of skeletal muscle oxidative capacity between groups, a component of peak oxygen consumption, the greater increase seen in aerobic capacity may be the result of enhanced cardiovascular adaptations such as improved stroke volume. Whatever the cause, the current evidence would suggest that, while improvements in markers of health do occur with HIT at 70%, there may be added benefit to exercising at higher intensities (i.e. 100%).

4.2 Overall Strengths of the Thesis

To our knowledge this is the only study to compare the impact of interval intensity during HIT with overweight and obese young adults. This is important as our study design may allow for improvement in exercise prescription by resolving an optimal intensity for interval training to stimulate health benefits. Further, having conducted this study in an overweight and obese population strengthens the applicability of the findings for such at-risk populations. As well, we believe the range of measures collected through this research represent a comprehensive overview of the layers of this project. Specifically, the use of such invasive measurements as muscle biopsies and blood

collection have allowed us to consider the effect of physiological mechanisms in interpreting our results.

4.3 Limitations of the Thesis

The small sample size involved with this study limited our ability to expand on our findings with regard to the relationship between interval intensity and the adaptive response. Ideally, more participants would have allowed us to create additional training groups at different intensities to strengthen our data regarding the intensity dependent impact of HIT including the possibility of an intensity threshold. Further, due to constraints surrounding the practical limits, both in number and size, of human muscle biopsies, our ability to contribute to the mechanisms underlying improvements in skeletal muscle oxidative capacity is restricted. Specifically, further examination of the relationship between interval intensity and the adaptations in skeletal muscle via mitochondrial biogenesis would be of particular interest.

4.4 Future Research Directions

There are still many questions that remain to be answered in resolving the optimal dose of HIT. Primarily, explanations as to why a higher intensity of HIT appears to increase cardiovascular function to a greater extent than lower intensity and the mechanisms that enable similar adaptation in mitochondrial content despite lower exercise intensity remain to be determined. Acute studies may help to reveal more detail regarding the mechanisms of improvement in markers of health with HIT. Research regarding the presence and location of intensity thresholds would be of additional interest

as there appears to be threshold values below 70% and above 100%, between which there is no added benefit to increasing exercise intensity in terms of mitochondrial content. Ultimately, randomized controlled trials involving overweight and obese young adults should be conducted to produce definitive evidence regarding the impact of interval intensity and existence of threshold intensity values that govern the health associated benefits of HIT.

4.5 Implications for Exercise Prescription

The practical implications of this research are that HIT does not need to be conducted at supramaximal or excessively demanding intensities in order to achieve the important health benefits of exercise. Participants in the 70% group were able to improve their aerobic capacity and exercise performance while demonstrating equivalent improvements to the 100% group in measures of skeletal muscle oxidative capacity. However, the greater improvements achieved by the 100% group in aerobic capacity and exercise performance indicate that there may be additional benefit to exercising at higher intensities. Importantly, there were no significant differences in measures of perceived enjoyment or self-efficacy between groups despite significantly greater decreases in affect during exercise for the 100% group. These findings indicate that, despite reduced affect during higher intensity intervals, adherence to such exercise programs may not suffer due to high intensity demands.

4.6 Summary of MSc Research Experiences

Over the course of my time in the Gurd lab I have learned many valuable skills. I am now able to perform a number of laboratory experiments in several types of biological samples (blood, muscle, saliva, etc.) and for a variety of measures (content, activity, extraction, etc.). As well, through countless hours reviewing academic literature and attempting to synthesize my thoughts I believe I have improved my ability to read, write, and think critically. From a practical perspective, I have learned a lot about problem solving; knowing where and how to look for answers when. Lastly, I have gained invaluable experience managing the responsibilities that accompany conducting a training study as well as supervising numerous participants and research assistants.

4.7 Conclusions

Interval exercise is an effective and efficient training modality with the potential to improve markers of health across a variety of populations. By working to optimize the training dose for an at-risk population we are creating a foundation for exercise prescription to maximize efficiency and reverse the trends toward disease.

Appendix A
Research Ethics Board – Informed Consent



School of Kinesiology and Health Studies
28 Division St., Kingston, Ontario
Canada, K7L 3N6

Consent to Volunteer for Participation in a Study

TITLE: Effect of exercise training at a variety of intensities on mitochondrial function in young lean and obese adults.

PRINCIPAL INVESTIGATOR: Brendon J. Gurd, PhD
Queen's University
School of Kinesiology and Health Studies
Kingston, Ontario, K7L 3N6
613-533-6000 ext. 79023

You are being invited to participate in a study examining the influence of different exercise protocols that vary in intensity (difficulty), duration (length) and mode (type) on mitochondrial function (the ability of your muscle to produce energy) and exercise capacity. This study will also compare the effects of these different exercise protocols in young adults who are either lean or overweight. You have been invited to participate in this

study because you are a young adult (20-40 years) who is either lean (waist circumference <86 cm) or overweight (waist circumference >94 cm). The following brief is intended to provide you with the details you should be aware of prior to your consent as a participant in this study. Please read the following information carefully and feel free to ask any question that you may have.

BACKGROUND INFORMATION

Exercise capacity (Fitness) is an important predictor of long term health. More specifically, the ability of your muscle's mitochondria to produce energy (mitochondrial function) is impaired with obesity and is a predictor of both further weight gain and development of type 2 diabetes. In healthy active populations interval training (repeated bouts of exercise separated by periods of recovery) is a potent, time effective stimuli for increasing mitochondrial function and exercise capacity. In addition, recreational activity is recommended as part of a healthy lifestyle, however its effects on mitochondrial function and exercise capacity are unknown. This study will examine the ability of different exercise training protocols and modes (cycling vs. whole body exercise vs. recreational games vs. ice hockey) to improve mitochondrial function and exercise capacity.

You will not be able to participate in the study if you have been diagnosed previously with any respiratory, cardiovascular (eg. High blood pressure, heart conditions), metabolic (eg. Diabetes), neurological or musculoskeletal

disease; or you are currently on medication; or you are a smoker; or you respond to the exercise protocol in an irregular manner (i.e. chest pains, dizziness, shortness of breath, excessive awareness of breathing)

EXPLANATION OF PROCEDURES

Participation

Participation in the study is voluntary. You may refuse to participate or withdraw from the study at any time with no effect on your academic or employment status.

Should you chose to participate you will take part in experimental procedures outlines below. These include exercise tests, physiological tests, and one of a selection of exercise training protocols. The investigator will explain to you, in detail how each of these procedures will be conducted in the study in which you have agreed to participate.

Exercise tests:

During each of the exercise tests you will be required to wear a nose-clip (to prevent you from breathing through your nose) and a rubber mouthpiece (similar to breathing through a snorkel or diving mask). This will enable us to measure the volume of air that you breathe in and out, and measure the gas concentration in that air. You may experience some initial discomfort from wearing the nose-clip and mouthpiece. You will also be required to wear a heart rate monitor around your chest during all tests. You will be asked to perform each of these tests on one occasion before and once occasion following exercise training.

Incremental exercise test: This test is performed on either a cycle ergometer (a stationary bike) or a treadmill and is designed to measure your fitness level. During this test the intensity of exercise increases gradually until you are physically unable to continue exercising because the intensity is either too high or too uncomfortable. The test will begin with the exercise intensity being very light and easy (very little resistance). After a few minutes the exercise intensity will gradually and continuously increase until you are unable to continue because of fatigue, or until you wish to stop.

Low-intensity exercise cycling test: This test is performed on a cycle ergometer (a stationary bike), and is designed to measure heart rate and your ability to burn fat during exercise. This test will last for 1 hour and will involve riding at a low-intensity (slow bike ride).

Exercise test of maximal power: This test is performed on either a cycle ergometer (stationary bike) or a treadmill and is designed to measure your ability to generate high levels of power during exercise. This test will involve either all out cycling or sprinting and will take between 30 and 60 seconds.

Physiological tests:

Blood sample: Both before and following training you will be asked to have a small sample of blood taken by a registered nurse. You may experience some minor discomfort when this small blood sample is drawn from a vein in your arm. The blood sampling may be painful and minor bruising is possible following venous blood sampling but generally fades within a few days.

Muscle Biopsy: Before and after training you will also be asked to have small amounts of muscle removed from your thigh muscle (quadriceps muscle) by means of a needle biopsy. The muscle biopsies will be taken by a medical doctor trained in this technique. While you are resting on a bed, an anesthetic will be applied locally to anesthetize the skin over your thigh muscle at the sites where the biopsies will be taken. A small incision (approximately 1 cm each) will be made through your skin and into your muscle at points approximately midway between your hip and knee. Small samples of muscle will be taken from each incision. This procedure is referred to as a biopsy.

There may be some discomfort associated with the biopsy procedure

(like someone pressing hard into your muscle) but you should experience no pain. Following the exercise there may be light bruising of the leg muscle but this will generally fade within a couple of days. There is also a slight risk of infection following a biopsy but proper sterilization of equipment and cleaning of the sampled area minimizes this risk. If the site of the muscle biopsy becomes more tender and redness and/or swelling develops in that area over the next five to seven days you should seek medical attention immediately. You should also report this change to the research person supervising your study as soon as possible. **Please refer to the Muscle Biopsy Information Sheet for more information regarding this procedure.**

Exercise Training Protocols:

Any exercise carries a slight risk of heart attack or may be uncomfortable if you are unfit or not used to exercise. The risk of a cardiac event (heart attack, dysrhythmias etc.) in a mixed subject population (healthy low risk and unhealthy high risk patients together) is approximately 6:10 000, however this risk decreases in a previously healthy (i.e. young, moderately active) population. There may be some minor discomfort during the exercise testing. You may experience increased awareness of breathing, muscle pain and/or fatigue, increased sweating, or a general feeling of fatigue or nausea, all of which are not unexpected consequences of exercise. You are being asked to participate in one of the following exercise training programs. The investigator will explain to you exactly what is involved in the specific

protocol you are being assigned to. Please initial beside the box that is checked.

- Low-intensity exercise training:** This protocol will involve riding a stationary bike for a period of up to 90 minutes at a low intensity similar to a leisurely bike ride. You will be asked to perform this protocol 3 times a week for a period of 6 weeks.
- Moderate-intensity exercise training:** This protocol involves riding a bike at a moderate-intensity, like a fast bike ride, for 4 minutes at a time (called an interval) followed by 2 minutes of rest. This interval will be repeated up to 10 times. You will be asked to perform this protocol 3 times a week for a period of 6 weeks.
- High-intensity exercise training:** This protocol involves riding a bike at a high-intensity, like an all out sprint, for 1 minutes at a time (called an interval) followed by 2 minutes of rest. This interval will be repeated up to 12 times. Alternatively you may be asked to perform a high-intensity exercise training protocol that involved whole body exercise (burpees, push-ups, squats, jumping jacks) for a maximum of 8 minutes at a time (20 second intervals separated by 10 seconds of rest). You will be asked to perform one of these protocols 3 times a week for a period of 6 weeks.
- Recreational activity:** This training program will require that you

report to a gym for an hour to take part in recreational games (basketball, floor hockey, handball, etc). You will be asked to participate in recreational activities 3 to 5 times per week for a period of 6 weeks.

- Recreational ice hockey:** This training program will require that you report to a local ice rink for an hour to take part in recreational ice hockey. You will be asked to participate in recreational ice hockey 3 times per week for a period of 6 weeks.

RISK OF INJURY

All exercise also carries a small risk of personal injury. Should any such injury occur during your participation in this study you will be initially cared for by the study administrators, all of whom are certified in first aid. Should further assistance be required you will be taken to the university health centre/hospital or emergency as required.

POTENTIAL BENEFITS OF PARTICIPATION

You will gain no direct benefit through participation in this study.

CONFIDENTIALITY

During the course of your participation in this study you will not be required to provide any personal information beyond your name and phone number (for study purposes only). All information obtained during the course of this study, including your name and fitness results, is strictly confidential and your anonymity will be protected at all times. Your information will be kept in locked files and will be available only to Dr. Brendon Gurd and those working within his laboratory. Your identity will not be revealed in any description or publication.

By signing this consent form, you do not waive your legal rights nor release the investigator(s) and sponsors from their legal and professional responsibilities.

VOLUNTARY CONSENT

I have been given an opportunity to ask any questions concerning the procedures. All of my questions regarding the research project have been satisfactorily answered. I understand that my test results are considered confidential and will never be released in a form that is traceable to me. I do understand that I am free to deny consent if I so desire, and may withdraw from the study at any time without any effect on my academic or employment status.

Should I have any questions about the study, I know that I can contact Dr. Brendon Gurd (613 533-9023), Dr. Jean Coté, Head, School of Kinesiology and Health Studies (613 533-6601), or Dr. Albert Clark, Chair, Queen's Health Sciences & Affiliated Teaching Hospitals Research Ethics Board (613 533-6081). A copy of this consent form will be provided me for my records. My signature below means that I freely agreed to participate in this study.

Volunteer's Signature

Date

STATEMENT OF INVESTIGATOR

I, or one of my colleagues, have carefully explained to the subject the nature of the above research study. I certify that, to the best of my knowledge, the subject understands clearly the nature of the study and demands, benefits, and risks involved to participants in this study.

Principal Investigator's Signature

Date

Appendix B

Muscle Biopsy Information Sheet

Muscle Biopsy Information Sheet

You have volunteered to take part in a research study that requires you to undergo a muscle biopsy. This is a commonly performed procedure in research studies and for the medical diagnosis of muscle disease. The procedure will be performed by a medical doctor trained to perform muscle biopsies or a specially trained researcher directly supervised by a medical doctor.

The muscle biopsy involves the removal of a small piece of muscle tissue from one of the muscles in your leg using a sterile hollow needle. The area over the outside of your lower thigh muscle (vastus lateralis muscle) will be carefully cleaned. A small amount of local freezing (anesthetic) will be injected into and under the skin. You will likely experience a burning sensation while the freezing is injected. Then a small, 4 – 5 mm incision will be made in your skin in order to create an opening for the biopsy needle. There is often a small amount of bleeding from the incision, but this is usually minimal.

The biopsy needle will then be inserted through the incision into the thigh muscle and a small piece of muscle (100 – 200 mg), about the size of a pencil eraser, will be quickly removed and the needle taken out. During the time that the sample is being taken (about 5 seconds), you may feel the sensation of deep pressure in your thigh and on some occasions this is moderately painful. However, the discomfort very quickly passes and you are quite capable of performing exercise and daily activities. There may be some minimal bleeding when the needle is removed which may require application of pressure for a few minutes.

Following the biopsy, the incision will be closed with sterile tape (steri-strips), and wrapped with a tensor bandage. You should refrain from excessive muscle use for the remainder of the day. Once the freezing wears off, your leg may feel tight and often there is the sensation of a deep bruise or "Charlie Horse". Pain killers such as Acetaminophen (e.g. Tylenol) or Ibuprofen (e.g. Advil) are acceptable if you experience pain associated with the biopsy. It is also beneficial to periodically apply an ice pack to the biopsy site the following day, as this will help to reduce any swelling and any residual soreness. The following day your leg may feel uncomfortable when going down stairs. The tightness in the muscle usually disappears within 2 days and subjects routinely begin exercising at normal capacity within 2 days. In order to allow the incisions to heal properly and minimize any risk of infection, you should avoid prolonged submersion in water for 4 days. Daily showers are acceptable, but baths, swimming, saunas, etc. should be avoided for at least 4 days following the biopsy procedure.

Potential Risks

- The local freezing will likely result in a burning feeling in the thigh at the time of the injection. This will last only 5 – 10 seconds. There is an extremely low risk of allergic reaction to the local injection (1 in 1 million).
- The chance of a local skin infection is less than 1 in 1000. Carefully cleaning the skin and keeping the area clean and dry until the skin heals will minimize this.
- Most subjects experience local soreness and stiffness in the leg for two or three days after the biopsy similar to a deep bruise or Charlie Horse. There is a very low risk of internal bleeding at the biopsy site which can result in more prolonged pain and stiffness in the leg.
- On occasion, a small lump of scar tissue may form under the site of the incision, but this normally disappears within 2-3 months, or within a few weeks if massaged. A small visible scar often remains from the biopsy incision.
- There is the possibility of a small area of numbness (about the size of a one dollar coin) around the biopsy site. This usually resolves over 5 – 6 months. There is a very low risk (estimated at less than 1/5000) of damage to a small nerve branch to the muscle. This would result in partial weakness of the vastus lateralis muscle (one of four muscles that straightens the knee) and would likely have no impact on day-to-day activities. Nerve injuries like this usually resolve in 8 – 12 months, but there is a theoretical risk of mild leg weakness.

Concerns or Problems

Infection can be serious, if you experience excessive redness, swelling or infection around the biopsy site or pain or stiffness in your leg you must contact Dr. Simpson right away. Dr. Simpson will be available 24 hours a day to answer any of your concerns or questions about the biopsy.

Dr. Craig Simpson: Cell Phone (613) 532-3371

However, if for some reason, you are not able to contact Dr. Simpson then you should contact your family doctor or go to the Emergency Department.

Please keep this Information Sheet until such time as your biopsy site has fully healed.

MUSCLE BIOPSY SUBJECT SCREENING FORM

To help us ensure your safety and wellbeing please answer the following questions.

1. Have you ever had a negative or allergic reaction to local freezing (e.g. during dental procedures)?

No Yes

2. Do you have any tendency toward easy bleeding or bruising (e.g with minor cuts or shaving)?

No Yes

3. Are you currently taking any medications that may increase the chance of bleeding or bruising (e.g. Aspirin, Coumadin, Anti-inflammatories, Plavix)?

No Yes

4. Have you ever fainted or do you have a tendency to faint when undergoing or watching medical procedures?

No Yes

5. Will you contact Dr. Craig Simpson directly if you have any concerns about the biopsy site including: excessive redness, swelling, infection, pain or stiffness of the leg?

No Yes

Subject Name (print) : _____

Subject Signature : _____

Date : _____

Signature of Person Conducting Assessment: _____

Appendix C

Post-training Survey

Please complete the following in with regard to your current feelings about exercise.

1.
 - a. How enjoyable is it for you to complete hard or very hard exercise of at least 30 minutes 3-days/week?

1	2	3	4	5	6	7
Not Enjoyable at All			Moderately Enjoyable			Extremely Enjoyable

- 2.

	Not at all Confident			Moderately Confident				Completely Confident		
How confident are you that you could schedule hard or very hard exercise of at least 30 minutes 3 days/ week?	1	2	3	4	5	6	7	8	9	10
How confident are you that you could physically complete hard or very hard exercise of at least 30 minutes 3 days per week?	1	2	3	4	5	6	7	8	9	10

Appendix D
11-Point Likert Affect Scale

What number best represents how you feel right now?

-5	-4	-3	-2	-1	0	1	2	3	4	5
Very Bad	Moderately Bad	Somewhat Bad	Somewhat Good	Moderately Good	Very Good					