INDIVIDUAL VARIATION IN VO$_2$PEAK RESPONSE FOLLOWING SPRINT INTERVAL TRAINING: THE ROLE OF PERIPHERAL ADAPTATION

by

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A thesis submitted to Kinesiology and Health Studies in conformity with the requirements for the Degree of Master of Science

Queen’s University
Kingston, Ontario, Canada
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Abstract

There is a large degree of heterogeneity in response to regular physical activity at the individual level, with some exhibiting no or very small improvements in VO2peak following highly controlled exercise training. The purpose of this thesis was to examine individual variation in VO2peak response to sprint interval training (SIT) in relation to individual responses to multiple measures of peripheral physiological adaptation. Specifically, VO2peak, capillary density, fibre-specific SDH content, and type I fibre % were measured in 23 young, healthy, recreationally active males before and after 4 weeks SIT (Tabata protocol 4 x per week). The key findings of this experiment included that, when separated into tertiles of VO2peak response, the high (HI) and low (LO) groups differed significantly in VO2peak change after training. Secondly, there was no difference between HI and LO groups for response in any of capillary density, fibre-specific SDH content, or fibre type %, with no correlation found between individual VO2peak response and changes in any measured peripheral variable. Together, these results confirm that individuals respond heterogeneously to SIT and suggest that this heterogeneity does not result from differences in individual changes in capillary density, fibre-specific SDH content or type I fibre %. It is speculated that some other combination of peripheral physiological adaptation must explain variability in VO2peak response to 4 weeks of SIT.
Co-Authorship

The manuscript presented in this thesis: Individual Variation in VO$_2$peak Response Following Sprint Interval Training: The Role of Peripheral Adaptation is the work of James Raleigh in collaboration with his supervisor Dr. Brendon J. Gurd, Matthew Giles and Matthew Nelms. James Raleigh contributed to: developing the research question, conducting background research, VO$_2$peak test administration, tissue preparation and analysis, statistical analysis, interpreting the results, and writing the initial draft of the manuscript. Dr. Brendon J. Gurd contributed to the design of the experiment, developing the research question, harvesting of tissues, provided guidance on the interpretation of results, and manuscript revision for intellectual content. Matthew Giles assisted in VO$_2$peak test administration and tissue preparation and analysis. Matthew Nelms assisted in tissue analysis.
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<th>Description</th>
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<tbody>
<tr>
<td>ATP</td>
<td>adenosine triphosphate</td>
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<tr>
<td>ADP</td>
<td>adenosine diphosphate</td>
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<td>ANOVA</td>
<td>analysis of variance</td>
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<td>AU</td>
<td>arbitrary units</td>
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<td>BMI</td>
<td>body mass index</td>
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<td>β-oxidation</td>
<td>beta oxidation</td>
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<td>BPM</td>
<td>beats per minute</td>
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<tr>
<td>BV</td>
<td>blood volume</td>
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<td>C</td>
<td>Celsius</td>
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<td>Ca</td>
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<td>cytochrome c</td>
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<td>endothelial nitric oxide synthase</td>
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<tr>
<td>ETC</td>
<td>electron transport chain</td>
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<td>ETS</td>
<td>electron transport system capacity</td>
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<td>GLUT 4</td>
<td>glucose transporter type 4</td>
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<tr>
<td>Hb mass</td>
<td>total body hemoglobin mass</td>
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<tr>
<td>HIIT</td>
<td>high intensity interval training</td>
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<td>HR</td>
<td>heart rate</td>
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<td>heart rate reserve</td>
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<tr>
<td>LDH</td>
<td>lactate dehydrogenase</td>
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<td>left ventricular mass</td>
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<tr>
<td>m</td>
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</tr>
<tr>
<td>MCT</td>
<td>mono-carboxylate transporter</td>
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<td>MHC</td>
<td>myosin heavy chain</td>
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<tr>
<td>MICT</td>
<td>moderate intensity continuous training</td>
</tr>
<tr>
<td>Min</td>
<td>minute</td>
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<tr>
<td>MSc.</td>
<td>Master’s of science</td>
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<tr>
<td>NADH</td>
<td>nicotinamide adenine dinucleotide</td>
</tr>
<tr>
<td>NR</td>
<td>nonresponse</td>
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<tr>
<td>P</td>
<td>oxidative phosphorylation capacity</td>
</tr>
<tr>
<td>PCr</td>
<td>phosphocreatine</td>
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<td>pyruvate dehydrogenase</td>
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<td>PDK</td>
<td>pyruvate dehydrogenase kinase</td>
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<tr>
<td>PFK</td>
<td>phosphofructokinase</td>
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PGC-1α - peroxisome proliferator activated receptor γ (PPARγ) coactivator 1α
mRNA- messenger ribonucleic acid
PV- plasma volume
pWR- peak work rate VO₂peak – peak oxygen consumption
Q- cardiac output
Qmax- maximum cardiac output
RER- respiratory exchange ratio
RNA- ribonucleic acid
RPM- revolutions per minute
s- second
SD- standard deviation
SEM- standard error of the mean
SDH- succinate dehydrogenase
SIT- sprint interval training
SNP- single nucleotide polymorphism
SR- sarcoplasmic reticulum
SV- stroke volume
TE- typical error
TTC- time to completion
wk- week
CHAPTER 1:  

Introduction

1.1 General Introduction

Low exercise aerobic capacity is associated with an increased risk of metabolic disorder and cardiovascular disease (Ortlepp et al. 2004, Church et al. 2005), as well as premature death (Wilmore et al. 2001). Physical activity is widely promoted to improve exercise capacity, along with numerous other risk factors and health outcomes (Bouchard and Rankinen 2001). While these beneficial effects of regular exercise are consistently seen at the group level, it has become increasingly clear that there is a large degree of heterogeneity in response to regular physical activity at the individual level, with some exhibiting no or very small improvements in aerobic capacity (VO₂peak) following highly controlled exercise training (Bouchard and Rankinen 2001, Scharhag-Rosenberger et al. 2012, Astorino and Schubert 2014). “Nonresponders” make up as much as 20 to 45% of exercise study participants, following both endurance (Sisson et al. 2009, Ross et al. 2015) and interval training protocols (Astorino and Schubert 2014, Gurd et al. 2016, Raleigh et al. 2016). While these individuals may represent a large portion of exercise study participants, the vast majority of published studies emphasize main effects and group differences while paying little attention, if any, to individual differences (Bouchard and Rankinen 2001). Of the over 100 studies published in the last decade examining the effects of HIIT/SIT on variables including VO₂peak, only 3 have directly examined individual responses to interval training.

1.2 Linking nonresponse to SIT

Results from numerous studies in recent years indicate that individual variability exists in the magnitude of response to endurance training. Bouchard and colleagues (1999) examined data
from the HERITAGE study, revealing a mean increase in VO$_2$peak of 0.4 L/min yet individual responses ranged from no improvement to 1.0 L/min. More recently, Scharhag-Rosenberger and colleagues (2012) found similar variability in change in VO$_2$peak (-0.38-0.87 L/min) and exercise HR (-22.0-2.0 bpm) following 1 year of endurance training, with 24% and 17% of participants respectively being classified as nonresponders to each of these outcomes.

Given the clear presence of these nonresponders to endurance exercise programs, further research has focused on identifying underlying factors for such individual variation in response. Genetics, age, sex and baseline cardiovascular function have all been found to influence an individual’s response to endurance training (Cunningham et al. 1987, Bouchard et al. 1999, Hautala et al. 2003, Sisson et al. 2009). It has also been hypothesized that an increase in exercise stimulus (i.e. intensity) may result in more widespread responses in individuals with low sensitivity to exercise training (Buford et al. 2013). Despite this claim, there is very little research examining individual responses to higher intensities of exercise such as submaximal, high-intensity interval training (HIIT) or supramaximal sprint interval training (SIT).

Astorino and Schubert (2014) retrospectively examined individual responses to each a SIT and a HIIT training protocol. SIT training consisted of 6 days of Wingate training (4, 30-sec. intervals at an “all-out” intensity), while the HIIT protocol involved 12 weeks of training between 60 and 90% of peak work rate (pWR) for 6-10 1-minute intervals. Individual changes in VO$_2$peak, exercising HR and fat oxidation were examined, with nonresponders present for all variables. Specifically, VO$_2$peak nonresponse was recorded at rates of 35% and 5% for SIT and HIIT respectively. Encouragingly, however, frequency of nonresponse within an individual to all three outcomes was low (1 in 40), indicating that HIIT and SIT each provide an adequate stimulus for widespread physiological adaptation. Similarly, Raleigh and colleagues (2016)
compared rates of VO\(_2\)peak response to HIIT and SIT protocols, finding that 20% of HIIT and 18% of SIT participants displayed nonresponse following 3 weeks of training. Gurd and colleagues (2016) also examined the incidence of nonresponders for maximal or submaximal performance following a variety of SIT protocols, with the overall rate of nonresponse for VO\(_2\)peak being 22%. Interestingly the rate of nonresponse declined to 0% in protocols where participants trained 4 times per week (n=18) compared to rates between 30 and 50% in those training 3 times per week (n=45). While each of these studies provide preliminary evidence for considerable heterogeneity in individual patterns of response following HIIT/SIT, it is evident that more research is required to further characterize how individuals respond to HIIT/SIT.

1.3 Mechanisms underlying nonresponse to HIIT/SIT: what is the role of peripheral adaptation?

VO\(_2\)peak is the most commonly used indicator of improvements in aerobic exercise capacity in exercise training literature, and furthermore it is the most used measure of individual response in studies that examine individual variation to exercise training (Hautala et al. 2006, Sisson et al. 2009, Ross et al. 2015). Following endurance training, improvements in VO\(_2\)peak are typically attributed to increases in cardiac function and oxygen delivery, or central adaptation (Grassi 2001). When it comes to SIT, however, improvements in VO\(_2\)peak following training have been credited more to peripheral adaptations (ie. increased skeletal muscle oxidative capacity, etc.) (MacPherson et al. 2011). Given that SIT-induced improvements in VO\(_2\)peak are primarily attributed to peripheral adaptations within skeletal muscle, it is possible that variability in individual peripheral responses to training may be partly responsible for heterogeneity in individual VO\(_2\)peak response to SIT. Preliminary evidence suggests that patterns of individual response in sarcoplasmic reticulum function (Ørtenblad et al. 2000), muscle oxidative capacity
(Gibala et al. 2006), and mitochondrial respiration (Granata et al. 2015) in response to SIT exists, but no research has specifically examined whether this individual variability in peripheral response can explain training-induced variability in VO₂peak response following SIT.

1.4 Thesis Focus and Experimental Approach

Exercise training of all varieties elicits a range of responses in individuals, with much of the current research rooted in examining individual response to endurance type exercise. While early evidence suggests individual patterns of response to SIT are also prevalent (Ørtenblad et al. 2000, Gibala et al. 2006, Granata et al. 2015), further research is required to define the relationship between VO₂peak and peripheral physiological adaptations following SIT training. At present, there is no study that compares individual changes in VO₂peak to multiple measures of peripheral adaptation following a SIT protocol. Therefore, the aim of this thesis is to investigate this relationship by running participants through a highly controlled SIT exercise protocol, and collecting both VO₂peak and multiple measures of muscular adaptation before and after training.

1.5 Thesis Objective

To test the hypothesis that SIT will induce group-level improvements to VO₂peak and peripheral factors, as well as higher levels of peripheral response in high VO₂peak responders compared to low VO₂peak responders, we will examine the relationship between individual responses in VO₂peak as well as multiple measures of peripheral adaptation (i.e. fiber type shift, oxidative capacity and capillary density) following chronic SIT.
1.6 Thesis Hypotheses

Specifically, we hypothesize that 1) there will be high and low responders in VO$_2$peak following training that will differ significantly in their magnitude of response, and 2) high individual responders to VO$_2$peak will also be high responders to measures of peripheral adaptation (fibre type, oxidative capacity and capillary density) following 4 weeks of SIT training, while low responders to VO$_2$peak will also be low responders to peripheral measures.
1.7 References


Granata, C., Oliveira, R.S.F., Little, J.P., Renner, K., and Bishop, D.J. 2015. Training intensity modulates changes in PGC-1α and p53 protein content and mitochondrial respiration, but


Chapter 2: Literature Review

2.1 Introduction

2.1.1 Interval Training Definitions

Interval training, by definition, involves repeated bouts of relatively intense exercise interspersed by periods of rest or lower-intensity recovery. Many acronyms have been used for interval training protocols, leading to some degree of confusion when comparing results from various studies. Recently, Weston and colleagues (2013) proposed a basic set of nomenclature for interval training protocols, that will be used in this review. These definitions include using high intensity interval training (HIIT) to describe interval training targeting intensities between 80-100% of peak heart rate (pHR) and sprint interval training (SIT) for protocols targeting intensities in excess of 100% of VO$_2$peak. It is also worth noting that moderate intensity continuous training (MICT) encompasses endurance exercise at 60-75% pHR.

2.1.2 Introducing Nonresponse to Exercise Training

At the group level, each MICT, HIIT and SIT consistently elicit improvements in VO$_2$peak, as well as numerous other physiological adaptations (Gist et al. 2006; Sloth et al. 2013; Gibala et al. 2006; Scribbans et al. 2014), however, it has become increasingly clear that there is a large degree of heterogeneity in response to regular physical activity at the individual level. Indeed, some individuals exhibit no or very small improvements in VO$_2$peak following highly controlled exercise training (Bouchard and Rankinen 2001). “Nonresponders” to MICT for VO$_2$peak have recently been found to be as high as between 20 and 45% (Sisson et al. 2009). Nonresponse to MICT has also been observed for indices of submaximal performance.

Recent evidence suggests that individual VO2peak responses to HIIT may be superior to that following MICT (Bacon et al. 2013), since higher intensities of exercise may induce adaptation in those with low sensitivity to MICT (Buford et al. 2013). Despite such speculation, nonresponse has still been shown following HIIT/SIT (Astorino and Schubert 2014, Gurd et al. 2016), highlighting a need to examine the rates of individual response to such protocols, as well as the underlying physiological mechanisms responsible for adaptation/nonresponse. Filling this knowledge gap may shed light on the optimal exercise program for eliciting benefits in the general population.

2.2 Overview of Literature Review

The following literature review will begin by exploring individual patterns of response, including the presences of nonresponders, to exercise training. This will be followed by an examination of potential factors which may contribute to the varying individual responses to said training. This section will be broken down into a discussion of the role of genetic as well as differences in subject characteristics (i.e. age, sex, race and initial fitness level) in individual response to training. The next section will then explore types of physiological adaptation or “response” (i.e. central vs. peripheral adaptations) to exercise, and how each may play a role in VO2peak response to training. The final section will focus upon individual variability in VO2peak response to HIIT/SIT, with a specific emphasis on the contribution of peripheral adaptive response.
2.3 Individual Patterns of Response to Exercise Training

2.3.1 Section Introduction

This section will begin by providing a general overview of the importance of examining individual variation as well as group response to exercise training programs. Given that nonresponders have been found in the majority of literature looking at individual responses, it is important to examine how nonresponders are identified and classified. This will then transition into an examination of potential factors that may play a role in individual patterns of response to exercise training. The section will finish with a discussion of the potential implications of individual patterns of response to exercise training.

2.3.2 Nonresponse to Exercise Training

It is well accepted that exercise training at the group level leads to improvements in aerobic capacity, as well as decreased risk for metabolic disorder and cardiovascular disease. Indeed, until fairly recently, the vast majority of published studies have focused solely upon main effects and group differences, without considering individual changes following exercise training (Timmons 2011). Bouchard and Rankinen (2001) were amongst the first to comprehensively examine individual variation to standardized exercise. In a very large randomized controlled trial (n=720), they noted “considerable heterogeneity in the responsiveness of physiological indicators of risk factors to regular physical activity” (Bouchard and Rankinen 2001) over a training period of 21 weeks, including some “nonresponders” who displayed no change in VO$_2$peak from baseline at follow-up. Subsequently, studies utilizing a range of protocols and study populations have examined individual variation in VO$_2$peak response to standardized exercise programs, with nonresponse commonly found in excess of
30% (Sisson et al. 2009, Astorino and Schubert 2014, Ross et al. 2015, Wolpern et al. 2015). It has become increasingly clear over the last two decades that individual VO$_2$peak response to exercise is highly heterogeneous, and thus, it is important to characterize individual patterns of response to exercise to help define the underlying mechanisms of this response.

2.3.3 How Are Nonresponders Classified and Identified?

While there is a clear heterogeneity in individual response to exercise, with some participants demonstrating greater adaptation in VO$_2$peak than others, it is important to clarify which criteria is used to identify a nonresponder. Researchers have utilized various cut-off points to determine the point at which an increase in a given variable in an individual is large enough to constitute a relevant physiological response. Sisson and colleagues (2009) used the basic strategy of classifying nonresponse by any individual with a change in VO$_2$peak < 0, however this method fails to account for biological variability associated with examining the rates of response to training (Gurd et al. 2016). More recently, several groups have classified a meaningful response to exercise training using previously published coefficients of variations (Scharhag-Rosenberger et al. 2012, Astorino and Schubert 2014, Wolpern et al. 2015). While this approach does account for biological variability, it fails to acknowledge equipment-specific technological variability, and thus is still not ideal.

Gurd and colleagues (2016) have since implemented an approach using typical error, calculated utilizing the following equation adapted from Hopkins (2000):

\[ TE = \frac{SD_{diff}}{\sqrt{2}} \]

where $SD_{diff}$ is the variance (standard deviation) of the difference scores observed between 2 repeated tests. A nonresponder for a given physiological variable was defined as an individual
who failed to demonstrate an increase or decrease that was greater than 2 x TE away from zero. The odds of an individual change that is greater than 2 x the TE being a true physiological change are 12:1 (Hopkins 2000). Gurd and colleagues (2016) importantly calculated their own TE of measurement for VO\(_2\)peak, lactate threshold and a 500 kcal time to completion (TTC) ride specifically for the equipment used to evaluate these variables in the 5 studies assessed and analyzed. This cutoff method provides a robust and conservative threshold for determination of individual response (Gurd et al. 2016), and future studies should also consider both biological variability and technical error of measurement observed within a given laboratory (Gurd et al. 2016).

2.3.4 How Participant Characteristics Influence Response to Exercise Training

Given the clear heterogeneity in individual response to exercise training, it is important to identify the underlying factors that may cause one individual to respond differently than another. Potential factors that have been examined extensively within the current literature include genetic factors as well as phenotypical differences amongst participants such as age, sex, race and initial fitness level.

One of the first major studies to examine genetic influence on VO\(_2\)peak response to exercise training was conducted by Bouchard and colleagues (1999), who examined 481 sedentary adults from 98 two-generation families completing 3 sessions of training each week over a period of 20 weeks. They found a maximal heritability estimate for VO\(_2\)peak response to training, adjusted for age and sex, reaching 47%, leading to the conclusion that a substantial component of the observed individual heterogeneity in response to exercise training can be attributed to genetics (Bouchard et al. 1999). This result led to others attempting to uncover the
specific genes that may be directly involved in this pathway. The intricacy of the human genome makes the exploration of a specific collection of genes daunting, particularly for what is likely a highly complex trait in individual VO_{2\text{peak}} response to exercise. A substantial body of research has been dedicated towards “candidate genes” such as peroxisome proliferator-activated receptor γ coactivator (PGC-1α). PGC-1α has been referred to as the “master regulator” of mitochondrial biogenesis in skeletal muscle, and is suggested to activate both nuclear and mitochondrial transcription factors for this adaptive process via feedforward signaling (Handschin et al. 2003). However, there is some debate as to how integral PGC-1α expression is as a predictor of VO_{2\text{peak}} response to exercise training, with one study showing that PGC-1α knockout mice can respond to endurance training and improve aerobic capacity (Leick et al. 2008). Identification of genomic markers for complex human traits have traditionally required enormous sample sizes, with each single-nucleotide polymorphism (SNP) seeming to contribute only weakly as a predictive marker for that trait (Frazer et al. 2009). With this in mind, Timmons and colleagues (2010) combined RNA profiling with single-gene DNA marker association analysis to develop a validated molecular predictor for VO_{2\text{peak}} response to exercise training, by measuring a ~30 gene RNA expression signature in muscle prior to training. They found that this model explained 23% of the variance in gains in VO_{2\text{peak}} in the HERITAGE study. While it is clear that genetics play an important role in predicting VO_{2\text{peak}} trainability, substantial future research is required to not only clarify the important genes and gene sequences that contribute to this trait, but to provide a clear understanding of how these genes impact the underlying physiology that results in small or large changes in VO_{2\text{peak}} following training.

Phenotypical differences between participants including age, sex, race and baseline VO_{2\text{peak}} have each also been examined in relation to VO_{2\text{peak}} response to training, with largely
equivocal results. When it comes to age, some research indicates no relationship with exercise trainability (Bouchard and Rankinen 2001), while others find it to be a predictor of nonresponse, such that older participants are less trainable than their younger counterparts (Hautala et al. 2003). Similarly, baseline VO$_2$peak has been suggested to play no role in determining VO$_2$peak response to training (Kohrt et al. 1991), while contradictory evidence has found a lower baseline to predict a larger magnitude of response (Cunningham et al. 1987). When it comes to differences between males and females, existing evidence suggests little or no difference between sexes in trainability of VO$_2$peak (Kohrt et al. 1991), or a tendency for women to be less trainable than men (Blumenthal et al. 1989). Existing evidence would seem to suggest no significant relationship between VO$_2$peak trainability and race (Skinner et al. 2001). Parker and colleagues (2010) employed a more complex approach, proposing age and sex as synergistic contributors to heterogeneity in exercise training response. They proposed that known differences in cardiovascular aging between men and women underlie an observed relative lack of response to exercise in older women compared to each young men, older men and young women. While the differences in hemodynamic aging seen between males and females that may lead to this relationship are beyond the scope of the current review, the proposed age-sex interaction as a contribution to heterogeneous individual response to exercise training (Parker et al. 2010) highlights the need for more innovative approaches to the question as to whether phenotypical differences between participants affect VO$_2$peak response to standardized exercise.

2.3.5 How Exercise Volume Affects Response to Training

It has been postulated that individuals with low sensitivity to generalized exercise training may require a greater stimulus (i.e., increased training dose or intensity) to achieve a
meaningful physiological response (Buford et al. 2013). Accordingly, increasing exercise volume may reduce the rate of nonresponse amongst these individuals (Gurd et al. 2016).

There are 3 recent studies that have examined the independent effects of increasing exercise volume on rates of individual response to exercise training. Based on their results, there appears to be a dose-response relationship, such that increasing exercise volume decreases nonresponse for VO$_2$-peak following training, however the relationship may be more complex than this. Sisson and colleagues (2009) were the first to directly examine the relationship, conducting a randomized controlled trial on sedentary post-menopausal women, each of which was randomized into one of 3 exercise groups of varying weekly energy expenditure (4, 8, or 12 kcal/kg/wk) for 6 months. They found greater volumes of exercise to be associated with lower probability of VO$_2$-peak nonresponse, such that the likelihood of not responding in the 12kcal/kg/wk group was 74% lower than in the 4kcal/kg/wk group (Sisson et al. 2009).

These results have since been corroborated by Ross and colleagues (2015), who found that increasing exercise time from ~150 to 300 min/wk resulted in a reduction in the rate of nonresponse from 38.5% to 17.6%. Interestingly, they also highlighted differences in time required for responders to reach their maximum improvement in VO$_2$-peak, with the low-dose group plateauing at 8 weeks, and the high-dose group plateauing at 16 weeks. This result suggests that those classified as nonresponders in exercise training studies, may actually be responders when allowed a sufficient training period to elicit maximal response to a given training stimulus. Given that many exercise training studies (particularly SIT studies) examine individual variation in response are 6 weeks or shorter (MacPherson et al. 2011, Gurd et al. 2016), this presents some doubt as to those classified as nonresponders in these studies.
Gurd and colleagues (2016) also made an important conclusion based upon their secondary analysis of several SIT training studies, finding that the incidence of VO\textsubscript{2peak} nonresponse for SIT 3 times per week was 37% (Scribbans et al. 2014b, Zelt et al. 2014), compared to a complete elimination of nonresponse when SIT was performed 4 times per week (Ma et al. 2013, Scribbans et al. 2014a). These results suggest a threshold for exercise training, such that when the training frequency per week reaches 4, nonresponders are minimized.

Given the research reviewed on the relationship between exercise training volume and rate of VO\textsubscript{2peak} nonresponders following training, there appears to be a dose-response relationship, such that increases in total volume per week results in greater response (Sisson et al. 2009). Additionally, consideration may need to be paid to the length of the training period and frequency of weekly sessions, as greater weekly volumes are associated with participants taking longer to reach their peak response (Ross et al. 2015) and more sessions per week have been linked to greater incidence of response (Gurd et al. 2016).

2.3.6 How Exercise Intensity Affects Response to Training

Increases in exercise intensity may provide adequate stimulus to elicit VO\textsubscript{2peak} adaptation for those with low sensitivity to generalized exercise training, independent of exercise volume (Buford et al. 2013). Exercise intensity, however, appears to have a more complex relationship with individual response to training than exercise volume. While only a few studies have examined differing intensities of training on individual variation in training response, a preliminary relationship can be established.

Wolperrn and colleagues (2015) found that increases in submaximal exercise from ~60-70\% of heart rate reserve (HRR) to ~70-80\% of HRR resulted in a decrease in nonresponse from
58% to 0% over a 12-week training period. The authors, however, point out that their sample size (n=12 per group) is lower than other major training studies and thus interpretation of results should take this into consideration. Ross and associates (2015) used a much larger sample size to compare individual responses in VO$_2$peak between submaximal intensities corresponding to ~50% of VO$_2$peak (n=51) and ~75% of VO$_2$peak (n=31). Following 24 weeks of training, the low-intensity group yielded nonresponse for VO$_2$peak in 17.6% of participants, whereas the high-intensity had zero recorded nonresponders. Thus, from limited evidence it would appear that increasing intensity of submaximal exercise yields increasing rates of response. Whether this relationship is conserved when intensity of exercise is further increased towards maximal and supramaximal intensities (i.e. HIIT and SIT) becomes the next important question.

Astorino and Schubert (2014) retrospectively examined individual responses to each a SIT and a HIIT training protocol. SIT training consisted of 6 days of Wingate training, while the HIIT protocol involved 12 weeks of training between 60 and 90% of peak work rate (pWR). Individual changes in VO$_2$peak nonresponse were recorded at rates of 35% and 5% for SIT and HIIT respectively. While these results provide preliminary evidence for individual patterns of response following HIIT/SIT, including what would appear to be a higher rate of nonresponse in SIT compare to HIIT, it is important to note that those in the SIT protocol performed much less exercise volume per session as well as a much shorter training period, thus not isolating the effects of intensity on individual responses.

Raleigh and colleagues (2016) examined 3 separate intensities of work-matched HIIT and SIT targeting 80% (LO), 115% (MID) and 150% (HI) of peak work rate (pWR) respectively. Due to the work-matched nature of these experimental groups and the fact that more than two conditions were compared allowed for the independent effect of intensity on the rate of
nonresponses to be examined, including a possible dose-response relationship. Our results showed the highest rate of responders in the MID condition (92%), followed by the HI (82%) and LO (67%) conditions. The increase in responders observed when increasing intensity from LO to the MID condition may represent a threshold beyond which no further increase in response is achieved with further increases in intensity (MID and HI conditions did not differ), however the sample size examined in this study is too small for anything beyond speculation to be possible (Raleigh et al., 2016).

Thus, preliminary evidence would suggest that increasing submaximal exercise intensities yields greater rates of responders for VO\textsubscript{2}\text{peak}. Beyond this point, it would appear that further increases in intensity (i.e. increasing intensity from HIIT to SIT) yields no further increases in responders, however more research examining work-matched conditions with large sample sizes are required to make more definitive claims.

2.4 Types of physiological adaptation or response to HIIT/SIT

2.4.1 Section Introduction

This section will begin by providing a general overview of each central, and peripheral physiological adaptations that accompany HIIT and SIT. This will be followed by a discussion of which specific adaptations may play a role in individual variability of VO\textsubscript{2}\text{peak} response to HIIT/SIT training.

2.4.2 Impact of HIIT and SIT on Central Adaptations to Exercise

Physiological adaptations to exercise can be characterized as either central or peripheral adaptations. Central adaptations involve improvements in the ability of the cardiovascular system
to deliver oxygen by convection to working tissue. This includes increased left ventricular function (e.g. augmented cardiac output (Q) and stroke volume (SV)), as well as increases in blood volume (BV), and therefore its constituents (e.g. plasma volume (PV) and total body hemoglobin mass (Hb\text{mass})). HIIT and SIT have traditionally been associated with improvements in peripheral rather than central factors (Jacobs et al. 2013), although recent research may suggest otherwise (Warburton et al. 2004, Daussin et al. 2007, 2008, Wisløff et al. 2007b, Matsuo et al. 2014).

Ricci and colleagues (1982) conducted one of the first studies to quantify central adaptation to interval training. Following 8 weeks of SIT, they found no increase in left ventricular (LV) mass among young male students, however, it should be noted that mean baseline VO\textsubscript{2}peak of participants was well above average (59.5 mL/kg/min). More recently, Jacobs and colleagues (2013) conducted a study on untrained adults (baseline VO\textsubscript{2}peak: ~43 mL/kg/min) to evaluate physiological adaptations, ranging from cardiovascular to skeletal muscle properties, following six sessions of HIIT. They found that the intervention induced no alterations in maximal cardiac capacity or blood characteristics, specifically oxygen-carrying capacity and total blood volume. While this study appears to corroborate the findings of Ricci and associates’ (1982), the extremely short nature of the intervention (6 sessions over 2 weeks) begs the question of the long-term effects of HIIT and SIT on markers of central adaptation to exercise.

There are multiple examples of research in the last decade that have found HIIT protocols to induce improvements in $Q_{\text{max}}$, namely through increases in SV (Daussin et al. 2007, 2008, Wisløff et al. 2007a). The effect of SIT on $Q_{\text{max}}$ is much more contested with some finding no effect and hypothesizing the short duration associated with SIT to be insufficient to affect cardiac
function (MacPherson et al. 2011), while others have found significant improvements in $Q_{\text{max}}$ following SIT, by mechanism of improvements in each maximal SV and $HR_{\text{max}}$ (Matsuo et al. 2014). Aside from changes to $Q_{\text{max}}$, there are other examples of central adaptations that have been exhibited following HIIT and SIT. Matsuo and associates (2014) compared 8 weeks of MICT, HIIT and SIT for changes in cardiac mass and found that LV mass increased significantly following each of HIIT and SIT. The authors noted that this result was contradictory to previous evidence (Ricci et al. 1982), however they highlighted that their investigation was the first of its nature to use cardiac MRI, which is considered to be the gold standard for assessing cardiac mass (Devereux et al. 1997). There is also research to suggest that HIIT can increase BV. Warburton and colleagues (2004) found that after 12 weeks of training, HIIT significantly increased BV, plasma volume (PV) and red cell volume. This was accompanied by significant changes in $Q$, SV, end diastolic volume, peak ejection rate and peak filling rate during maximal exercise. It is interesting to note that all studies to find significant central adaptations following HIIT/SIT have been 8 weeks in length or longer.

Thus, in light of evidence largely uncovered in the last decade, it would appear that HIIT and SIT protocols at least 8 weeks in duration are capable of improving a multitude of central physiological adaptations (i.e. $Q_{\text{max}}$, LV mass, BV), though the precise mechanisms underlying how HIIT and SIT influence these outcomes remains ill understood and warrants further investigation.

### 2.4.3 Impact of HIIT and SIT on Peripheral Adaptation to Exercise

In contrast to central adaptations, peripheral physiological adaptations to exercise are associated with the ability of muscle to utilize oxygen once it has been delivered convectively
via the cardiovascular system. These adaptations may include changes in muscle fibre type, proteins associated with increased oxidative capacity, microvasculature and morphology response, and shifts in cellular metabolism. HIIT and SIT are each known to influence many markers of peripheral physiological adaptation following training.

Interval training elicits increases in skeletal muscle mitochondrial content. A study by Little and colleagues (2010) utilized a protocol of 6 sessions over 2 weeks, each involving 8-12 x 60 s intervals at ~100% peak power interspersed with 75 s of recovery, and found increases in protein content and activity of COX and CS by ~30% and ~20%, respectively. HIIT causes a transition in skeletal muscle fiber type, with Joanisse and colleagues (2013) finding an increase in hybrid fibers and reduction in type II fibers, likely indicating a fast-to-slow transition, or a change towards greater oxidative capacity. Similar results have been observed following SIT, with our own lab showing that 6 weeks of SIT causes an increased proportion of type I fibers concomitant with a reduction in type IIA fibers (Scribbans et al. 2014a). Preliminary evidence would suggest that HIIT can bring about a skeletal muscle microvasculature response, in the form of the growth of new capillary beds (Scribbans et al. 2014a), as well as elevation of endothelial nitric oxide synthase (eNOS) (Cocks et al. 2013). Each of these adaptations contribute to improvements in endothelial function.

Notable changes to proteins involved in carbohydrate (CHO) and fat metabolism have also been observed following HIIT/SIT studies. When it comes to CHO metabolism, elevated levels of phosphofructokinase (PFK), pyruvate dehydrogenase kinase (PDK), lactate dehydrogenase (LDH) and pyruvate dehydrogenase (PDH) have been found following HIIT (Parra et al. 2000, Burgomaster et al. 2008), as well as muscle transport proteins associated with import of glucose and export of by-products such as GLUT 4 and mono-carboxylate transporters.
respectively (MCT 1 and 4) (Thomas et al. 2012). Multiple proteins implicated in fat oxidation also appear to be upregulated by HIIT/SIT, including regulators of transport across the sarcoplasmic reticulum (SR) and mitochondria (Spriet 2014), as well as controllers of intramuscular triglyceride lipolysis (Shepherd et al. 2013) and long-chain fatty acid β-oxidation (Parra et al. 2000, Burgomaster et al. 2008). Despite upregulation of enzymes involved in glycolysis and glycogenolysis following HIIT/SIT, this type of training is more likely to cause a shift towards oxidative phosphorylation during exercise (Burgomaster et al. 2006). This is supported by less phosphocreatine (PCr) breakdown, glycogenolysis and glycolysis during performance tests (Burgomaster et al. 2006, 2008), as well as a higher reliance on β-oxidation during submaximal exercise (Perry et al. 2008). The result of such changes is a preservation of glycogen stores, and higher ability to meet adenosine triphosphate (ATP) demand oxidatively.

Based on a rich collection of research, it is clear that each HIIT and SIT are effective in eliciting significant improvements to many measures of peripheral adaptation including those associated with structure and function of muscle tissue, as well as the surrounding microvasculature.

2.4.4 Physiological Factors Contributing to Adaptations in VO₂peak Following HIIT/SIT

VO₂peak is the most commonly utilized indicator of improvements in aerobic-exercise capacity in exercise-training literature (Raleigh et al. 2016). It is well-established that HIIT and SIT induce similar improvements in VO₂peak compared to MICT (Eddy et al. 1977, Burgomaster et al. 2005, Cocks et al. 2013, Scribbans et al. 2014a), however the mechanism for how each modality of training specifically elicits this shift in VO₂peak is less clear.
Traditionally, HIIT and SIT were postulated to improve VO_{2peak} primarily, or even exclusively, by means of peripheral adaptations (Jacobs et al. 2013), however, given the numerous aforementioned central adaptations that can accompany HIIT/SIT this is may not be the case. Indeed, Daussin and colleagues (2008) found that 8 weeks of HIIT led to improvements in each capillary density, mitochondrial function and Q, suggesting that the accompanying increase in VO_{2peak} is likely the result of a combination of peripheral and central factors. The relative contributions of these factors towards improvements in VO_{2peak} likely differs depending on the training protocol (i.e. intensity, volume, length of protocol, etc.). What may actually be the case is that HIIT/SIT’s capacity to influence cardiac and haematological factors increases over time, with short training protocols being insufficient to evoke significant increases. This posit is supported by the fact that those studies to implement HIIT/SIT for 6 weeks or fewer and see improvements in VO_{2peak} found no changes in central factors (MacPherson et al. 2011, Jacobs et al. 2013), whereas similar protocols extending 8 weeks or longer found significant changes to VO_{2peak} as well as multiple measures of central adaptations (i.e. Q_{max}, LV mass, BV) (Warburton et al. 2004, Daussin et al. 2008, Matsuo et al. 2014). In contrast, peripheral factors appear to respond more quickly to interval training, with appreciable increase in skeletal muscle respiratory capacity and mitochondrial content occurring after just 6 sessions of HIIT over 2 weeks (Jacobs et al. 2013). The rapid increases in peripheral markers of adaptation to HIIT/SIT may reflect that fluctuations in oxygen (O_{2}) demand and uptake induce repeated disturbances of cellular homeostasis, which plays a major role in controlling adaptations of muscular mitochondrial function (Daussin et al. 2008).

Thus, it would appear that the relative contributions of central and peripheral factors in contributing to increases in VO_{2peak} with HIIT/SIT are temporally dependent, with VO_{2peak}
largely increasing due to peripheral factors with short protocols (< 6 weeks), and shifting towards a combination of peripheral and central factors over longer training periods (> 8 weeks). Clearly, further investigation of this relationship is warranted, including an in depth investigation into the relative roles of specific central and peripheral factors in improving VO$_2$peak with HIIT/SIT protocols of varying length and in a variety of participant populations.

2.5 Individual Patterns of Response to SIT and the Role Peripheral Factors

2.5.1 Section Introduction

Earlier, the considerable variability in individual VO$_2$peak response following SIT was reviewed. This section will begin by examining role of specific peripheral factors following SIT on this heterogeneity in VO$_2$peak response. This will be followed by acknowledgment of current gaps in the literature and directions for needed future research.

2.5.2 The Influence of Individual Peripheral Adaptations on VO$_2$peak Response to HIIT/SIT

While it is clear that individual patterns of response exist for VO$_2$peak following MICT (Sisson et al. 2009, Ross et al. 2015), there is considerably less research examining the variation in individual VO$_2$peak response to HIIT/SIT protocols and the potential underlying factors for this variability. Of those who have investigated individual VO$_2$peak response to HIIT/SIT, even fewer have done so in concert with peripheral adaptation to exercise.

Granata and colleagues (2015) implemented 4 weeks of SIT and HIIT, finding that, at the group level, SIT and not HIIT significantly increased mitochondrial respiration. When it comes to individual responses however, participant-specific changes in these variables were only depicted graphically, with no accompanying data or written discussion included. Thus, no
concrete claims can be drawn from individual response to these SIT and HIIT protocols, including any potential relationship between individual VO$_2$peak change, and response of peripheral factors. This is not the only study in which this issue is present. There are similar examples of HIIT and SIT studies in which individual responses are graphically depicted for skeletal muscle mitochondrial oxidative capacity (Daussin et al. 2008), COX I, COX II, COX IV, muscle buffering capacity, resting muscle glycogen (Gibala et al. 2006), submaximal energy metabolism and lactate accumulation (Burgomaster et al. 2006), as well as sarcoplasmic reticulum function (Ørtenblad et al. 2000), with no further mention or analysis brought forward. It is problematic that these examples of research have not communicated their results fully, as in their current state they do not add appreciably to our knowledge of how individual peripheral responses contributes to VO$_2$peak change following HIIT/SIT. To our knowledge, only two studies to date have specifically explored individual patterns of response following HIIT/SIT.

The first of these retrospectively examined results from each a HIIT and a SIT protocol, aiming to identify individual responders and nonresponders to VO$_2$peak as well as whole-body fat oxidation derived from respiratory exchange ratio (RER) (Astorino and Schubert 2014). Responders were classified as those participants exhibiting changes greater than 1 coefficient of variation (CV) for a given variable. HIIT consisted of training 3 d/wk at 60-80% WR$_{peak}$ or 80-90% WR$_{peak}$ for 12 weeks. Following HIIT, VO$_2$peak and fat oxidation each increased significantly with 95% (19/20) and 65% (13/20) rates of response respectively. There were no participants who did not respond to one of the two measures. Meanwhile, SIT consisted of 6 sessions of Wingate training over a period of 2 weeks. Following training, SIT participants also saw significant increases in each VO$_2$peak and fat oxidation levels with 65% (13/20) and 60% (12/20) rates of response respectively. Ten % of participants (2/20) did not respond to either
measure. Given that the SIT protocol consisted of just 6 days of training over 2 weeks, significant changes in energy utilization at the cellular level (i.e. increased whole-body lipid oxidation) in 60% of participants is interesting, particularly since 12 weeks of HIIT induced a very similar response rate (65%). Group means would suggest that the HIIT was much more effective at inducing fat oxidation responders than SIT (~16% and ~8% increases respectively), when in reality, they are closer to equal, highlighting the importance of reporting individual response in such training studies.

Another important study was conducted by Gurd and colleagues (2016), who examined the incidence of nonresponse in VO₂peak as well as lactate threshold across a range of SIT protocols, spanning from 3-6 weeks. Responders in this study were characterized as those with a change exceeding 2 x TE. The overall rate of responders for VO₂peak across all participants studied was 78% (49/63), with no significant correlation observed between individual changes in VO₂peak and lactate threshold ($r = 0.17; p = 0.44$). Interestingly, the authors also found that 23% could be classified as “global nonresponders”, or individuals who failed to increase in both VO₂peak and lactate threshold following SIT performed 3 times per week. These results suggest that individuals respond highly heterogeneously in not only VO₂peak, but in lactate threshold as well.

The current body of knowledge, while limited, does provide some insight into individual whole-body response following HIIT/SIT. Importantly, individual patterns of response in VO₂peak, as well as lipid oxidation (Astorino and Schubert 2014) and lactate threshold (Gurd et al. 2016) illuminate the benefits to measuring and reporting multiple variables of response to training, so that those who do not respond in a specific variable (such as VO₂peak) can optimize training based on their adaptation tendencies.
2.5.3 Future Directions

It is clear that individuals vary greatly in their physiological responses to standardized exercise. Heterogeneous response amongst participants to interval training (ie. HIIT and SIT), however, remains an understudied area in the field, particularly when it comes to comparing indicators of response other than VO\textsubscript{2\text{peak}}. Indeed, only two studies to date have comprehensively examined individual response to multiple variables following HIIT/SIT (Astorino and Schubert 2014, Gurd et al. 2016) with each failing to examine measures of peripheral or central adaptation in addition to VO\textsubscript{2\text{peak}}. Given the evidence suggesting that peripheral adaptations are primarily responsible for VO\textsubscript{2\text{peak}} gains in shorter HIIT/SIT studies (ie. ~6 weeks or fewer), the first logical target for study should involve examining individual response to multiple peripheral measures of adaptation following a short HIIT/SIT protocol and determining how observed variability in these responses relate to changes in VO\textsubscript{2\text{peak}}. This would ideally include the measurement of a spectrum of peripheral adaptations (i.e. fiber type, oxidative capacity, capillary density) in order to effectively elucidate a complete picture of the relative contributors to individual VO\textsubscript{2\text{peak}} response following short-term HIIT/SIT. The physiology for why we hypothesize each of these peripheral variables will contribute to increased VO\textsubscript{2\text{peak}} in high responders is illustrated in Figure 1. Only once these relationships have been established should further research be devoted towards examining longer HIIT/SIT studies (i.e. 8 weeks or longer). This is due to the fact that for training periods of this length, central adaptations in addition to peripheral adaptations are likely to influence VO\textsubscript{2\text{peak}}, thus adding a further layer of complexity in uncovering specific physiological influences on individual VO\textsubscript{2\text{peak}} response following interval training.
Such research will contribute to the growing awareness of individual heterogeneity in response to exercise, including the presence of nonresponders, as well as further our understanding of why they exist. In doing so, this may allow for optimization of HIIT/SIT training protocols in order to minimize nonresponse, and thus bring the greatest potential health benefits to the greatest possible number of people in the general population.
Fig. 1 (Adapted from Gurd et al. 2007): Schematic of oxidative phosphorylation and the delivery of oxidative substrate to the electron transport chain (ETC) and mitochondrial ATPase from the myofilament ATPase (ADP), muscle perfusion (O$_2$) and carbohydrate metabolism (NADH via glycolysis, pyruvate dehydrogenase (PDH) and the tricarboxylic acid (TCA) cycle)
at baseline (top left). This figure is then altered to show how individuals may increase VO₂peak by means of increased capillary density (top right), SDH content (bottom left) and type I fibre % (bottom right). Briefly, capillary density could improve VO₂peak through increased number of capillaries (1), leading to greater surface area (i.e. conductance) for diffusion into the muscle (2) and therefore greater mass action for O₂ consumption at the ETC. Increased SDH content could improve VO₂peak through increased number of TCA cycles (1) leading to increased conversion of acetyl CoA to NADH (2), as well as increased number of ETCs (3), ultimately leading to greater mass action and conduction for O₂ consumption at the ETC (4). Increased type I fibre % could improve VO₂peak through increased total number of mitochondria (1) leading to greater conductance for O₂ consumption (2).
### Table 1. Participant and training program characteristics of HIIT/SIT studies to examine individual VO\(_2\)peak nonresponse rates

<table>
<thead>
<tr>
<th>Study</th>
<th>Group size (n) (Sex; M/F)</th>
<th>Participant Characteristics</th>
<th>Training Program Characteristics</th>
<th>Interval Protocols</th>
<th>Nonresponse cutoff criteria</th>
<th>Rate of nonresponse (%) (n/total participants)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Astorino &amp; Schubert (2014)</td>
<td>20 (0/20)</td>
<td>24 30 HIIT 3</td>
<td>60-90 12-20 12 6-10 60:60</td>
<td>&lt;1 CV</td>
<td>5% (1/20)</td>
<td></td>
</tr>
<tr>
<td>Astorino &amp; Schubert (2014)</td>
<td>20 (unknown)</td>
<td>24 43 SIT 3</td>
<td>~250 22-33 2 4-6 30:300</td>
<td>&lt;1 CV</td>
<td>35% (7/20)</td>
<td></td>
</tr>
<tr>
<td>Boyd et al. (2013)</td>
<td>9 (9/0)</td>
<td>23 35 HIIT 3</td>
<td>100 16-20 3 8-10 60:60</td>
<td>&lt;2 TE</td>
<td>0% (0/9)</td>
<td></td>
</tr>
<tr>
<td>Ma et al. (2013)</td>
<td>8 (8/0)</td>
<td>21 41 SIT 4</td>
<td>~170 4 4 8 20:10</td>
<td>&lt;2 TE</td>
<td>0% (0/8)</td>
<td></td>
</tr>
<tr>
<td>Scribbans et al. (2014a)</td>
<td>10 (8/2)</td>
<td>21 48 SIT 4</td>
<td>~170 4 6 8 20:10</td>
<td>&lt;2 TE</td>
<td>0% (0/10)</td>
<td></td>
</tr>
<tr>
<td>Scribbans et al. (2014b)</td>
<td>14 (14/0)</td>
<td>22 50 SIT 3</td>
<td>70 4 4 8 20:10</td>
<td>&lt;2 TE</td>
<td>31% (4/14)</td>
<td></td>
</tr>
<tr>
<td>Raleigh et al. (2016)</td>
<td>13 (6/7)</td>
<td>20 42 SIT 4</td>
<td>~115 16-24 3 8-12 60:60</td>
<td>&lt;2 TE</td>
<td>8% (1/13)</td>
<td></td>
</tr>
<tr>
<td>Raleigh et al. (2016)</td>
<td>11 (5/6)</td>
<td>20 40 SIT 4</td>
<td>~150 12-20 3 6-10 60:60</td>
<td>&lt;2 TE</td>
<td>18% (2/11)</td>
<td></td>
</tr>
<tr>
<td>Zelt et al. (2014)</td>
<td>12 (12/0)</td>
<td>22 44 SIT 3</td>
<td>~250 20-30 4 4-6 15:285</td>
<td>&lt;2 TE</td>
<td>33% (4/12)</td>
<td></td>
</tr>
<tr>
<td>Zelt et al. (2014)</td>
<td>10 (10/0)</td>
<td>23 50 SIT 3</td>
<td>~250 20-30 4 4-6 30:270</td>
<td>&lt;2 TE</td>
<td>50% (5/10)</td>
<td></td>
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</tbody>
</table>
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CHAPTER 3:

3.1 Introduction

While the beneficial effects of regular exercise are consistently seen at the group level, it has become increasingly clear that there is a large degree of heterogeneity in response to regular physical activity at the individual level, with some exhibiting no or very small improvements in aerobic capacity following highly controlled exercise training (Bouchard and Rankinen 2001, Scharhag-Rosenberger et al. 2012, Astorino and Schubert 2014). Individuals who fail to improve VO2peak following training, or “nonresponders”, make up as much as 20 to 45% of exercise study participants, following both endurance (Sisson et al. 2009, Ross et al. 2015) and interval training protocols (Astorino and Schubert 2014, Gurd et al. 2016, Raleigh et al. 2016). While these individuals may represent a large portion of exercise study participants, the vast majority of published studies emphasize main effects and group differences while paying little attention, if any, to individual differences (Bouchard and Rankinen 2001). Of the over 100 studies published in the last decade examining the effects of high intensity interval training (HIIT) or sprint interval training (SIT) on variables including VO2peak, only 3 have directly examined individual responses to interval training.

Astorino and Schubert (2014) retrospectively examined individual responses in VO2peak, to each HIIT and SIT protocols, finding VO2peak nonresponse at rates of 5% and 35% respectively. Similarly, Raleigh and colleagues (2016) compared rates of VO2peak response to HIIT and SIT protocols, finding that 20% of HIIT and 18% of SIT participants displayed nonresponse following 3 weeks of training. Gurd and colleagues (2016) also investigated the incidence of nonresponders following a variety of SIT protocols, with the overall rate of nonresponse for VO2peak being 22%. Interestingly the rate of nonresponse declined to 0% in
protocols where participants trained 4 times per week (n=18) compared to rates between 30 and 50% in those training 3 times per week (n=45). Together, these studies provide evidence of considerable heterogeneity in individual VO$_2$peak response following interval training including substantial levels of nonresponse, however the specific central and/or peripheral physiological mechanisms that determine this heterogeneity remains unknown.

Traditionally, HIIT/SIT was postulated to improve VO$_2$peak primarily, or even exclusively, by means of peripheral adaptations (Jacobs et al. 2013), yet recent evidence suggests that protocols >8 weeks are capable of eliciting improvements in central measures including maximal cardiac output ($Q_{\text{max}}$), cardiac mass (Matsuo et al. 2014), total blood volume, and end diastolic volume (Warburton et al. 2004). However, in HIIT/SIT protocols <6 weeks, no changes to central factors have been found (MacPherson et al. 2011, Jacobs et al. 2013). Thus, peripheral factors appear to respond more quickly to interval training, with appreciable increase in skeletal muscle respiratory capacity and mitochondrial content occurring after just 6 sessions of HIIT over 2 weeks (Burgomaster et al. 2006, Gibala et al. 2006, Jacobs et al. 2013). These rapid increases in peripheral markers of adaptation to HIIT/SIT may be because fluctuations in O$_2$ demand and uptake induce repeated disturbances of cellular homeostasis, that play a role in controlling adaptations of muscular mitochondrial function (Daussin et al. 2008). Thus, if peripheral adaptations are primarily responsible for improvements in VO$_2$peak at the group level, differences in individual VO$_2$peak response following HIIT/SIT may also be explained by differences in such factors.

At present, there is no study that compares individual changes in VO$_2$peak to multiple measures of peripheral adaptation following a SIT protocol. Therefore, the aim of this study was to investigate this relationship following a 4-week SIT exercise protocol, by collecting both
VO₂peak and multiple measures of peripheral adaptation before and after training. Specifically, capillary density, fibre-specific succinate dehydrogenase (SDH) content, and type I fibre percentage were measured before and after SIT. These factors were chosen, as capillary density is indicative of surface area for diffusion of O₂ into working muscle tissue (Tschakovsky and Hughson 1999), SDH content has been shown to be strongly correlated to overall mitochondrial content (Larsen et al. 2012), and type I fibre type % is a marker for whole-muscle mitochondrial content and oxidative capacity (Lin et al. 2002). We hypothesized that 1) there would be high and low responders in VO₂peak following training that differed significantly in their magnitude of response, and, 2) high individual responders to VO₂peak would also be high responders to measures of peripheral adaptations following SIT, while low responders to VO₂peak would also be low responders to peripheral measures.
3.2 Methods

3.2.1 Experimental Design

To study the effect of chronic SIT on the relationship between individual changes in VO$_2$peak and peripheral adaptations to training, participants completed 4 weeks of training (4 times/week). VO$_2$peak was determined with incremental VO$_2$peak tests performed during the pre-training and post-training weeks. Additional pre and post-measures included a fasted muscle biopsy and anthropometric measures (height, weight, and waist circumference). Participants in this study also completed testing for the determination of peak cardiac output and muscle deoxygenation, as well as having venous samples drawn during pre and post testing, however the results of these analyses were not included as part of the current analysis.

3.2.2 Participants

A total of 50 participants were assessed for eligibility. Four participants were assessed to be too active for participation. Fifteen participants withdrew prior to pre-testing, and 8 withdrew at some point between pre-testing and post-testing for a variety of personal reasons. A total of 23 healthy, recreationally active men completed all 6 weeks of the study. All participants were between 18-30 years old, while participating in no more than 150 minutes per week of structured physical activity at enrollment, as determined by a 7 Day PAR questionnaire (Sallis 1997). Participant characteristics are presented in Table 1. Participants were instructed to maintain their regular exercise habits throughout the training period. All experimental procedures were approved by the Health Sciences Human Research Ethics Board at Queen’s University and conform to the Declaration of Helsinki. Verbal and written explanation of the experimental
protocol and associated risks was provided to all participants prior to obtaining written informed consent.

3.2.3 Pre and Post-Training Testing

Pre and post-testing included identical measures. Participants reported to the lab having fasted for 12 hours on the mornings of Day 1 of pre and post-testing. Upon arrival at the lab, participants gave venous blood samples followed immediately by a muscle biopsy. Resting muscle biopsies were conducted with the Bergstrom needle biopsy technique (Bergstrom 1975) as we have done previously (Boyd et al. 2013, Edgett et al. 2013). 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 mounted with ProlongGold Antifade Reagent (Life Technologies, Burlington, Ont. Canada) and stored at -80°C until analysis.

Immediately following the resting muscle biopsy participants were fed a standardized breakfast (toasted bagel [181 kcal; 1 g fat, 36 g carbohydrate, 7 g protein] with 15 g of cream cheese [44 kcal; 4 g fat, 1 g carbohydrate, 1 g protein]), and 250mL of Minute Maid orange juice [56 kcal; 0g fat, 13g carbohydrate, 1g protein]). This was promptly followed by measurement of anthropometric values (height [cm], weight [kg], and waist circumference [cm]).

Thirty minutes after consumption of the standardized breakfast participants completed an incremental VO\(_2\) peak test. VO\(_2\) peak tests were conducted on a Monark, Ergomedic 874E stationary cycle ergometer (Vansbro, Sweden). The VO\(_2\) peak ramp protocol, has been described previously (Edgett et al. 2013). Briefly, it consists of a five-minute loadless warm-up followed by a step increase to 80 W for one minute and subsequent increases in work rate 25 W per minute until participant reached volitional fatigue or when the participant was unable to maintain
a minimum cadence of 70 RPM). Gas exchange and heart rate were measured continuously with a metabolic cart (Moxus AEI Technologies, Pittsburgh, PA), calibrated before each test using a two-point gas calibration (known gas and atmospheric air) and a 3L syringe for volume calibration. Relative VO$_2$peak and absolute VO$_2$peak, were selected as the highest value of continuous 30 s averages for each measure during the protocol. RPM was collected continuously throughout VO$_2$peak tests and peak work rate (WRpeak) was calculated using the average WR from the highest value of continuous 30 s averages for WR.

3.2.4 Training Protocol

Training was performed 4 times per week for 4 weeks, for a total of sixteen sessions of SIT. All training sessions consisted of a 5-minute warmup of loadless cycling, followed by eight 20-second intervals at 170% of VO$_2$peak WR, separated by 10 seconds of rest, for a total of 9 minutes. Participants were instructed to maintain a cadence of 80 RPM throughout all training sessions including warm-ups and loadless rest periods between intervals. HR and RPM were recorded at the completion of each interval. Rate of perceived exertion (RPE) was collected immediately following the completion of the final interval of each training session.

3.2.5 Immunofluorescent and Histochemical Analysis

Immunofluorescent analysis of myosin heavy chain isoforms was performed as we have done previously (Bloemberg and Quadrilatero 2012, Scribbans et al. 2014) using primary antibodies against myosin heavy chain (MHC) I (BA-F8), MHCIIa (SC-71), and MHCIIx (6H1) (Developmental Studies Hybridoma Bank, Iowa City, IA, USA). In addition, sections were incubated with a primary antibody against dystrophin [MANDYS1 (3b7), Developmental
Studies Hybridoma Bank] to identify the muscle membrane. Fibre types were identified by isotype-specific fluorescent secondary antibodies (type I, blue; type IIa, green; type IIX, red; as well as type IIA/IIX hybrid fibres, IIAX). For all immunofluorescent procedures, sections were mounted with Prolong Gold Antifade Reagent (Life Technologies, Burlington, ON, CA) and imaged the following day. Quantification of capillary density was conducted as done previously in our lab (Scribbans et al. 2014). Briefly, sections were fixed in 4% paraformaldehyde for 10 minutes, followed by permeabilization with 0.5% TritonX-100 for 30 minutes, and then blocked in 10% goat serum for 30 minutes. Sections were incubated overnight in 5% goat serum with the appropriate primary antibodies specific for the endothelium (PECAM) and sarcolemma (dystrophin) (Developmental Studies Hybridoma Bank, Iowa City, IA, USA). After 3x5 minute washes in PBS, sections were incubated for 1 hour in 5% goat serum with the appropriate fluorescent secondary antibodies (Life Technologies, Burlington, ON, CA) before imaging.

All sections imaged for fibre type and capillary density were visualized with an Axio Observer Z1 microscope (Carl Zeiss, Jena, TH, Germany). Individual images were taken across the entire muscle cross-section and assembled into a composite panoramic image using AxioVision software (Carl Zeiss). Panoramic images stained for fibre type were examined and labeled, such that the same 40 individual type I, type IIA and type IIX fibres were marked for each ‘pre’ sample, and the same number of matched fibres were marked from each ‘post’ sample. Any potential shift in fibre from ‘pre’ to ‘post’ were then quantified. Panoramic images stained for capillary density were analyzed by first ensuring that 3 rectangular sections ~100 μm² in area, spanning exclusively cross-sectional fibres, were labeled on every image. Within each labeled box, illuminated green capillaries were counted. Capillary density was then quantified for
each box, and thus for each image, based upon known areas of labeled sections and then these values were compared between ‘pre’ and ‘post’ conditions.

Histochemical staining for succinate dehydrogenase (SDH) (Bloemberg and Quadrilatero 2012) was determined as a general indicator of oxidative potential. Histochemical staining of all muscle portions for a given participant was performed simultaneously. Images were acquired with a brightfield Nikon microscope linked to a PixeLink digital camera. Individual images were taken across the entire muscle cross-section and assembled into a composite panoramic image using Microsoft Image Composite Editor (ICE) (Microsoft, Redmond, WA, USA). Image analysis was performed in ImageJ and calculated by subtracting background staining. Compiled images were matched to fibre-type images and the 40 of each labeled fibre type were analyzed. Data is expressed relative to the values obtained in type I fibres, which is assigned a reference value of 1.0, and reported as mean optical density in arbitrary units (AU).

Fibre counts, SDH, and capillary density are reported as group means +/- SD based on individual participant means/values.

3.2.6 Statistical Analysis

Mean changes in all measured variables (pre- to post-training) were assessed using paired t-tests (significance accepted at p < .05; see Table 1). Pearson correlations were performed between individual change scores (change from pre- to post-training) of VO$_2$peak, and changes in capillary density, fibre-specific SDH content, and fibre type %.

Individual participants who completed the study (N=23) were awarded a score between 1 and 23, corresponding to their ranking in descending order for the magnitude of change in VO$_2$peak, and each peripheral measure of adaptation following training (i.e. the participant with
the highest magnitude of change in a variable was awarded a score of 1, while the participant with the lowest magnitude of change was awarded a score of 23). These rankings were then used to form 3 tertiles of VO$_{2\text{peak}}$, capillary density, fibre-specific SDH and type I fibre % response: LO (1-8), MID (9-15) and HI (16-23). These data has been compiled in order of cumulative peripheral response (Table 2), as well as in order of VO$_{2\text{peak}}$ response (Table 3). A 2-way repeated measures analysis of variance (ANOVA) was then used to examine whether changes in peripheral variable responses are different between individuals with a low vs. a high VO$_{2\text{peak}}$ response.

All statistical analysis was performed and all figures were made using GraphPad Prism v 7.0 (GraphPad Software Inc., La Jolla, CA, USA). Statistical significance is accepted at $p < 0.05$, while the strength of correlational relationships was defined as weak, moderate and strong ($r = \pm 0.10$ to $\pm 0.29$ $r = \pm 0.30$ to $\pm 0.59$ $r = \pm 0.60$ to $\pm 1.0$, respectively). All data is presented as means +/- SD, unless otherwise indicated.
3.3 Results

3.3.1 Individual VO$_{2peak}$ Response to SIT

TE was $\pm$ 53.5 (2 x TE = 107) mL/min for absolute VO$_{2peak}$, as determined previously (Gurd et al. 2016). Based on this criterion, the overall rate of nonresponders across all participants studied was 26% (6/23). Interestingly, 1 adverse responder (individual whose VO$_{2peak}$ decreased by more than 2 x TE) was observed. Individual VO$_{2peak}$ responses from Pre to Post are illustrated in Figure 2.

3.3.2 Group Responses to SIT

Group responses following SIT are summarized in Table 1. Relative and absolute VO$_{2peak}$ increased significantly following training (p<0.01) as did WRpeak (p<0.05). Capillary density also increased significantly following training (p<0.01); there were no significant changes to type I or type IIA fibre SDH content, or type I/IIA fibre %.

3.3.3 Comparisons between Individual VO$_{2peak}$ and Peripheral Responses After SIT

Baseline measures for all variables measured were compared between LO and HI groups, using paired t-tests (significance accepted at p < .05; see Table 2), with significant differences (p<0.05) seen for each absolute VO$_{2peak}$ as well as type IIA fibre SDH content, such that baseline was lower for each variable in the HI group. No differences existed at baseline between groups for any other measure. Group-specific baseline values are represented in Table 2.

Representative images are presented in Figure 3 for all immunofluorescent and histochemical analyses. Two-way repeated-measures ANOVAs were conducted to compare the level of VO$_{2peak}$ response (LO vs. HI groups) and training (Pre and Post) on VO$_{2peak}$ and all peripheral physiological measures obtained. Two-way repeated-measures ANOVAs were also
conducted to compare level of VO\textsubscript{2}peak response (LO vs. HI groups) and characteristics of individual VO\textsubscript{2}peak tests (Pre and Post) for each peak respiratory exchange ratio (RER) and HRpeak achieved during pre and post VO\textsubscript{2}peak tests. A significant interaction (p<0.05) and main effect of training (p<0.05) was observed for VO\textsubscript{2}peak, with post-hoc results indicating that those in the HI had a significantly greater increase in VO\textsubscript{2}peak from baseline (Pre) to follow-up (Post) compared to the LO group (Fig. 4A). A main effect of training (p<0.05) was observed for capillary density with no interaction effect observed (Fig. 4B). A main effect of group (p<0.05) was observed for change in SDH activity in type IIA fibres, such that the HI group had higher type IIA SDH activity at Pre and Post, with no observed interaction or training effect (Fig. 3D). There was no interaction effect or effect of training for type I SDH activity (Fig. 4C), type I fibre % (Fig. 4E) or type IIA fibre % (Fig. 4F). There was no interaction effect or effect of VO\textsubscript{2}peak test for either peak RER or HRpeak achieved during pre and post VO\textsubscript{2}peak tests.

No significant correlations were observed between changes in VO\textsubscript{2}peak and capillary density (r = 0.02; p = 0.92) (Fig. 5A), type I fibre SDH content (r = -0.04; p = 0.87) (Fig. 5B), type IIA fibre SDH content (r = -0.04; p = 0.85) (Fig. 5C), and type I fibre % (r = 0.36; p = 0.09) (Fig. 5D).

Individual participant (N=23) ranking of change scores for peripheral physiological characteristics following 4 weeks of training are presented in Table 3. The mean rank score for individual participants ranged from 3-19, with SEM’s ranging from 1.4-10.2. The sum (Σ) of rank scores for all 4 muscle characteristics ranged from 12 to 74. Only 1 participant scored in one tertile (high, moderate or low responder) for all peripheral variables, while 11 participants ranked in two tertiles and 11 participants scored in all 3 tertiles. Visually, this table appears to show a trend where most of the high individual responses for all 4 peripheral measures are
grouped on the left side, and most of the low response is grouped on the right. These same individual rankings in peripheral response are represented in Table 4, with participants being re-ranked in descending order of VO$_2$peak response (Individual 1 is the highest VO$_2$peak responder and individual 23 is the lowest VO$_2$peak responder). When arranged in this manner (i.e., in relation to VO$_2$peak response), there does not appear to be any visible trend in individual peripheral response.
Fig. 2 Individual participant responses to 4 weeks of SIT (4 x per week) with 2x the typical error (TE) illustrated using dashed lines. Nonresponders have a peak oxygen uptake (VO$_2$peak) response that falls within the 2x the TE (107 mL of O$_2$/min) (shaded region). Individuals have been classified as having a low response (black bars, n=8), moderate response (striped bars, n=7), and high response (white bars, n=8).
**Fig. 3** Representative slides of serial sections of capillarization (green dots are individual capillaries), fibre type specific SDH content, and immunofluorescent fibre-type analysis (blue fibres are type I, green are type IIA) before (Pre) and after (Post) 4 weeks of SIT training (4 x per week).
Fig. 4 Changes in VO\textsubscript{2peak} (A), capillary density (B), type I fibre SDH content (C), type IIA fibre SDH content (D), type I fibre % (E), and type IIA fibre % (F) from Pre and Post 4 weeks of SIT (4 x per week) in LO and HI groups. * Significant (p<0.05) interaction and training effect. † Significant (p<0.05) training effect. α Significant (p<0.05) group effect.
Fig. 5 The relationships between individual changes in VO$_2$peak and capillary density (A), type I fibre SDH content (B), type IIA fibre SDH content (C) and type I fibre % (D). The relations are established after a period of 4 weeks of SIT (4 x per week).
Table 2. Participant Characteristics (N=23) pre and post 4 weeks of SIT

<table>
<thead>
<tr>
<th></th>
<th>Pre</th>
<th>Post</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>20.43 (1.75)</td>
<td>___</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>182.23 (6.54)</td>
<td>___</td>
</tr>
<tr>
<td>Body mass (kg)</td>
<td>78.52 (10.01)</td>
<td>78.41 (10.27)</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>23.72 (2.43)</td>
<td>23.68 (2.48)</td>
</tr>
<tr>
<td>Absolute VO₂peak (mL/min) *</td>
<td>3806.24 (616.52)</td>
<td>4160.13 (530.26)</td>
</tr>
<tr>
<td>Relative VO₂peak (mL/kg/min) *</td>
<td>48.65 (6.39)</td>
<td>53.39 (5.99)</td>
</tr>
<tr>
<td>Peak WR (W) *</td>
<td>278.78 (40.94)</td>
<td>306.81 (45.10)</td>
</tr>
<tr>
<td>Type I fibre %</td>
<td>50.67 (11.20)</td>
<td>47.58 (12.32)</td>
</tr>
<tr>
<td>Type IIA fibre %</td>
<td>44.18 (10.98)</td>
<td>48.59 (10.86)</td>
</tr>
<tr>
<td>Type I SDH content (AU)</td>
<td>34.35 (7.08)</td>
<td>34.93 (8.80)</td>
</tr>
<tr>
<td>Type IIA SDH content (AU)</td>
<td>24.14 (5.86)</td>
<td>26.26 (7.01)</td>
</tr>
<tr>
<td>Capillary density (cap/mm²) *</td>
<td>468.25 (87.02)</td>
<td>533.26 (70.01)</td>
</tr>
</tbody>
</table>

* Significant difference following training (p < 0.05). cm, centimeters; kg, kilograms; m, meters; mL, milliliter; min, minutes; AU, arbitrary units; cap, capillaries; mm, millimeters; W, Watts; WR, work rate
Table 3. Baseline values for LO and HI groups

<table>
<thead>
<tr>
<th></th>
<th>LO</th>
<th>HI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>20.75 (1.49)</td>
<td>19.75 (1.58)</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>179.63 (6.64)</td>
<td>180.56 (6.57)</td>
</tr>
<tr>
<td>Body mass (kg)</td>
<td>76.49 (9.72)</td>
<td>72.69 (7.68)</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>23.68 (2.41)</td>
<td>22.28 (1.79)</td>
</tr>
<tr>
<td>Absolute VO₂peak (mL/min) *</td>
<td>3995.83 (573.92)</td>
<td>3348.92 (458.34)</td>
</tr>
<tr>
<td>Relative VO₂peak (mL/kg/min)</td>
<td>52.21 (3.37)</td>
<td>46.49 (7.92)</td>
</tr>
<tr>
<td>Peak WR (W)</td>
<td>281.83 (47.63)</td>
<td>263.66 (30.08)</td>
</tr>
<tr>
<td>Type I fibre %</td>
<td>53.47 (8.78)</td>
<td>54.62 (13.51)</td>
</tr>
<tr>
<td>Type I SDH content (AU)</td>
<td>37.81 (6.77)</td>
<td>31.46 (6.75)</td>
</tr>
<tr>
<td>Type IIA SDH content (AU) *</td>
<td>28.66 (4.28)</td>
<td>19.79 (4.76)</td>
</tr>
<tr>
<td>Capillary density (cap/mm²)</td>
<td>505.40 (113.85)</td>
<td>453.70 (68.35)</td>
</tr>
</tbody>
</table>

* Significant difference at baseline between groups (p < 0.05). cm, centimeters; kg, kilograms; m, meters; mL, milliliter; min, minutes; AU, arbitrary units; cap, capillaries; mm, millimeters; W, Watts; WR, work rate
Table 4. Ranking of individual participants (N=23) change scores of skeletal muscle characteristics following 4-weeks of SIT 4x per week.

| Overall Rank of Individual Participants | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 | 21 | 22 | 23 |
| **Σ Rank Scores**                       | 12| 24| 24| 32| 33| 39| 40| 42| 44| 46| 49| 51| 52| 54| 55| 55| 56| 58| 59| 63| 69| 73| 74 |
| **Mean Rank**                           | 3 | 6 | 6 | 8 | 8 | 10| 10| 11| 11| 12| 12| 13| 13| 14| 14| 14| 14| 15| 15| 16| 17| 18| 19|
| **StDev**                               | 1 | 7 | 3 | 7 | 4 | 4 | 4 | 9 | 9 | 4 | 3 | 7 | 9 | 5 | 9 | 7 | 10| 5 | 6 | 5 | 4 | 5 | 5 |

| Cap Density | 1 | 3 | 1 | 3 | 1 | 1 | 2 | 2 | 3 | 1 | 2 | 1 | 2 | 3 | 1 | 3 | 3 | 3 | 2 | 2 | 2 | 2 | 2 |
| Type I SDH  | 1 | 1 | 1 | 1 | 2 | 2 | 1 | 1 | 1 | 2 | 3 | 3 | 3 | 2 | 3 | 2 | 3 | 2 | 1 | 2 | 3 | 3 | 3 |
| Type IIA SDH| 1 | 1 | 1 | 1 | 2 | 1 | 1 | 1 | 2 | 3 | 2 | 2 | 3 | 3 | 3 | 2 | 3 | 2 | 2 | 1 | 3 | 3 | 3 |
| Type I %    | 1 | 1 | 2 | 2 | 1 | 2 | 2 | 2 | 3 | 1 | 1 | 2 | 3 | 1 | 1 | 2 | 1 | 2 | 3 | 3 | 3 | 3 | 3 |

Note: Black, high responders; dark grey, moderate responders; White, low responders; Rank scores, individual participants were awarded a score between 1 and 23, which corresponded to their ranking in descending order for each muscle variable (i.e. the participant with the highest magnitude of change was awarded a score of 1, while the participant with the lowest magnitude of change was awarded a score of 23). The sum (Σ) of rank scores represents the sum of the rank scores for all 4 muscle characteristics (maximum score possible = 92, minimum score possible= 4); Cap Density, capillary density; Type I SDH, succinate dehydrogenase activity in type I muscle fibres; Type II SDH, succinate dehydrogenase activity in type II muscle fibres; Type I %, % of fibre distribution of type I fibres.
Table 5. Ranking of individual participants (N=23) VO$_2$peak responses in relation to their change scores of skeletal muscle characteristics

<table>
<thead>
<tr>
<th>Overall Rank of Individual Participant VO$_2$peak change</th>
</tr>
</thead>
<tbody>
<tr>
<td>1  2  3  4  5  6  7  8  9  10  11  12  13  14  15  16  17  18  19  20  21  22  23</td>
</tr>
<tr>
<td>Σ Rank Scores</td>
</tr>
<tr>
<td>Mean Rank</td>
</tr>
<tr>
<td>StDev</td>
</tr>
<tr>
<td>55  24  39  56  63  46  32  59  51  44  69  40  12  54  52  49  24  55  33  74  58  73  42</td>
</tr>
<tr>
<td>14  6  10  14  16  12  8  15  13  11  17  10  3  14  13  12  6  14  8  19  15  18  11</td>
</tr>
<tr>
<td>7  3  4  10  5  4  7  6  7  9  4  4  1  5  9  3  7  9  4  5  5  5  9</td>
</tr>
<tr>
<td>Cap Density</td>
</tr>
<tr>
<td>Type I SDH</td>
</tr>
<tr>
<td>Type IIA SDH</td>
</tr>
<tr>
<td>Type I %</td>
</tr>
<tr>
<td>3  1  1  1  2  1  3  3  1  3  2  2  1  3  2  2  3  1  1  2  3  3  2  2  3  3  3  2</td>
</tr>
<tr>
<td>2  1  2  3  2  2  1  2  3  1  3  1  1  2  3  3  1  3  2  3  3  1  3  1</td>
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<tr>
<td>2  1  1  3  3  3  1  1  2  2  3  1  1  3  3  2  1  3  2  3  2  2  1</td>
</tr>
<tr>
<td>1  2  2  2  3  1  1  3  3  1  3  2  1  1  1  2  1  2  2  3  3  3  3  3</td>
</tr>
</tbody>
</table>

Note: Black, high responders; dark grey, moderate responders; White, low responders; Rank scores, individual participants were awarded a score between 1 and 23, which corresponded to their ranking in descending order for each muscle variable (i.e. the participant with the highest magnitude of change was awarded a score of 1, while the participant with the lowest magnitude of change was awarded a score of 23). The sum (Σ) of rank scores represents the sum of the rank scores for all 4 muscle characteristics (maximum score possible = 92, minimum score possible= 4); Cap Density, capillary density; Type I SDH, succinate dehydrogenase activity in type I muscle fibres; Type II SDH, succinate dehydrogenase activity in type II muscle fibres; Type I %, % of fibre distribution of type I fibres.
3.4 Discussion

3.4.1 Overview

This study examined individual changes in VO$_2$peak, as well as multiple peripheral measures of adaptation to test the hypothesis that 4 weeks of SIT would induce higher levels of peripheral response in high VO$_2$peak responders compared to low VO$_2$peak responders. The major findings from the current analysis were that: 1) there were high (HI group, n=8) and low responders (LO group, n=8) for VO$_2$peak, who differed significantly in their VO$_2$peak response following 4 weeks of SIT; 2) there was no difference between HI and LO VO$_2$peak response groups for any peripheral factors examined (capillary density, fibre-specific SDH content, fibre type distribution); 3) individual changes in VO$_2$peak did not correlate significantly with any of our measured peripheral variables. Together, these results suggest that while individual patterns of response exist for VO$_2$peak following 4 weeks of SIT, this heterogeneity in individual response cannot be explained by differences in any of the peripheral adaptations measured in this experiment.

3.4.1 Individual VO$_2$peak Response to SIT

Following 4 weeks of SIT (4 x per week), VO$_2$peak improved significantly in the HI group, while no change was observed for those in the LO group. Interestingly, baseline absolute VO$_2$peak was lower in the HI group than the LO group, which agrees with previous research that has shown higher VO$_2$peak trainability in those with a lower baseline VO$_2$peak (Cunningham et al. 1987). All 8 HI individuals were responders while only 2 LO individuals were responders. The incidence of nonresponse in the current study was determined using TE (Hopkins 2000), which considers both the biological variability and technical error of measurement within our laboratory (Gurd et al. 2016), while also providing a robust and conservative threshold for
determination of individual response (Bouchard et al. 2012). The nonresponse rate for this study was 26% (6/23). This rate falls in line with previous literature, which has shown non-response to exercise training in the range of 20-45% (Astorino and Schubert 2014, Gurd et al. 2016).

Additionally, the heterogeneous range of VO2peak responses depicted in Figure 1 is comparable to that shown in previous studies to report individual VO2peak response (Astorino and Schubert 2014, Gurd et al. 2016). Given the fact that peak RER and HRpeak achieved during each VO2peak test did not differ from baseline in either group, or between HI and LO groups, we have no reason to believe that differences in effort put forward during VO2peak ramp tests between groups explains observed differences in individual VO2peak response.

3.4.2 Justifying Our Hypothesis

It was hypothesized that changes in individual VO2peak following 4 weeks of SIT could be explained by corresponding changes to measures of peripheral physiological adaptation. This is to say that the highest VO2peak responders (HI group) would also be high responders in peripheral response (capillary density, fibre-specific SDH content, fibre type distribution). This hypothesis is supported by studies that previously implemented HIIT/SIT for 6 weeks or fewer and reported that improvements in VO2peak are accompanied only by improvements in peripheral factors (MacPherson et al. 2011, Jacobs et al. 2013), with adaptations in central measures of response to exercise only being found in interval training studies extending 8 weeks or longer (Warburton et al. 2004, Daussin et al. 2008, Matsuo et al. 2014). It is postulated that this may be the case due to differences in mitochondrial quality and density in untrained subjects compared to their trained counterparts (Jacobs and Lundby 2013), as evidenced by the observation that individuals with elite aerobic capacities have superior mitochondrial respiratory capacity compared to those classified as only active or well-trained. Thus this lower baseline
may allow for more rapid adaptation to the repeated disturbances of cellular homeostasis induced by interval-type exercise (Daussin et al. 2008). If this is the case, it is logical to examine individual changes to markers of mitochondrial content and whole-muscle oxidative capacity in relation to individual VO\textsubscript{2}peak response following SIT.

Of those to implement HIIT/SIT protocols for 6 weeks or fewer, significant improvements have been seen in each protein content and activity of COX and CS (Little et al. 2010), type I fibre % and capillary density (Scribbans et al. 2014), and ability to meet ATP demand oxidatively (Burgomaster et al. 2006, 2008, Perry et al. 2008). Thus, for a SIT study of 4 weeks, it is reasonable to hypothesize that changes in VO\textsubscript{2}peak may largely be governed by changes in such peripheral factors. The peripheral factors chosen for the current study included capillary density, fibre-specific SDH content and type I fibre %. Capillary density was chosen because it is reflective of surface area for diffusion of P\textsubscript{cap}O\textsubscript{2} into working muscle tissue (Tschakovsky and Hughson 1999), and has been identified as a key component in the adaptive response in systemic VO\textsubscript{2}peak (Hepple et al. 1997). SDH content was measured, as it involved in both the mitochondrial respiratory chain and the citric acid cycle (McPhee et al. 2010), and it has been shown to be strongly correlated to overall mitochondrial content (Larsen et al. 2012). Fibre type % was obtained, as a shift towards a greater proportion of type I (slow-twitch) fibres is indicative of much higher mitochondrial content and oxidative capacity than type II (fast-twitch) fibres (Lin et al. 2002).

### 3.4.3 Individual Capillary Density Response

Capillary density increased significantly following training in both the LO and HI group with no differences observed between groups. There was also no correlation found between individual changes in VO\textsubscript{2}peak and capillary density. Therefore, individual changes in capillary
density following SIT appear not to explain individual variation in VO$_2$peak observed between high responders (HI) and low responders (LO) in the current study. This result is contrary to previous literature, which has implicated capillary density as a key determinant in VO$_2$peak response following training (Ingjer 1978). Given the fact that the LO group, which included 6 nonresponders, saw a significant increase in capillary density following SIT, other factors must explain differences in VO$_2$peak response between groups. This may reflect that provision of O$_2$ to muscle cells, through diffusion from surrounding capillaries, is in excess of the mitochondria’s ability to utilize it, and thus an increase in capillarization with SIT does not independently increase VO$_2$peak. This line of thinking is in disagreement with arguments presented by Saltin (2006), who contends that the bulk of experimental evidence gathered in the last 80 years argues in favour of cardiac output and oxygen delivery setting the limit. We would tend to agree with Wagner’s counterpoint (2006), however, which advocates for a more integrated approach to what may limit VO2peak, particularly where it comes to the role of diffusing capacity at the muscle. Indeed, more recent evidence would suggest that mitochondrial capacity may limit O$_2$ consumption during maximal exercise in untrained subjects (Gifford 2015). Alternatively, there is evidence to suggest that changes in VO$_2$peak following training are related to changes in the size of the capillary-to-fibre interface rather than capillary density (Hepple et al. 1997). If this is the case, then perhaps changes to individual capillary-to-fibre interfaces would provide more information regarding VO$_2$peak differences following SIT.

3.4.4 Individual SDH Content and Fibre Type Responses After SIT

No differences in response were observed between the HI and LO groups for any of type I SDH content, type IIA SDH content, or type I fibre %. There were also no significant correlations between individual responses in each of these factors and VO$_2$peak. Baseline type
IIA fibre SDH content was lower in the HI group than the LO group, but given that type IIA SDH content did not increase in either group, this is unlikely to have affected VO$_2$peak response. Thus, similar to capillary density, neither fibre-specific SDH content nor type I fibre % can explain differences in individual VO$_2$peak response following 4 weeks of SIT. The fact that both the LO and HI group did not show improvements in these factors is in disagreement with past research, which has consistently shown mitochondrial density and content (Holloszy 1967, Hoppeler et al. 1985) and skeletal muscle oxidative capacity (Pesta et al. 2011) to increase with training at the group level. It is worth noting, however, that these training interventions were END rather than SIT. While an increase in SDH and a shift towards a higher percentage of type I fibres would be indicative of such changes, differences in individual VO$_2$peak responses may be attributable to qualitative rather than quantitative changes to mitochondria. In addition to quantitative changes to mitochondria (ie. density and total content), qualitative adaptations, such as functional modifications in respiratory control and capacity may also playing an important role in VO$_2$peak (Gnaiger 2009). This is supported by Jacobs and Lundby (2013), who recently demonstrated that improvements in mitochondrial respiratory capacity (specifically oxidative phosphorylation capacity (P) and electron transport system capacity (ETS)) correspond with whole body aerobic capacity independently of mitochondrial content.

3.4.5 Interpreting Our Results

Absolute VO$_2$peak was found to be significantly lower at baseline in the HI group compared to the LO. While VO$_2$peak trainability has been shown to be higher in those with a lower baseline (Cunningham et al. 1987), the physiological mechanisms which may cause these individuals to increase VO$_2$peak to a greater degree remains largely unknown. It has been found previously that untrained exercise study participants are more likely to improve VO$_2$peak by
means of augmented \( \text{O}_2 \) utilization (ie. peripheral factors) compared to trained individuals who improve \( \text{VO}_2\text{peak} \) by means of improved \( \text{O}_2 \) supply (ie. central factors) (Jacobs et al. 2013, Gifford 2015). Given this, the less fit HI group may have been predisposed to improve \( \text{VO}_2\text{peak} \) more than the LO group, particularly following a SIT protocol in which only peripheral measures of adaptation were expected to improve (MacPherson et al. 2011, Jacobs et al. 2013).

Production of ATP by oxidative phosphorylation requires the presence of \( \text{O}_2 \), NADH, ADP and Pi (Wilson 1994). Mitochondrial oxidative phosphorylation, unlike other metabolic pathways that are regulated over such a wide range of rates, is regulated primarily by mass action (ie. provision of substrate) (Wilson 1994). Thus, an increase in these substrates during maximal exercise would likely lead to an increase in the rate of oxidative phosphorylation and therefore oxygen consumption. Changes in the peripheral factors examined in the current study would be expected to affect provision of these substrates, and thus could potentially explain individual changes to \( \text{VO}_2\text{peak} \).

An increase in capillary density in an individual following training would be indicative of a greater surface area (ie. conductance) for diffusion of \( \text{O}_2 \) into the cell (Tschakovsky and Hughson 1999), and thus greater provision of \( \text{O}_2 \) to the ETC for oxidative phosphorylation. Given that capillary density increased in the LO group, which saw no increase in \( \text{VO}_2\text{peak} \), it is unlikely that the increase in \( \text{VO}_2\text{peak} \) in the HI group came as a result of increased \( \text{O}_2 \) provision to the ETC. This is strengthened by the lack of correlation seen between individual changes in \( \text{VO}_2\text{peak} \) and capillary density. An increase in fibre-specific SDH content (Larsen et al. 2012) and/or type I fibre % (Lin et al. 2002) following training would suggest increases in mitochondrial content and oxidative capacity. This would include greater provision of NADH to the ETC through more citric acid cycling, as well as a greater number of total ETCs consuming
O_2 (ie. increased VO_2peak). Given that neither fibre-specific SDH content nor type I fibre %
changed in LO or HI responders, this suggests that increases in mitochondrial content did not
cause the significant VO_2peak response seen in the HI group. Assuming that central adaptations
do not occur in SIT protocols of this length (MacPherson et al. 2011, Jacobs et al. 2013), some
other peripheral adaptation must be at the root of the increased VO_2peak response seen in HI
participants compared to LO participants.

Citric acid cycle enzymes isocitrate dehydrogenase and 2-oxoglutarate dehydrogenase are
regulated by mitochondrial Ca^{2+} concentration (Cerretelli et al. 1979), with changes in
mitochondrial Ca^{2+} levels, therefore, determining the supply of reducing equivalents (ie. NADH)
to the ETC as well as mitochondrial respiration rate (Wilson and Rumsey 1988, Moreno-Sánchez
et al. 1990). Therefore, individual response in sarcoplasmic reticulum function may account for
individual differences in VO_2peak response. Additionally, measures of mitochondrial respiratory
capacity (specifically oxidative phosphorylation capacity (P) and electron transport system
capacity (ETS)), may help to explain VO_2peak variation, given that mitochondrial respiratory
capacity corresponds with whole-body aerobic capacity independent of mitochondrial content
(Jacobs and Lundby 2013) and has been shown to dissociate from mitochondrial content
following HIIT and SIT training (Granata et al. 2015).

3.4.6 Conclusions and Limitations

Following 4 weeks of SIT, the HI but not the LO group significantly increased in
VO_2peak, while no differences in response were found between groups for any of capillary
density, fibre-specific SDH content or type I fibre %. These experiments provide evidence of
considerable heterogeneity in VO_2peak response to such training, and provide evidence that
neither microvasculature response (ie. capillary density) nor increases in markers of
mitochondrial content/oxidative capacity (ie. SDH content and fibre type %) contribute appreciably to individual variation in VO\textsubscript{2} peak response following 4 weeks of SIT. With this knowledge, future research should focus on examining other potential peripheral adaptations that may contribute to this individual heterogeneity in VO\textsubscript{2} peak response. Such factors may include measures of mitochondrial respiratory capacity (ie. P and ETS), sarcoplasmic reticulum function or capillary-to-fibre interface.

A potential limitation to the current study derives from its relatively small sample size. While 23 participants completed the study, designation of participants into tertiles of VO\textsubscript{2} peak response only allowed for comparisons between 8 LO group and 8 HI group participants. Moreover, of the participants in the LO group, only 6 of 8 would be classified as nonresponders. Future studies would ideally include a larger sample size in order to elicit more true nonresponders and thus strengthen comparisons made with high responders. Another potential limitation to this study lies in the assumption that an individual muscle biopsy sample is representative of the adaptation of the entire muscle. Duplicate biopsy studies have found that the CV for determination of fibre distribution is ~10% (Simoneau et al. 1986), and as high as 20% for measurement of enzyme activity (ie. SDH) and capillarization (Coggan 1995). Thus, future studies should attempt to minimize the influence of this variability with large sample sizes and multiple biopsies, where possible.
3.5 References


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CHAPTER 4

General Discussion

4.1 Summary of Key Findings

The purpose of this thesis was to test the hypothesis that SIT would induce high and low levels of VO2peak response, and that high individual responders to VO2peak would also be high responders to measures of peripheral adaptations. This was done by conducting a training study in which 23 young, healthy, recreationally active males participated in 4 weeks of SIT (4 x per week), with baseline measures of VO2peak, as well as multiple measures of peripheral physiological response (capillary density, fibre-specific SDH content, type I fibre %) being measured at baseline (Pre) and following (Post) training. The key findings of this experiment included that 4 weeks of SIT induced varying levels of VO2peak response (nonresponse rate of 26%), as well as that when separated into tertiles of VO2peak response, the HI and LO groups differed significantly in VO2peak change after training. The second key finding was that there was no difference between HI and LO groups for response in any of capillary density, fibre-specific SDH content, or fibre type %. In addition, individual changes to VO2peak did not correlate significantly with any of our measured peripheral variables. In concert, these results confirm that individuals’ VO2peak responds quite heterogeneously to SIT and that these results cannot specifically be explained by individual changes in capillary density, fibre-specific SDH content or type I fibre %.

4.2 Reflecting on my Thesis Project

This project was a very large, collaborative undertaking, made possible by many individuals throughout our department. This study involved work from Dr. Brendon Gurd, a
PhD. student, 4 MSc. students (including myself), as well as numerous interns and undergraduate trainers. Despite our efforts, there are some definite limitations to our work that are worth noting.

The original goal for participants in this study was 30, a number which we thought both realistic and ideal for the statistical comparisons we planned. 23 individuals completed the duration of the study, however 50 were initially screened. A variety of reasons led to this original 50 being decreased to 23, including some being too active for inclusion (more than 150 minutes of moderate/vigorous weekly PA), lack of time, being uncomfortable with the muscle biopsy, as well as other personal reasons. One can only speculate as to whether the final crop of participants is a representative sample of the population we chose to examine, as we were obviously unable to determine responsiveness of VO$_2$peak/peripheral factors to SIT in those who did not fully complete the study.

An additional major limitation to this study stems from the assumption that the single muscle biopsies taken before and after training provided a representative sample of adaptations that may have occurred in the entire muscle. Ethical limitations prevent large muscle samples from being drawn, so the reality is that a very small piece of muscle must be used as an indication of what is going on in the entire vastus lateralis. Aspects of our protocol, such as rotating the biopsy needle to obtain a 360° spectrum of tissue in a given biopsy, are designed to obtain a more comprehensive picture of what is going on in the muscle. Despite this, there is a range of error associated with variables obtained from a sample of this nature, and therefore we can only be so confident as to how close our measured value is to the true level of adaptation. This range of error is compounded when you take a Pre and a Post sample. Indeed, duplicate biopsy studies have found that the CV for determination of fibre distribution is ~10% (Simoneau et al. 1986), and as high as 20% for measurement of enzyme activity (ie. SDH) and
capillarization (Coggan 1995). While obtaining multiple biopsies at each time point may have helped to reduce this error, ethical and logistical reasons made doing so unrealistic for this study. Thus, it would seem that our results pertaining to muscle characteristics (ie. SDH content, fibre type, and capillary density) should be interpreted with some caution.

Following the last individual completing post-training data collection, myself and an undergraduate intern completed the majority of my analysis. This included mounting 10 micron slices of muscle tissue for each participant onto glass slides, which were then brought to Dr. Joe Quadrilatero’s lab at the University of Waterloo. It was there that all histochemical staining and imaging for capillary density, fibre-specific SDH content and type I fibre % took place. Quantification of individual participant images was done back at Queen’s in the subsequent weeks. This study as a whole was a success, but that is not to say that there was no room for improvement. If I were to approach this same study again (perhaps without the same restraints of time, money, resources, etc.), there are a few things that I would like to do differently.

The first of these is perhaps the most obvious, in that ideally we would have been able to examine many more peripheral factors in this study. The factors that we chose to examine were capillary density, fibre-specific SDH content and type I fibre %. While these are all valuable measures, which I would collect again, obtaining an additional measure of mitochondrial content (such as citrate synthase) would only have strengthened our conclusions. Previous interval training studies have also examined direct measures of mitochondrial function, rather than content (Daussin et al. 2008). If I were to conduct this study again, I would also be interested in obtaining something like mitochondrial respiratory capacity (specifically oxidative phosphorylation capacity (P) and electron transport system capacity (ETS)) which corresponds with whole body aerobic capacity independently of mitochondrial content (Jacobs and Lundby
2013). Additional measures such as sarcoplasmic reticulum function, proliferator-activated gamma coactivator 1 alpha (PGC-1α) mRNA expression and resting muscle glycogen content are all potential peripheral factors that could help provide the best chance of explaining individual variation in VO₂peak following SIT. The reason we chose the factors that we did stemmed from the fact that they all could be quantified using histochemical techniques I have previously conducted (Edgett et al. 2016), as well as the fact that a limited sample of human muscle tissue is available from a single muscle biopsy. If we had been ethically/logistically able to take a second muscle biopsy at each time point (ie. Pre and Post), this would have made it possible to take more peripheral measures.

While 23 participants is a good number of participants to complete a training study of this length and commitment, the LO group (N=8) that we used for the sake of comparing varying levels of VO₂peak response, was not entirely made up of nonresponders (6/8). In future studies, we would ideally use a population large enough such that similar comparisons could be made with a LO group made entirely of nonresponders, while still using enough participants to detect statistical differences between groups.

4.3 Practical Implications of My Thesis

While it would seem that many of the major takeaways from this study are rooted in molecular mechanisms associated with physiological adaptation to exercise, there are practical implications as well that may be communicated to the lay reader. This mainly stems from the highly heterogeneous response observed for VO₂peak amongst participants following 4 weeks of SIT. Given the range of responses, including a 21.7% rate of nonresponse (5/23) and an adverse response rate of 4.3% (1/23), it is clear that individuals respond diversely to this type of exercise.
Buford and colleagues (2013) have discussed the idea of shifting exercise prescription to a more personalized approach, given the fact that nonresponders are so prevalent in END studies to look at individual changes in VO$_2$peak. They highlight the potential for tailored exercise strategies for targeted outcomes in specific population groups (Buford et al. 2013). Given the present results, of similar heterogeneity to SIT exercise, it is clear that such approaches would be valuable for interval training interventions as well. Before such approaches are developed, what individuals who intend to improve their VO$_2$peak with SIT should do is monitor how well they respond to training. Given that individuals who do not respond to one form of exercise (ie. SIT) may not be any less likely to respond to another (ie. HIIT, END or resistance exercise) (Hautala et al. 2006), changing up your exercise regimen may help to provide an individual with a greater chance of improving VO2peak. Alternatively, a multimodal exercise program (ie. a combination of multiple training modalities performed regularly), may provide the general population with the best opportunity for widespread response to training (Buford et al. 2013). It is hoped that studies targeting this type of approach to exercise prescription at the individual level will continue to emerge in the near future.

4.4 Overall Research Experience

My graduate experience in my Master of Science in Kinesiology and Health Studies began with coursework. Each course I took provided me with a unique opportunity to learn and develop skills that would prove integral to the rest of my degree, under the close guidance of instructors from our department. This included statistical methods, researching literature, scientific writing, data presentation, study design, and approaching research with a critical lens. These skills, in part, helped me to publish my first primary authorship in Applied Physiology, Nutrition and Metabolism with the assistance of my supervisor Dr. Brendon J. Gurd.
Over the past two years I have had the chance to hone my exercise training skills, as well as learn a variety of techniques in our “wet lab”. This includes VO_{2}\text{peak} ramp tests, protein assays, Western blots, tissue homogenization, tissue mounting, and blood extraction. These skills have allowed me to perform tissue analysis of my own thesis as well as collaborate on other projects. This includes two secondary authorships I recently gained in collaboration with other members of our lab, each of which are now published in Applied Physiology, Nutrition and Metabolism. Briefly, these experiments included one in which I conducted a series of exercise tests on young, healthy men and women to establish a typical error value for a 500 kcal time to completion test that we have used previously in our lab. This typical error value was then used retrospectively to establish nonresponse rates to previous training studies conducted in our lab. The second of these experiments, involved performing histochemical analysis on muscle tissue from before and after participants completed a 48 hour fast. My analysis allowed comparison between fed and fasted states for fibre-specific changes in resting glycogen and intramuscular triglyceride levels. I have also had the chance to work on other projects, including Western blot analysis of muscle from rats with kidney disease, and pilot work on the effects of sodium bicarbonate supplementation on extracellular buffering during SIT. Working on these projects has also provided me with the opportunity to attend and present at multiple regional conferences.

Twice over the last two years I have had the opportunity to collaborate with Dr. Joe Quadrilatero’s lab at the University of Waterloo. Each time I have collaborated with another MSc. student on histochemical staining and imaging, including the analysis of muscle used in my thesis (staining for capillary density, SDH content and type I fibre %). This experience has given me a chance to learn from students in a different field, as Dr. Quadrilatero’s lab specializes in cell apoptosis.
My time here has been a valuable learning experience, allowing me to constantly develop and exercise leadership, organizational skills and critical thinking skills. One of the most valuable aspects of my thesis and MSc. as a whole has been recruiting and working with younger undergraduate students who have volunteered in the lab. This specifically includes a group of 4 students who started volunteering for my fourth year thesis in 2013 and are each now preparing to conduct their own thesis project this Fall. Each of the skills and experiences I have described, along with guidance from my supervisor Dr. Brendon J. Gurd, have helped me complete this thesis project.
4.5 References


Appendices

Appendix A: Ethics Approval

QUEEN'S UNIVERSITY HEALTH SCIENCES & AFFILIATED TEACHING HOSPITALS RESEARCH ETHICS BOARD

March 22, 2010

Dr. B. Gurd
School of Kinesiology and Health Studies
Queen's University

Dear Dr. Gurd,

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

The members of the Queen's University Health Sciences & Affiliated Teaching Hospitals Research Ethics Board have examined the protocol, questionnaires and the revised consent form for your project (as stated above) and consider it to be ethically acceptable. This approval is valid for one year from the date of the Chair's signature below. Please attend carefully to the following list of ethics requirements you must fulfill over the course of your study:

- Reporting of Amendments: If there are any changes to your study (e.g. consent, protocol, study procedures, etc.), you must submit an amendment to the Research Ethics Board for approval. (see http://www.queensu.ca/vpr/reb.htm).

- Reporting of Serious Adverse Events: Any unexpected serious adverse event occurring locally must be reported within 2 working days or earlier if required by the study sponsor. All other serious adverse events must be reported within 15 days after becoming aware of the information.

- Reporting of Complaints: Any complaints made by participants or persons acting on behalf of patients must be reported to the Research Ethics Board within 7 days of becoming aware of the complaint. Note: All documents supplied to participants must have the contact information for the Research Ethics Board.

- Annual Renewal: Prior to the expiration of your approval (which is one year from the date of the Chair's signature below), you will be reminded to submit your renewal form along with any new changes or amendments you wish to make to your study. If there have been no major changes to your protocol, your approval may be renewed for another year.

Yours sincerely,

[Signature]
Chair, Research Ethics Board

Date: March 28, 2010

Study Code: PHE-100-10

Investigators please note that if your trial is registered by the sponsor, you must take responsibility to ensure that the registration information is accurate and complete.
Appendix B: Ethics Amendment

QUEEN’S UNIVERSITY HEALTH SCIENCES & AFFILIATED TEACHING HOSPITALS
RESEARCH ETHICS BOARD (HSREB)

HSREB Amendment Acknowledgment/Ethics Clearance

January 06, 2016

Dr. Brendon Gurd
School of Kinesiology and Health Studies
Queen’s University

ROMEO/TRAQ: #6003260
Department Code: PHE-100-10
Study Title: Effect of exercise training at a variety of intensities on mitochondrial function in young lean and obese adults
Review Type: Delegated
Date Ethics Clearance Issued: January 06, 2016

Dear Dr. Gurd,

The Queen’s University Health Sciences & Affiliated Teaching Hospitals Research Ethics Board (HSREB) has reviewed the amendment application and granted ethics clearance/acknowledgement for the following:

- Addition of non-invasive measurement methods for muscle oxygenation and cardiac output – to be performed at Dr. Neder Serafini’s Laboratory of Clinical Exercise Physiology (LACEP) at the Kingston General Hospital.
- Revised information/consent form

Yours sincerely,

[Signature]
Chair, Health Sciences Research Ethics Board

The HSREB operates in compliance with, and is constituted in accordance with, the requirements of the TriCouncil Policy Statement: Ethical Conduct for Research Involving Humans (TCPS 2); the International Conference on Harmonisation Good Clinical Practice Consolidated Guideline (ICH GCP); Part C, Division 3 of the Food and Drug Regulations; Part 4 of the Natural Health Products Regulations; Part 3 of the Medical Devices Regulations, Canadian General Standards Board, and the provisions of the Ontario Personal Health Information Protection Act (PHIPA 2004) and its applicable regulations. The HSREB is qualified through the CTO REB Qualification Program and is registered with the U.S. Department of Health and Human Services (DHHS) Office for Human Research Protection (OHRP). Federalwide Assurance Number: FWA#: 00004184, IRB#: 00001173

HSREB members involved in the research project do not participate in the review, discussion or decision.
Appendix C: Subject Recruitment and Information Forms

Sample Recruitment form displayed in bulletin boards and television monitors around Queen’s University Campus. Including the School of Kinesiology and Health Studies, Athletic and Recreation Centre, and John Deutsch University Centre.

The MEGA Study

The Muscle Biochemistry Lab at Queen’s is interested in measuring health outcomes after Sprint Interval Training.

Are you?

- Recreationally Active (Less than 3hrs of structured physical activity)
- Lean male between the ages of 18-30 (Pant size less than 36)
- No history of cardiovascular and metabolic diseases (stroke, hypertension, type II diabetes)
- Not currently taking prescribed medication
- A non-smoker

$100 compensation and Free personal training to meet your New Years Resolutions!

This study involves four weeks of supervised training, muscle biopsies, and Cardiovascular Fitness Testing.

Email Matthew Giles at 9mdg2@queensu.ca for more information!
Physical Activity Readiness Questionnaire was completed by all subjects prior to subject screening.
Consent to Volunteer for Participation in a Study

TITLE: Effect of central and peripheral adaptations on the variability of response to aerobic capacity following sprint interval training

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 heart and cardiovascular system to deliver oxygen, and your muscle’s mitochondria to produce energy (mitochondrial function) can be
impaired with obesity and is a predictor of both further weight gain and development of type II diabetes. Further, fat cells also contain mitochondria, and decreased mitochondrial function in fat is also associated with weight gain and the development of disease. 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 the heart, the cardiovascular system and mitochondrial function and exercise capacity are unknown. This study will examine the ability of different exercise training protocols and modes (cycling, running, whole body exercise or recreational games [i.e. sport]) to improve heart, cardiovascular system and mitochondrial function and exercise capacity. We will also ask you a series of questions designed to increase our understanding of what type of exercise you would prefer and/or are likely to perform during your normal daily routine.

You will not be able to participate in the study if you have been diagnosed previously with any respiratory, cardiovascular (e.g. High blood pressure, heart conditions), metabolic (e.g. 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 choose 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.

The majority of the procedures described below, and all exercise training will take place within the Muscle Physiology Lab at the School of Kinesiology and Health Studies. Measurement of muscle oxygenation and cardiac output will be performed in the Laboratory of Clinical Exercise Physiology located within Kingston General Hospital.
**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 one 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.

**Psychological Questionnaires:**
On the first visit, prior to the VO$_2$peak test, you will be required to fill out a series of questionnaires designed to determine the amount of physical activity you regularly perform and to predict whether or not you will enjoy high intensity exercise. It is expected that these questionnaires will take less than 30 minutes to complete.

In addition, during training sessions of this study you will be asked a series of questions designed to evaluate how you are feeling towards the exercise you are performing.

Finally, following the exercise protocol you will be asked to fill out a series of questionnaires designed to determine how much you enjoyed the exercise and how likely you are to part-take in exercise in the future. These final questionnaires should also take less than 30 minutes to complete.

All results from these questionnaires will be kept private and will be recorded in an anonymous fashion (i.e. by subject number rather than by name).

**Physiological tests:**
**Blood sample:** Both before and following training you will be asked to have a small sample of blood taken. 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 or by an individual trained in the technique under the supervision of a medical doctor. 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 (less than 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.**

**Measurement of Muscle Oxygenation:** Before and after training you will be asked to have the changes in muscle oxygenation measured during an incremental exercise test (described above). Muscle oxygenation, a measure of how much blood is being delivered by your cardiovascular system to your muscle, is measured by a sensor that will be strapped to your leg and that uses infra-red light (near infra-red spectroscopy) measure tissue oxygenation. This process is a non-invasive, optical (light-based) method with no reported side-effects related to its use in humans.

**Cardiac Output Evaluation:** Before and after training you will be asked to have the amount of blood pumped by your heart each beat (cardiac output) measured during an incremental exercise test (described above). To measure cardiac output a mixture of gases (oxygen, hexafluoride, and nitrous oxide) will be given to you through the same mouthpiece used to measure oxygen uptake at pre-selected points during exercise. These gases are harmless and they do not change your blood oxygen levels, cause discomfort, or cause shortness of breath. We will advise you when these gases will be switched on
and you will breathe them for 5 breaths. You will also know when you are breathing this mixture because a bag in front of you will inflate and deflate as you exhale and inhale. Minimal coaching on breathing rate ("breathe a little faster" or "breathe a little slower") may be provided during this measurement. There are no reported complications or side effects from completing 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.

- **High-intensity exercise training:** This protocol involves riding a bike at a high-intensity, like an all-out sprint, for 20 seconds at a time (called an interval) followed by 10 seconds of rest. This interval will be repeated 8 times. You will be asked to perform these protocols 4 times a week for a period of 4 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 center/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 (613 533-6000, ext 79023), Dr. Jean Coté, Head, School of Kinesiology and Health Studies (613 533-6601). If you have any concerns about your rights as a research participant please contact Dr. Albert Clark, Chair for the Queen’s University 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