

Muscle Oxygenation and Aerobic Metabolism During High-Intensity Interval
Training Bodyweight Squat Exercise in Comparison to Continuous Cycling

by

Andrew Kates
B.Sc., Dalhousie University, 2011

A Thesis Submitted in Partial Fulfillment
of the Requirements for the Degree of

MASTER OF SCIENCE

in the School of Exercise Science, Physical and Health Education

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Abstract

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The purpose of this study was to evaluate muscle oxygenation, cardiorespiratory, and blood lactate responses to an acute bout of a high-intensity interval training (HIIT) bodyweight squat protocol (HIIT-squats) in comparison to (continuous) moderate intensity cycling exercise (MOD). On separate days, within a two week period, 15 recreationally active males (28 (4.6) years) performed: 1) incremental test to exhaustion on a cycle ergometer, 2) 30-minutes of moderate intensity cycling (MOD; 65% VO_2max), and 3) HIIT-squats consisting of eight x 20 seconds of bodyweight squats performed at maximal cadence with 10-s rest intervals. During each exercise condition, oxygen consumption (VO_2) and heart rate were monitored continuously, and muscle oxygenation (tissue saturation index, TSI) at the left vastus lateralis muscle was measured for 2 minutes pre-, throughout, and for 5 minutes post-exercise using Near-Infrared Spectroscopy (NIRS; Portalite, Artinis Medical Systems, Netherlands). Blood lactate was measured at pre- and one, three, and five minutes post-exercise. Mean and peak changes in TSI were similar in both HIIT-squats (mean = -14.6 (5.3)%, peak = -19.7 (5.2)%; $p > 0.05$) and MOD (mean = -13.2 (5.6)%, peak = -18.2 (7.6)%; $p > 0.05$), with peak changes in TSI occurring significantly faster in HIIT-squats (71.2 (95.2) seconds (s) after onset of exercise) than in MOD (1452.9 (647.8)s; $p < 0.05$). The half time of TSI recovery following HIIT-squats ($T_{1/2\text{TSI}} = 25$ (7.9)s) was not significantly different post-MOD (25 (9.6)s). Mean VO_2 during HIIT-squats (31.48 (4.58) $\text{ml}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$) was similar to MOD (33.76 (5.71) $\text{ml}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$), however minute ventilation (V_E), respiratory exchange ratio (RER) and all post-exercise blood lactate concentrations were significantly higher in HIIT-squats compared to MOD ($p < 0.05$). Despite the different durations of HIIT-squats and MOD, mean and peak changes in aerobic metabolism during and after exercise were similar. Results provide evidence of

both aerobic and anaerobic contributions to energy metabolism in response to HIIT-squats, and highlight possible mechanisms for the commonly reported improvements in aerobic power following chronic HIIT.

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Acknowledgments

I would first like to thank my family; Dad, Mom, David and Jenn for always supporting me. Even though I'm far from home, I think about you all every day and I know that none of this would be possible without your support. Thank you for everything!

I would like to say a big thank you to my supervisor Dr. Kathy Gaul for everything you have taught me during our time working together. When I first came to Victoria two years ago, I could not have imagined having such a great working relationship with anyone. All of your help throughout this degree has been greatly appreciated and I am honoured to have had the chance to work with you.

Also, thank you to my committee member Dr. Lynneth Stuart-Hill for your contributions to this thesis and to Dr. Shawn Davison for your assistance and contributions as external examiner. A big thank you to Greg Mulligan, particularly for all of your help in the lab, and teaching me many of the basics of exercise physiology testing. As well, thank you to the many research assistants who helped to make data collection run smoothly.

And finally, this thesis would truly not have been possible without the support of my Victoria family. Thank you from the bottom of my heart to Charlotte Miglin, Emily Peroni, Jason Poucher, Sammy Weiser Novak, Emery Prette and Barry Luksenberg. We've been through it all together, and the memories we've made here will last a life time. I love you guys and thank you for making Victoria a true home!

Dedication

The hard work that went into this thesis is dedicated to Barry Luksenberg. Barry, we miss you every single day. From Whistler to Victoria, we really lived it up on the West coast! I can easily say these were the best times of my life. When you greeted me on my first day in Victoria, and we walked along Dallas road and caught up, we had no idea about the incredible times that lay ahead. But we really made the most of our time here, and it will never be the same without you. Your passion to conquer everything from snowboarding to squash, guitar, chess, cooking, language, work, travel, and many more things has been and will continue to be an inspiration to me. Thank you for that and thank you for being a brother to me.

Chapter 1 Introduction

Despite the overwhelming evidence supporting the health benefits of regular physical activity/exercise (PA), Canadian adults are insufficiently active, with most failing to meet the recommended guidelines of 150 minutes of moderate-to-vigorous PA (MVPA) each week (Blair, 2009; Blair et al., 1989; Colley et al., 2011; Tremblay et al., 2011). Recent accelerometry data has shown that only 15% of adults were meeting recommended levels of weekly PA, with only 5% meeting guidelines by participating in regular purposeful exercise throughout the week (Colley et al., 2011). Furthermore, 63% of adults accumulate at least 15 minutes of MVPA at least one day per week, meaning that 37% fail to even meet this unexceptional level of activity (Colley et al., 2011). Although there may be a variety of reasons why people fail to participate in regular PA, “lack of time” has consistently been identified as the number one barrier (Godin et al., 1994; Trost, Owen, Bauman, Sallis, & Brown, 2002). Clearly, innovations in exercise promotion and prescription are needed in order to overcome this barrier and increase PA participation amongst Canadians. Promotion of PA for improvements in both aerobic fitness and muscular performance (i.e. muscular strength and endurance) are of importance for attaining these beneficial effects (Brill, Macera, Davis, Blair, & Gordon, 2000; Warburton, Nicol, & Bredin, 2006), and time efficient exercise programs are of particular interest.

High-Intensity Interval Training (HIIT)

Recently, there has been increased interest in High-Intensity Interval Training (HIIT) as a means for individuals to achieve the health benefits of endurance training (END), with a diminished time and volume commitment. HIIT involves repeated bouts of brief intermittent exercise performed at a maximal level of intensity and interspersed with periods of rest or low-intensity exercise (Gibala, 2009). In young, healthy individuals, HIIT has been shown to induce improvements similar to END in maximal aerobic power (VO_{2max}) (Burgomaster et al., 2007, 2008), insulin sensitivity (Babraj et al., 2009; Metcalfe, Babraj, Fawkner, & Vollaard, 2012; Richards et al., 2010), cardiovascular and autonomic function (Heydari, Boutcher, & Boutcher, 2013), and body composition (Heydari, Freund, & Boutcher, 2012; Trapp, Chisholm, Freund, & Boutcher, 2008).

Moreover, despite common misconceptions about the generalizability of high-intensity exercise, HIIT research has not been restricted to young healthy individuals. Different forms of HIIT have been used in studies with various at-risk populations including overweight/obese individuals (Gillen, Percival, Ludzki, Tarnopolsky, & Gibala, 2013; Heydari et al., 2012; Whyte, Gill, & Cathcart, 2010), middle-age sedentary adults (Hood, Little, Tarnopolsky, Myslik, & Gibala, 2011), patients with coronary artery disease (Currie, Dubberley, McKelvie, & MacDonald, 2013) and individuals living with type 2 diabetes (Little et al., 2011). The encouraging results from these diverse study populations clearly illustrate the prominence of HIIT research and the many potential benefits of researching and promoting HIIT. Most commonly, HIIT-related research has focused on exercise at

or near maximal intensity, often involving repeated 30-second cycle sprints interspersed with long rest intervals (traditional HIIT). Moving forward, research involving novel and diverse HIIT protocols is warranted, in order to maximize the efficiency and effectiveness of exercise prescription involving HIIT (Gillen & Gibala, 2014).

Low-Volume HIIT

Recently, a number of studies have reported on a low-volume HIIT protocol (LV-HIIT) involving eight x 20-seconds (s) maximal effort exercise intervals, interspersed with 10-s rest intervals, resulting in a four minute exercise protocol that is much shorter than both END and traditional HIIT (Ma et al., 2013; McRae et al., 2012; Tabata et al., 1996). Originally, Tabata et al. (1996) reported that when participants completed the LV-HIIT protocol on a cycle ergometer four days per week for six weeks, they improved maximal aerobic power to an equal extent as a group training five days per week, for 30 minutes, at an intensity of 70% VO_{2max} (Tabata et al., 1996). Additionally, improvements were observed in anaerobic exercise capacity following LV-HIIT, but not following the higher volume cycling protocol. The authors concluded that LV-HIIT could improve both the aerobic and anaerobic energy releasing systems, with a minimal time commitment compared to traditional END exercise programs (Tabata et al., 1996).

More recently, there has been further attention given to LV-HIIT, as researchers explore the potential of time-efficient exercise programs as a means to improve cardiorespiratory and metabolic fitness. The results observed by Tabata et

al (1996) have been replicated using a similar four day x four week program with a weekly training volume of only 16 minutes (Ma et al., 2013). Following the training program, eight active male participants significantly improved their maximal aerobic power (VO_{2max} ; $p < 0.05$) and Wingate mean and peak power ($p < 0.05$). Furthermore, skeletal muscle mitochondrial proteins (i.e. COX, COX IV) were elevated post-training, supporting previous findings that improved aerobic power following HIIT may result from “peripheral” adaptations within the exercising muscle (Macpherson, Hazell, Olver, Paterson, & Lemon, 2011).

LV-HIIT has also been adapted to include exercises typically associated with resistance training (RT) or calisthenics. McRae et al. (2012) designed a four day x four week LV-HIIT program involving burpees, mountain climbers, jumping jacks and squat thrusts performed at maximal cadence during each 20-s interval. Results of the training program were compared with those assessed in a group of participants who completed 30 minutes of treadmill running at $\sim 85\%$ HR_{max} . Upon completion of the training programs, VO_{2max} improved to the same degree in both groups. Furthermore, LV-HIIT also improved anaerobic exercise capacity, lower-body, upper-body, and core muscular endurance while the running program had no effect (McRae et al., 2012).

These results suggest that adaptations to aerobic health and fitness can be achieved with a much shorter duration of exercise than the 150 minutes that is currently recommended (Tremblay et al., 2011), provided that intensity is sufficiently high. The very minimal time commitment of LV-HIIT would certainly overrule the common “lack of time” excuse and could play a strong role in the

optimization of individual health and fitness. Research involving the acute metabolic and physiological demands of LV-HIIT, will increase the understanding of how a single exercise session eventually leads to the significant health benefits that have been previously observed, and will inform future PA prescription. To our knowledge, the metabolic and physiological demands of LV-HIIT and END-type exercise have not been reported concurrently within the same participants. Given the current understanding that the physiological adaptations associated with HIIT likely occur primarily at the peripheral level (Macpherson et al., 2011), further investigation of the acute peripheral responses to LV-HIIT is warranted.

Near-Infrared Spectroscopy

Near-Infrared Spectroscopy (NIRS) is a tool which allows for continuous and non-invasive monitoring of oxygenation in the microvasculature of skeletal muscles during exercise (Bhambhani, 2004). Relative concentrations of oxyhemoglobin/oxy-myoglobin (O_2Hb) and deoxyhemoglobin/deoxy-myoglobin (HHb) can be assessed in real time by the absorption of near-infrared (NIR) light from the 650- to 950-nm wavelength (Wolf, Ferrari, & Quaresima, 2007). These concentrations can then be used to calculate O_2Hb saturation (Tissue Saturation Index; TSI%), which reflects the dynamic balance between O_2 supply and O_2 consumption in the investigated muscle (Ferrari, Muthalib, & Quaresima, 2011). The validity of NIRS for measuring muscle oxygen saturation in vivo has been established (Belardinelli, Barstow, Porszasz, & Wasserman, 1995b; Lin, Lech, Nioka, Intes, & Chance, 2002; Mancini et al., 1994), and NIRS has previously been used to

explore muscle physiology in HIIT (Buchheit, Abbiss, Peiffer, & Laursen, 2012) and squatting exercise (Hoffman et al., 2003). Further review of HIIT and NIRS literature can be found in Appendix A. Using NIRS to further investigate the muscle oxygenation responses that occur during HIIT may provide greater insight into the acute metabolic requirements and physiological mechanisms which contribute to the optimization of health (Coffey & Hawley, 2007).

Purpose and Rationale of Study

The purpose of this study was to investigate the metabolic and physiological demands of a LV-HIIT bodyweight squat protocol (HIIT-squats) by measuring the associated muscle oxygenation and cardiorespiratory responses in healthy, active males. A secondary purpose was to compare these responses with those measured during an acute bout of continuous moderate intensity exercise on a cycle ergometer and the responses measured during a stepwise incremental cycling test to exhaustion.

Research Questions

The following research questions were addressed in this study:

1. What are the physiological and muscle oxygenation responses to an acute bout of LV-HIIT bodyweight squats (HIIT-squats)?
2. How do the physiological and muscle oxygenation responses observed during an acute bout of HIIT-squats, compare to the responses observed during 30 minutes of continuous moderate intensity cycling (MOD)?

3. How do the physiological and muscle oxygenation responses observed during an acute bout of HIIT-squats, compare to the responses observed during a stepwise incremental test to exhaustion (VO_{2max})?

Delimitations

Participants were apparently healthy, recreationally active adult males (22-36 years old) living in Victoria, BC.

Limitations

1. The HIIT-squats protocol used in this study was unfamiliar to some participants. This could have limited the performances observed during HIIT-squats in these participants (i.e. less squats performed compared to a participant who is more familiar with the exercise and the feeling of working at maximal effort).
2. The light absorption and metabolic properties of fat and muscle differ considerably. Therefore, adipose tissue has the potential to interfere with the NIRS signal as demonstrated by reduced tissue absorbancy of NIR light with increasing levels of adipose tissue thickness (Homma, Fukunaga, & Kagaya, 1996).
3. Due to the similar light absorption properties of hemoglobin and myoglobin at the near infrared level, NIRS is not able to distinguish between these two chromophores. Therefore the contribution of hemoglobin/myoglobin to the NIRS signal is unknown.

4. Although limiting practical interpretation of our results, no physiological calibration (i.e. arterial occlusion) of the NIRS device was performed in order to stay consistent with previous studies which have investigated muscle oxygenation trends during HIIT. Nevertheless, we are confident that a low-oxygenation reference point would have been similar in both HIIT and MOD, and therefore would not have altered our conclusions (Smith & Billaut, 2010).
5. Although cycling and squatting involve some similar movements and muscle groups, they are two distinct exercises, thus limiting the extent of direct comparisons that can be made between the two exercise conditions.

Assumptions

1. Participants exerted maximal effort during the HIIT-squats protocol and did not adapt a pacing strategy.

Operational Definitions

- ***High-Intensity Interval Training (HIIT)***: Repeated bouts of brief intermittent exercise performed at a maximal level of intensity and interspersed with periods of rest or low-intensity exercise.
- ***Tissue Saturation Index (TSI)***: the concentration of oxyhemoglobin/oxymyoglobin (O_2Hb), in relation to total hemoglobin/myoglobin (tHb; $(O_2Hb/(HHb+O_2Hb))$).

- **Muscle Deoxygenation:** The decrease in TSI in the microvasculature of the interrogated muscle during exercise.
- **Muscle Reoxygenation:** The increase in TSI in the microvasculature of the interrogated muscle during post-exercise recovery.
- **Baseline TSI_{mean} (%)** – Mean TSI during the 2 minute rest period immediately preceding exercise.
- **Exercise TSI_{mean} (%)** – Mean TSI during the course of an entire bout of exercise.
- **ΔTSI_{mean} (%)**– Mean Change of TSI. The difference between Baseline TSI_{mean} and Exercise TSI_{mean}.
- **TSI_{min} (%)** – The minimum TSI value observed during exercise.
- **ΔTSI_{min} (%)** – The largest observed change in muscle oxygenation between rest and exercise. The difference between Baseline TSI_{mean} and TSI_{min}.
- **TSI End Exercise (%)**- TSI measured during the final 1 second of exercise.
- **Recovery TSI_{peak} (%)** – The highest TSI value measured during the first 3 minutes of post-exercise recovery.
- **T_{1/2TSI} (s)** – TSI Half Time Recovery. The time required for TSI to reach 50% recovery as defined by the halfway point between TSI End Exercise and Recovery TSI_{peak}.

Chapter 2 Methods

Research Design

All testing was conducted in the Exercise Physiology laboratory at the University of Victoria in Victoria, British Columbia, Canada. Data collection occurred exclusively between September 2013 and December 2013.

A within-subjects repeated measures design was employed to address the primary and secondary purposes of this study. Participants attended the lab on three different occasions to perform three distinct exercise protocols. The first day involved a familiarization to the study followed by a stepwise incremental cycling test to exhaustion (VO_{2max}). The second day involved 30 minutes of continuous moderate intensity exercise on a cycle ergometer (MOD). Participants returned for a third day to complete the high-intensity interval training bodyweight squats protocol (HIIT-squats). HIIT-squats consisted of eight x 20-second intervals of bodyweight squats, interspersed with 10-second rest intervals, for a total exercise session time of four minutes. The total time commitment for participants was approximately 2.5 hours: one hour for the VO_{2max} test, one hour for the MOD session and 30 minutes for the HIIT-squats session. Time between exercise protocols was standardized as much as possible for all participants. A minimum of 48 hours separated each exercise test, and participants were asked to complete all testing within a two-week time-frame in order to avoid a training effect over time. Each participant completed all three exercise conditions, and therefore the research design allowed for within-subject comparisons.

Participants were directed to refrain from vigorous physical activity, smoking, and alcohol consumption on all testing days, and were asked to attend the lab in a hydrated state. Upon arrival at the lab on the first visit, the purpose, nature, and possible risks of the experiment were explained to the participant who then provided written informed consent (Appendix B). Participants were also asked to fill out a physical activity readiness questionnaire (PAR-Q; see Appendix C) to assess overall health/fitness and to determine if it was safe for them to participate in the study (Thomas, Reading, & Shephard, 1992). During all sessions, the principle investigator was present at all times, along with a minimum of one laboratory assistant for both data collection and safety purposes. The study received ethical approval from the University of Victoria Human Research Ethics Board (HREB) and Biohazard Safety Committee prior to participant recruitment.

Participants

A total of fifteen (n=15) male participants volunteered and completed all aspects of the study. Participant recruitment was accomplished by seeking out volunteers from local training facilities, including the university fitness and weight training centre, locally-owned gyms, and also via word of mouth. Those who responded to the lead researcher with interest in the study were contacted via email to determine eligibility for participation in the study. In order to meet inclusion criteria, participants had to be apparently healthy with no known musculoskeletal or cardiorespiratory disease, and recreationally active. Participants were deemed to be recreationally active at the time of recruitment if they regularly performed

between one and three hours of structured aerobic activity per week (McRae et al., 2012). Furthermore, all participants were required to have current and regular involvement in resistance training, including lower body exercises, for a minimum of the past six consecutive months. These activity criteria helped to ensure that participants were able to complete all experimental procedures fully and with minimal risk of injury. The investigation was conducted exclusively with male participants due to convenience sampling and to minimize variations by gender, particularly with regard to the NIRS data. Most of the NIRS literature available involves male participants, thus allowing for direct comparisons with previous studies (McKay, Paterson, & Kowalchuk, 2009; Neary et al., 2001).

Data Collection

Anthropometric Data

Height (cm) was measured to the nearest 0.1cm using a wall-mounted stadiometer (Tanita Corporation of America, Arlington Heights, Illinois). Body mass (kg) was measured to the nearest 0.1kg in the clothing to be worn during exercise, minus footwear, using a Health-O-Meter kilo-pound beam (Congenital Scale Corporation, Bridgeview, Illinois). Body mass measurements were collected prior to each experimental session to account for any small changes in participant body mass that may have occurred over the course of their involvement in the study.

Skinfold measurements were collected using Harpenden calipers at the following sites: triceps brachii, biceps brachii, subscapularis, iliac crest, and medial calf, according to the Canadian Physical Activity, Fitness and Lifestyle Approach

(CPAFLA) specifications. An additional skinfold, over the left vastus lateralis muscle, the area of investigation of the NIRS device as described below, was also measured. Skinfold data were collected to characterize the body composition of the subject population and also to ensure that differences in the NIRS signal were minimally affected by adipose tissue thickness (ATT). To date, NIRS muscle research has been generally restricted to lean participants since the clinical applicability of muscle NIRS in patients with high ATT is limited (Ferrari et al., 2011; Homma et al., 1996). Skinfold measurements were collected upon arrival at the laboratory for the HIIT exercise session due to time considerations.

VO₂ and HR Data

Expired gases were collected and analyzed using a Rudolph valve collection system with a TrueOne 2400 Parvo Medics Metabolic Measurement System (MMS-2400, Parvomedics, Sandy, Utah) and OUSW computer software program (Parvomedics, Sandy, Utah). Prior to all exercise tests, the metabolic cart was calibrated with known standard gas concentration (oxygen 16% and carbon dioxide 4%), and flow was calibrated with a 3.0 L syringe. Nose clips were used to ensure that all breaths were taken from the mouth and all expired gases were collected.

Heart rate (HR) was continuously sampled by telemetry using a chest strap Polar HR monitor (T31, Polar Electro, Kempe, Finland). Participants were fitted with the HR monitor after all their anthropometric data were collected for that day. During the exercise testing sessions, HR and VO₂ data were collected continuously for two minutes at rest and throughout exercise. Data were averaged every 10s and

exported for analysis. The main variables of interest for analysis were absolute VO_2 ($\text{l}\cdot\text{min}^{-1}$), relative VO_2 ($\text{ml}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$), respiratory exchange ratio (RER) and minute ventilation (VE ; $\text{l}\cdot\text{min}^{-1}$). Due to inconsistencies with HR collection during HIIT-squats, HR data were not used in analysis.

Muscle Oxygenation Data

Muscle oxygenation data were collected by a NIRS device (Portalite, Artinis Medical Systems, Netherlands) using a 58x26mm optical probe with three LED light sources, each transmitting two wavelengths (± 760 nm and ± 850 nm). The source-detector distances (distances between the receiver and transmitters) were 30mm, 35mm, and 40mm. The NIRS probe was positioned over the left vastus lateralis, approximately 10-15cm from the knee joint (see Figure 1a), as described previously (Buchheit et al., 2012; Nagasawa, 2013; Smith & Billaut, 2010). For application of the device, participants were asked to fully extend their leg at the knee which served to activate the vastus lateralis, exposing the outline of the muscle and allowing for accurate placement of the NIRS probe on the muscle belly. The probe was then traced with a permanent marker to ensure that no movement occurred during exercise, and to facilitate accurate placement in subsequent exercise testing sessions. A piece of clear plastic wrap was used to protect the NIRS probe and to prevent distortion of the signal by sweat during exercise (Neary et al., 2001). The probe was secured with athletic tape to prevent movement during exercise, and covered with a black nylon sheath and a black cotton strap to prevent contamination from ambient light. Participants were asked to confirm that the

probe was attached securely without being so tight as to restrict blood flow or movement of the limb. The battery pack/transmitter, connected to the NIRS probe by a single electrical wire, was fed through the shorts and shirt of the participant, out the sleeve, and secured to the left arm using an arm band commonly used for securing an mp3 device during exercise (Figure 1b).

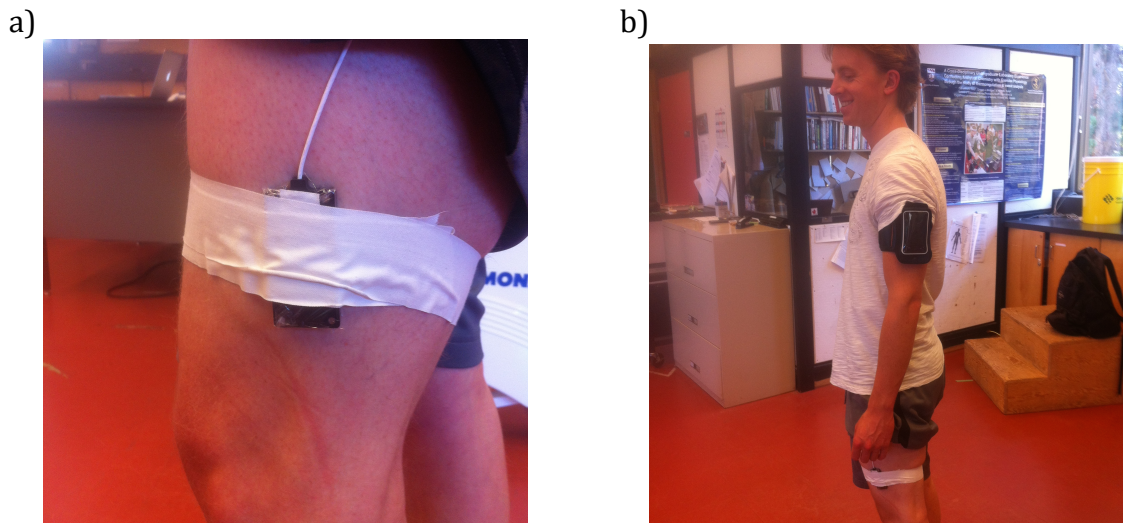


Figure 1

a) Setup of The NIRS Probe at the Left Vastus Lateralis Muscle, and b) Placement of the NIRS Battery Pack/Transmitter to the Left Arm

The NIRS device measures relative concentration changes of intramuscular oxyhemoglobin/oxy-myoglobin (O_2Hb) and deoxyhemoglobin/deoxy-myoglobin (HHb) at the site of investigation. Total hemoglobin/myoglobin is also given as the sum of O_2Hb and HHb concentrations (tHb; $O_2Hb + HHb$). Because NIRS cannot discern between hemoglobin (Hb) and myoglobin (Mb) chromophores, the extent of the contribution which Hb and Mb make to the NIRS signal is presently unclear, and the abbreviations HbO_2 , HHb and tHb refer to the combined signal of Hb and Mb.

Additionally, the NIRS device measures tissue oxygen saturation (StO_2) which is the concentration of O_2Hb , in relation to tHb ($O_2Hb/(HHb+O_2Hb)$) and is an absolute parameter. The Tissue Saturation Index (TSI), the estimation of StO_2 as a percentage, reflects the dynamic balance between O_2 supply and O_2 consumption at the area of investigation. Thus, an increase in TSI can be interpreted as enhanced oxygenation (increased O_2Hb relative to tHb) and a decrease in TSI% can be interpreted as reduced oxygenation (decreased O_2Hb relative to tHb). TSI is independent of the pathlength of the near infrared (NIR) photons in the muscle tissue, and thus is not prone to the considerable measurement error seen in O_2Hb , HHb and tHb concentrations due to the influence of scatter factors caused by adipose thickness and muscle tissue (Ferrari et al., 2011; Nagasawa, 2013). Therefore, TSI alone was used for analysis. During all testing protocols, NIRS data were collected continuously for two minutes at rest, throughout exercise, and during the first five minutes of recovery (Neary et al., 2001). Only fourteen ($n=14$) full NIRS data sets were available from the VO_{2max} test, due to a computer malfunction following the completion of one of the VO_{2max} tests. This test could not be repeated due to unforeseen scheduling events. The full fifteen ($n=15$) NIRS data sets were available for each of the HIIT-squats and MOD exercise sessions.

Blood Lactate Data

Blood lactate was measured pre-test and at one, three, and five minutes post-test using a lancet (Accu-Chek Safe-T-Pro Plus, Mannheim, Germany) and lactate analyzer (Arkray Lactate Pro, Japan). The protocol for collecting blood lactate is

described in Appendix D. The serial post-test blood lactate collection protocol was used to ensure accurate peak values were collected. Although blood lactate was measured at all time points for all participants, some values were excluded due to device malfunctions. Fourteen samples (n=14) are reported for the one and five minutes post-exercise blood lactate measurements following HIIT-squats and for the one minute post-exercise measurements following MOD. Twelve samples (n=12) are reported for the five minutes post-exercise blood lactate following MOD. All other blood lactate measurements yielded fifteen samples (n=15).

Exercise Testing Protocols

Prior to all testing protocols, participants were asked to select a comfortable seat height on the cycle ergometer and warm-up at a self-paced low-to-moderate intensity for five minutes (Smith & Billaut, 2010). Following the warm-up, participants were given five minutes of passive rest before the onset of exercise. Resting VO_2 , HR, and NIRS data were collected during the two minutes immediately preceding exercise and throughout all exercise protocols. Immediately upon exercise termination, the Rudolph valve used for the collection of expired gases was removed while NIRS data continued to be collected for five minutes of recovery. Post-exercise blood lactate measurements were also collected at this time.

Stepwise Incremental Cycle Test to Exhaustion (VO_{2max})

The protocol for VO_{2max} was as follows

- 1) Initial work rate was set at 100-150 Watts (W)

- 2) Work rate was increased by 50 W increments every two minutes until RER was > 1.00 or the participant began to show signs of physical discomfort
- 3) At this time the work rate was increased by 25 W until the criteria for $VO_{2\max}$ was met

At least two of the following criteria were met for determination of $VO_{2\max}$:

- 1) Attainment of predicted maximum HR (220-age)
- 2) A rise in VO_2 of less than two $\text{ml}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$ with a consistent increase in workload
- 3) RER > 1.15
- 4) Volitional exhaustion

HIIT-squats

Each participant completed a set of HIIT-squats consisting of eight \times 20-s work intervals separated by 10-s of rest. Participants were asked to complete as many bodyweight squats as possible within each 20-s interval, while maintaining proper form. During 10-s rest intervals, participants were asked to remain standing on the floor in the place where they were completing the squats and to refrain as much as possible from moving. Performance criteria used were similar to those in previous research involving parallel squat exercises (Robergs, Gordon, Reynolds, & Walker, 2007). Briefly, participants were instructed to begin the squat by pushing the hips posteriorly and simultaneously flexing at the hip and knee joints. The thighs had to reach a position parallel to the floor in the bottom of the squat. Once

full depth was achieved, upward movement occurred and the participant had to return to a fully upright position.

Participants were provided with a target which would make contact with the dorsal part of the leg when full squat depth was reached. Participants were encouraged, but not required, to use the target. In the case that the target was not used by the participant, it was used as a visual cue to aid the lead researcher in determining that full squat depth was achieved (Figure 2). The research team provided constant feedback regarding the quality of the squats. The number of acceptable repetitions performed during each set was recorded.

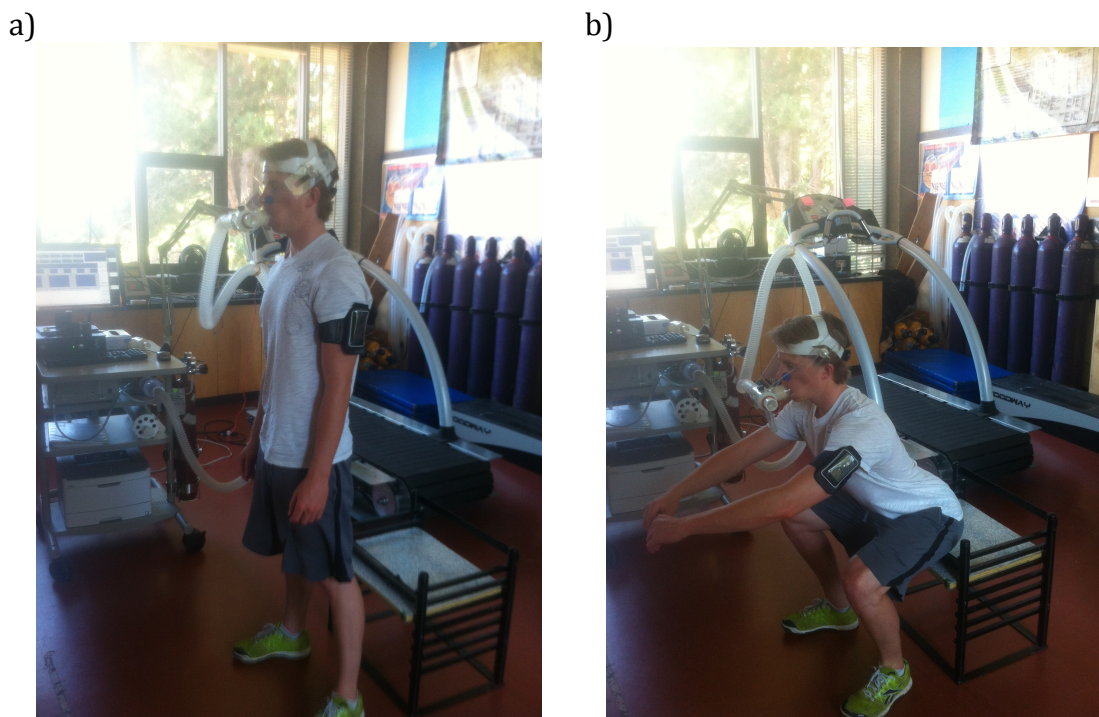


Figure 2

Setup for HIIT-squats Exercise Showing the Top (a) and Bottom (b) of the Squat and the Target Used to Ensure Sufficient Depth at the Bottom of the Squat.

A timing application for iPhone (WOD Version 2.1.3, © 2009-2014 Modal Domains) was used to keep time during exercise and to count the work (descending from 20 to 0 seconds) and rest intervals (descending from 10 to 0 seconds) as well as the number of sets completed. The timer was made visible to the participant and the lead researcher, and gave audible cues when work and rest intervals began and ended. All participants were familiarized with the timer prior to the beginning of exercise in order to avoid potential confusion.

Continuous Moderate Intensity Cycling (MOD)

For the MOD protocol, participants completed 30 minutes of continuous exercise on a cycle ergometer at 65% of their previously measured VO_{2max} . Work rate corresponding to this intensity level was determined prior to initiation of exercise. Wattage (W) was adjusted accordingly throughout the 30 minutes of exercise to ensure that the specified VO_2 was maintained as closely as possible. Exercise was initiated at 100-150W and increased by 50W each minute until the target work rate was achieved, which occurred within three minutes of the start of exercise for all participants.

Statistical Analysis

All NIRS data were filtered using a rolling average filter provided in the Portalite software (Portasoft 2.0.5.12, Artinis Medical Systems, Netherlands) before being exported for statistical analysis. %TSI data were averaged over one second intervals in order to calculate %TSI_{min}, %TSI End Exercise, and %TSI_{peak}. To

calculate $T_{1/2\text{TSI}}$, half of the difference between %TSI End Exercise and %TSI_{peak} was identified, and $T_{1/2\text{TSI}}$ was defined as the time from the completion of exercise to this halfway recovery point (Nagasawa, 2013). VO_2 data were averaged over 10 second intervals and exported. All data were organized in Microsoft Excel (Version 14.4.1, 2011, Microsoft Corp., Seattle WA) and analyzed via one-way repeated-measures analysis of variance (ANOVA), using SPSS statistical software (version 21.0, 2012, SPSS Inc., Chicago IL) to examine potential differences in physiological responses between all exercise tests. Significant main effects were assessed for statistical significance between groups using the Tukey's post-hoc test. Additionally, a Pearson correlation was used to describe the relationship between SO5S and vastus lateralis skinfold thickness. NIRS data were further analyzed via analysis of covariance (ANCOVA), with SO5S and vastus lateralis skinfold thickness as covariates in separate analysis to determine if correcting for adiposity or local skin fold thickness modified the NIRS findings. Since the addition of these covariates did not alter the results, the ANCOVA results are not reported. All data are presented as mean (SD). Statistical significance was set at an alpha of < 0.05.

Chapter 3 Results

Participant Characteristics

Fifteen apparently healthy, recreationally active males participated in this study and completed all three exercise tests. Table 1 provides a description of participant characteristics, including anthropometric measures as well as maximal aerobic performance variables. As body mass did not change significantly between any of the sessions, a mean value for body mass was obtained by averaging the measurements collected at each of the three sessions. Mean (SD) skinfold thickness at the vastus lateralis was 7.7 (4.4) mm and had a significant positive correlation with SO5S ($r = .83, p < 0.01$)

Table 1

Anthropometric Measures and Maximal Aerobic Performance Variables (n=15).

Variable	Mean (SD)	Range
Age (years)	28 (4.6)	22 – 36
Height (cm)	181.3 (4.5)	168.3 – 186.8
Body Mass (kg)	81.2 (9.8)	66.0 – 100.1
SO5S (mm)	46.8 (21.1)	20.6 – 91.5
VO _{2max} (ml·kg ⁻¹ ·min ⁻¹)	57.2 (9.9)	38.5 – 75.8
HR _{max} (b·min ⁻¹)	188 (8)	167 – 198

Exercise Characteristics

For HIIT-squats, the mean number of squats performed during each interval and an overall total number of squats are presented in Table 2, along with mean TSI (%) during each interval. The TSI responses in a representative participant over the

course of HIIT-squats and MOD exercise sessions are shown in Figure 3a and 3b respectively. During HIIT-squats, TSI decreased immediately at the onset of exercise and remained low for the duration of the four minute exercise protocol. This can be seen in Figure 3a and is supported by the mean TSI values for each set of exercise, displayed in Table 2. Mean (SD) resting TSI across all participants was 70.4 (4.9)% and mean TSI during exercise was 55.8 (5.3)%. During each of the 10-s rest intervals, TSI tended to increase slightly, however mean oxygenation levels during the course of HIIT-squats were not significantly different when considered with (55.8 (5.3)%) and without (55.5 (5.2)%) the rest intervals ($p > 0.05$), and therefore analysis of HIIT-squats data refers to the mean of the entire four minute protocol, including rest intervals. When exercise ceased and recovery began, TSI increased rapidly and an “overshoot” above pre-exercise resting values was consistently observed. The mean of peak TSI values observed during recovery (Recovery TSI_{peak}) from HIIT-squats was 78.2 (4.3)%.

During MOD exercise, TSI also decreased upon initiation of exercise. Mean (SD) TSI across all participants was 70.8 (5.7)% during pre-exercise rest and 57.6 (5.4)% during exercise. The decline in TSI was significantly slower than that observed in HIIT-squats ($p < 0.001$), since peak deoxygenation was observed at a mean time of 71.2 (95.2) seconds after the onset of HIIT-squats and 1452.9 (647.8) seconds after the onset of MOD. Similar to HIIT-squats, when MOD ended and recovery began, TSI increased rapidly and an “overshoot” above pre-exercise resting values was consistently observed. The mean TSI_{peak} value observed during recovery from MOD was 77.0 (5.2)%.

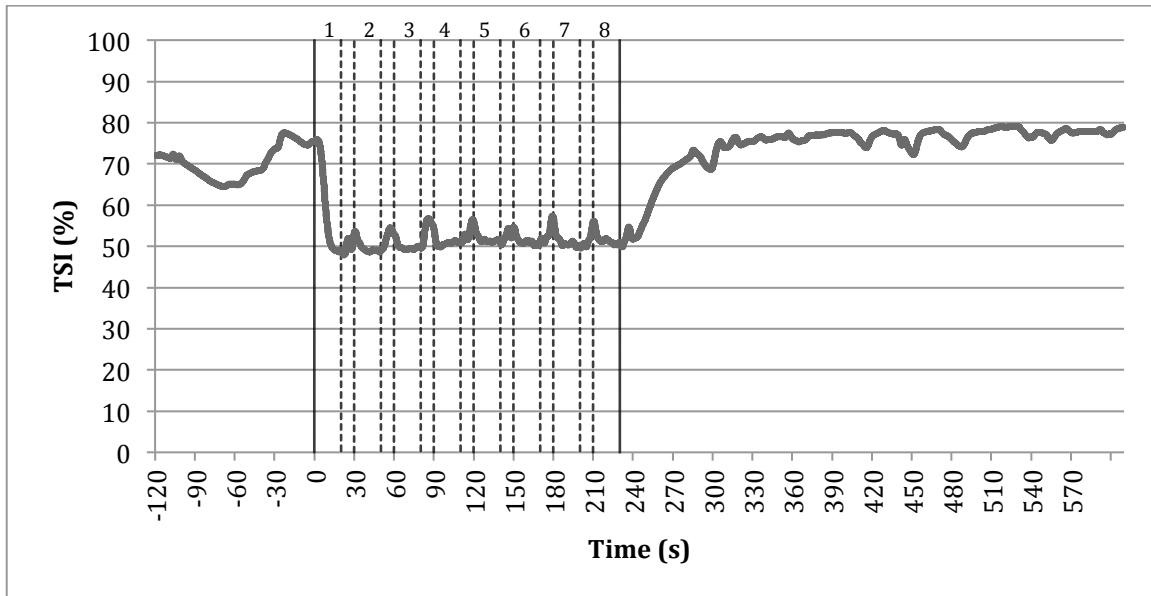
Table 2

Mean (SD) and Total Number of Squats Performed and TSI (%) During Each of Eight 20s Intervals (n=15)

Interval Number	Squats in 20s	TSI (%)
1	20 (2.2)	60.5 (4.0)
2	19 (2.2)	53.6 (5.8)
3	19 (2.1)	53.9 (5.4)
4	18 (2.2)	54.6 (5.3)
5	18 (2.3)	55.4 (5.8)
6	18 (2.3)	55.5 (5.8)
7	17 (2.6)	55.6 (6.1)
8	18 (2.6)	54.7 (5.8)
Total	146 (17.3)	55.8 (5.3)

Figure 4a and 4b show oxygen consumption ($\text{ml}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$) over the course of both the HIIT-squats and MOD exercise, respectively, in a single representative participant. Mean (SD) VO_2 was not significantly different between HIIT-squats ($31.4 (4.5) \text{ ml}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$) and MOD ($33.7 (5.7) \text{ ml}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$; $p > 0.05$). It is important to note that VO_2 was continuously monitored during MOD, and workload was adjusted to maintain intensity as close as possible to $65\% \text{VO}_{2\text{max}}$ throughout exercise. Actual mean workload during MOD was $59.1 (2.7)\% \text{VO}_{2\text{max}}$.

a)



b)

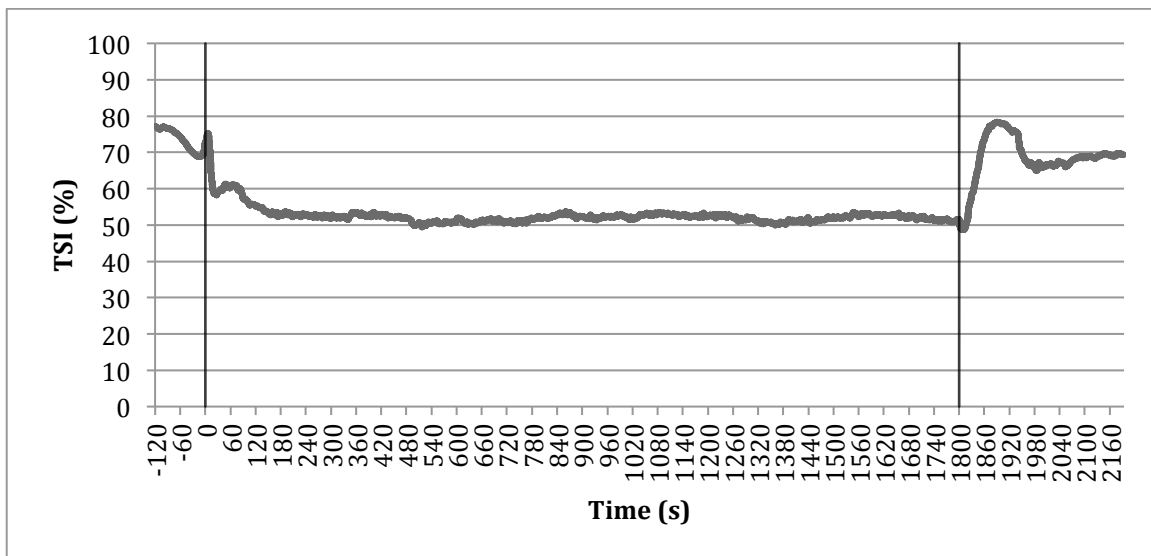


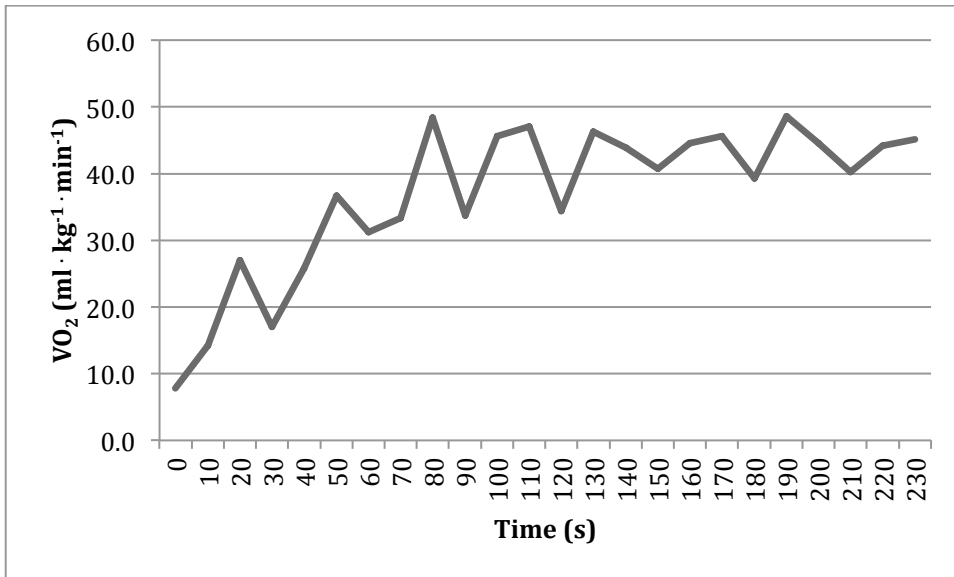
Figure 3

TSI (%) Response to (a) HIIT-squats and (b) MOD in a Representative Participant.

Exercise begins at 0 seconds on the horizontal axis. Solid lines represent the start and end of exercise, and dashed lines separate work and rest intervals in HIIT-squats.

Work Intervals during HIIT-squats are labeled 1-8.

a)



b)

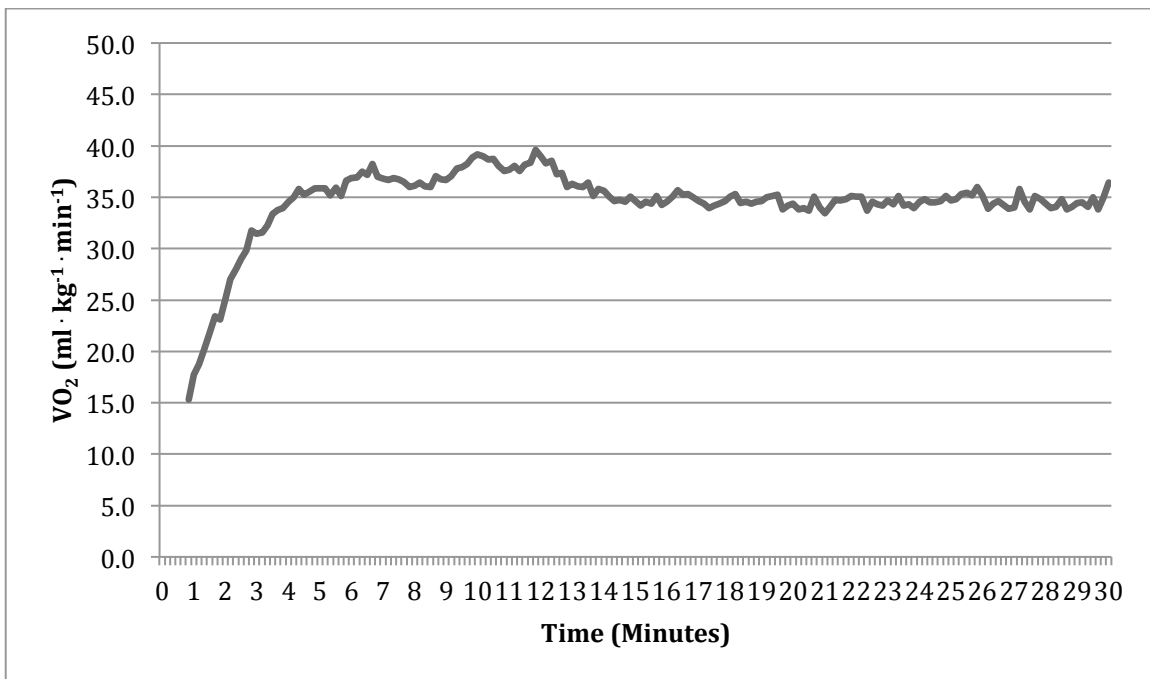


Figure 4

VO₂ Response to (a) HIIT-squats and (b) MOD in a Representative Participant.

Muscle Oxygenation

Exercise Response

Vastus lateralis muscle oxygenation responses to HIIT-squats and MOD exercise are reported in Table 3. Statistical analysis revealed no significant difference in baseline TSI_{mean} prior to the start of MOD and HIIT exercises ($p > 0.05$). The mean change in oxygenation (ΔTSI_{mean}) was -14.6 (5.3)% in HIIT and -13.2 (5.6)% in MOD and did not differ significantly between exercise conditions. The largest observed change in vastus lateralis muscle oxygenation relative to resting values (ΔTSI_{min}) was -19.7 (5.2)% during HIIT and -18.2 (7.6)% for MOD and was not significantly different between exercise conditions. The TSI during the final one second of exercise (TSI End Exercise) was also not significantly different between HIIT and MOD.

In comparison to tissue oxygenation assessed during $VO_{2\text{max}}$ ($n = 14$), ΔTSI_{min} tended to be greater in $VO_{2\text{max}}$ (-22.2 (7.7)%) than in HIIT (-19.7 (5.2)%) although the difference was not significant ($p > 0.05$). TSI End Exercise was also not significantly different between HIIT (54.0 (5.9)%) and $VO_{2\text{max}}$ (55.0 (7.7)%).

Table 3

Mean (SD) Muscle Oxygenation Responses to HIIT-squats, MOD, and VO_{2max} Exercise (n=15 unless otherwise noted).

Variable	HIIT-squats	MOD	VO_{2max}¹
Baseline TSI _{mean} (%)	70.4 (4.9)	70.8 (5.7)	73.3 (4.3)
Exercise TSI _{mean} (%)	55.8 (5.3)	57.6 (5.4)	-
Δ TSI _{mean} (%)	-14.6 (5.3)	-13.2 (5.6)	-
TSI _{min} (%)	50.7 (5.6)	52.6 (7.4)	51.1 (4.9)
Δ TSI _{min} (%)	-19.7 (5.2)	-18.2 (7.6)	-22.2 (7.7)
TSI End Exercise (%)	54.0 (5.9)	55.9 (6.5)	55.0 (7.7)
Recovery TSI _{peak} (%)	78.2 (4.3)	77.0 (5.2)	77.4 (7.9)
T _{1/2TSI} (s)	25 (7.9)	25 (9.6)	27.7 (10.7)

¹(n=14)

Muscle Reoxygenation Response

Vastus lateralis muscle reoxygenation responses after the end of HIIT-squats and MOD exercise are displayed in Table 3. Main variables of interest were peak TSI observed during the first three minutes of post-exercise recovery (TSI_{peak}) and the time required for half of the peak TSI recovery following exercise (T_{1/2TSI}). No significant differences in TSI_{peak} or T_{1/2TSI} were observed between HIIT-squats and MOD exercise ($p > 0.05$). Additionally, TSI_{peak} (77.4 (7.9)%) and T_{1/2TSI} (27.7 (10.7)s) in the VO_{2max} test (n = 14) were not significantly different from those observed following HIIT-squats.

Cardiorespiratory Responses to Exercise

Mean values for absolute and relative VO_2 , minute ventilation (VE) and respiratory exchange ratio (RER) during HIIT-squats and MOD are displayed in Table 4. No differences were found between HIIT-squats and MOD for either absolute VO_2 or relative VO_2 ($p > 0.05$). However, both VE ($p = 0.001$) and RER ($p = 0.001$) were significantly higher in HIIT-squats compared to MOD. Mean VO_2 during HIIT-squats was 55.8 (8.2)% VO_{2max} , and the mean of the highest VO_2 corresponded to 79.9 (9.4)% VO_{2max} .

Table 4.

Mean (SD) Cardiorespiratory Responses During HIIT-squats and MOD Exercise (n=15).

Variable	HIIT-squats	MOD	p-value
VO_2 ($l \cdot \text{min}^{-1}$)	2.5 (0.3)	2.7 (0.4)	.19
VO_2 ($\text{ml} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$)	31.5 (4.6)	33.8 (5.7)	.24
VE ($l \cdot \text{min}^{-1}$)	87.6 (18.5)	66.6 (9.65)	.001*
RER	1.07 (.06)	0.92 (.04)	.001*

* $p < 0.05$

Blood Lactate Response to Exercise

Blood lactate values assessed pre-, and one, three, and five minutes post HIIT-squats and MOD exercise are shown in Table 5. Blood lactate levels did not differ significantly between the two exercise conditions at baseline, but were

significantly higher in HIIT-squats at one minute ($p = 0.003$), three minutes ($p = 0.001$) and five minutes ($p = 0.001$) post-exercise.

Table 5

Mean (SD) Blood Lactate Data Measured Immediately Pre- and One, Three, and Five Minutes Post HIIT-squats and MOD Exercise. (n=15 unless otherwise noted)

Measure (mmol·L⁻¹)	HIIT-squats	MOD	p-value
Pre	2.3 (1.8)	1.9 (1.2)	.52
1 min Post	8.4 (3.9) ¹	4.4 (2.3) ¹	.003*
3 mins Post	9.5 (2.9)	3.8 (2.5)	.001*
5 mins Post	9.0 (4.4) ¹	2.9 (1.3) ²	.001*

* $p < 0.05$; ¹(n=14); ²(n = 12)

Chapter 4 Discussion

The purpose of this study was to investigate the metabolic and physiological demands of a HIIT bodyweight squat protocol (HIIT-squats) by measuring the associated muscle oxygenation and cardiorespiratory responses in healthy, active males. A secondary purpose was to compare these responses with those measured during an acute bout of continuous moderate intensity exercise on a cycle ergometer (MOD) and the responses measured during a stepwise incremental cycling test to exhaustion (VO_{2max}). The main findings were that HIIT-squats were supported by increased aerobic metabolism, as demonstrated by elevated whole body oxygen consumption (VO_2) and decreased muscle oxygenation (TSI) at the vastus lateralis muscle. Markers of aerobic energy metabolism including VO_2 , changes in TSI during exercise, and post-exercise reoxygenation ($T_{1/2TSI}$) were similar for both HIIT-squats and MOD. Furthermore, $T_{1/2TSI}$ and peak changes in TSI were similar between HIIT-squats and VO_{2max} . Evidence of significant anaerobic energy metabolism, including elevated blood lactate and RER, was observed in HIIT-squats, but not MOD. These results suggest that HIIT-squats involved a combination of both aerobic and anaerobic metabolic contributions, while MOD relied primarily on aerobic energy production.

Training programs involving different variations of HIIT have been shown to induce health benefits that are similar to those seen following longer duration endurance-type training (END) (Gibala et al., 2006; McRae et al., 2012). The HIIT-squats protocol investigated in this study has become a commonly used component of strength and conditioning programs in recent years. These programs often utilize

movements traditionally associated with resistance training or calisthenics (i.e. squats) performed at a very high intensity, but for a short duration, to stress multiple energy systems, and improve both aerobic power and muscular fitness (i.e. strength & endurance) simultaneously (Smith, Sommer, Starkoff, & Devor, 2013). Indeed, others have previously reported significant improvements in maximal aerobic power, muscular endurance, and anaerobic exercise capacity following training programs involving the unique low volume HIIT protocol (LV-HIIT) popularized by Tabata et al. (1996) and used in the present study (Ma et al., 2013; McRae et al., 2012). Despite these findings, data are currently limited on the acute physiological responses and energy metabolism that occur during HIIT, and particularly during LV-HIIT (Buchheit et al., 2012). A greater understanding of these responses is required to comprehend how single bouts of LV-HIIT eventually lead to the important health and fitness adaptations that have previously been observed. To our knowledge, the current study is the first to measure the acute metabolic requirements and physiological responses associated with HIIT-squats.

Muscle Oxygenation Responses

i. HIIT-squats Exercise Response

TSI reflects the dynamic balance between O_2 supply and O_2 consumption, and provides an accurate measure of the oxygenation changes at the anatomical area of investigation (Ferrari et al., 2011). During HIIT-squats, TSI began to decrease upon the initiation of exercise, reached minimum values at a mean time of 71.2 (95.2) seconds, and remained low for the duration of exercise (Figure 3a and Table 2). It

has been previously demonstrated that as exercise intensity increases, O_2 consumption in the exercising muscles exceeds O_2 supply, resulting in a decreased oxygenation level in muscle tissue (Belardinelli, Barstow, Porszasz, & Wasserman, 1995a; Bhambhani, 2004). Regarding high-intensity exercise specifically, muscle oxygenation begins to decrease at the onset of exercise, and remains low as long as the intensity of exercise is maintained. The degree of muscle deoxygenation, and therefore the degree to which Hb and Mb become desaturated, testifies to the imbalance of O_2 supply and O_2 demand. Greater deoxygenation indicates a strong O_2 demand and therefore aerobic metabolism (mitochondrial ATP production), even at the onset of very low volume, high intensity exercise (Chance, Dait, Zhang, Hamaoka, & Hagerman, 1992; Nioka et al., 1998).

A unique aspect of our study was the application of the bodyweight squat to a LV-HIIT protocol. The squat is typically considered a resistance training exercise, and although NIRS data on resistance training is limited, the oxygenation response to squats has previously been explored. During four sets of either low-rep, high-load squats (four repetitions at 90% one-repetition maximum (1-RM)) or high-rep, low-load squats (15 repetitions at 60% 1-RM) performed by resistance trained men, Hoffman et al. (2003) observed significant deoxygenation at the vastus lateralis. However, no significant difference was observed between the two squat protocols ($p > 0.05$). The present results demonstrate that squats performed with no external load, but at maximal cadence, also lead to substantial tissue deoxygenation at the vastus lateralis muscle. Hoffman et al. (2003) were the first to quantify the extent of muscle deoxygenation during squatting exercise, and noted that deoxygenation

values were similar to those previously observed during performance of a 30-second Wingate test (Nioka et al., 1998), a test which is often performed repeatedly in traditional HIIT protocols.

Muscle oxygenation patterns have been previously described during cycle- and run-based traditional HIIT protocols (Buchheit et al., 2012; Racinais et al., 2007; Smith & Billaut, 2010). Similar to these reports, our data showed that HIIT-squats were associated with substantial changes in TSI at the vastus lateralis, which occurred rapidly at the onset of exercise and plateaued across sets (Figure 3a). Buchheit et al. (2012) described the muscle deoxygenation at the vastus lateralis during traditional HIIT involving six repeated 30-second sprints on a cycle ergometer, interspersed with two minutes of passive recovery. They observed peak tissue deoxygenation during sprints (-26.5 (4.9)%) that was greater than that observed during HIIT-squats ($\Delta\text{TSI}_{\text{min}} = -19.7$ (5.2)%). One reason for this difference may have been the reoxygenation to above pre-exercise resting levels during two minute recovery periods, seen by Buchheit et al. (2012). The reoxygenation was likely partially responsible for the increasing trend in tissue oxygenation changes across sprints, with deoxygenation during the sixth sprint being significantly greater than during the first and second sprints ($p < 0.05$). Nevertheless, both HIIT-squats and traditional HIIT were associated with substantial peak changes in muscle deoxygenation, indicating a high degree of O_2 demand relative to O_2 supply. These results suggest a strong contribution from aerobic energy metabolism, despite the short duration of the exercise bouts.

In contrast to peak changes in TSI, mean Δ TSI throughout the entire exercise session was greater during HIIT-squats than during the previously reported traditional HIIT protocol, likely as a result of the shorter rest intervals. A unique aspect of HIIT-squats was the 2:1 work-to-rest ratio and the 10-s rest intervals popularized by Tabata et al. (1996) and utilized in LV-HIIT research (Ma et al., 2013; McRae et al., 2012). The 10-s rest intervals were much shorter than the two minute intervals used by Buchheit et al. (2012) and the four minute intervals often prescribed in traditional HIIT (Gibala & McGee, 2008). Although TSI tended to increase during the 10-s rest intervals (Figure 3a), mean TSI over the entire course of exercise was not significantly different with (55.8 (5.3)%) or without (55.5 (5.2)%) the rest intervals. On the contrary, Buchheit et al. (2012) reported mean TSI for the entire session, with rest intervals included, as 63.1 (4.6)% compared to mean TSI without rest intervals of 42.8 (5.8)%. These differences between HIIT-squats and traditional HIIT can be explained by the large discrepancy in the duration of the rest intervals used.

Compared to the three minutes of work in the 13 minute protocol presented by Buchheit et al., (2012), the short rest intervals between sets of the HIIT-squats in the current study allowed participants to complete two minutes and 40 seconds of work in a four minute session. Furthermore, the two minutes of active recovery used by Buchheit et al. (2012) was actually half the recovery time commonly used in traditional HIIT research studies (Gibala & McGee, 2008; Gibala et al., 2006). It is likely that similar peak TSI levels are reached in HIIT-squats compared to traditional HIIT, but with a much shorter time commitment (i.e. four minutes vs. 14-

23 minutes). Therefore, the 2:1 work-to-rest ratio is one possible reason why similar aerobic improvements have been observed after LV-HIIT (McRae et al., 2012; Tabata et al., 1996) in comparison to traditional HIIT protocols with long rest intervals (Gibala & Little, 2010).

Although we cannot speculate on a training effect following HIIT-squats, it is generally understood that adaptations following HIIT exercise programs occur at the muscular (or peripheral) level (Macpherson et al., 2011). Since the importance of local hypoxia at the working muscle appears to be necessary for inducing mitochondrial biogenesis (Hoppeler, Vogt, Weibel, & Flück, 2003) and improving muscular oxidative capacity (Daussin, Zoll, Dufour, et al., 2008), the low oxygenation levels observed in response to HIIT-squats may be an important mechanism for inducing physiological adaptations at the muscular level. The shorter rest intervals used for HIIT-squats, relative to traditional HIIT, allow for a similar amount of time spent exercising, and sustained hypoxia of a comparable magnitude throughout exercise. These findings based on a single session of HIIT-squats may help to explain the similar adaptations observed between the two modes of exercise, despite the lower time commitment of LV-HIIT.

ii. HIIT-squats Recovery Response

During high intensity exercise, the respiratory rate of muscle tissue is high due to the metabolic demand for PCr and ATP resynthesis (Chance et al., 1992). Upon completion of exercise, the balance between O₂ supply and O₂ demand begins to change in favour of O₂ supply, allowing for intramuscular PCr and ATP levels to

be restored. The resaturation of Hb and Mb, shown by increased TSI, signifies reoxygenation of the muscle tissue following exercise, and is represented by the half time to maximal recovery ($T_{1/2\text{TSI}}$) (Chance et al., 1992). In the current study, recovery of vastus lateralis muscle oxygenation was monitored for five minutes following HIIT-squats. Upon completion of exercise, TSI increased from exercising levels to values that exceeded those measured during pre-exercise rest (Figure 3a), indicating that O_2 supply to the recovering tissue was greater than O_2 consumption following exercise (McCully & Hamaoka, 2000).

Mean $T_{1/2\text{TSI}}$ following HIIT-squats was similar to that previously observed after 30-s of maximal intensity cycling in male long distance runners with similar aerobic fitness ($VO_{2\text{max}}$ approximately $60 \text{ ml}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$) to the participants of our study (Nagasawa, 2013). On the contrary, $T_{1/2\text{TSI}}$ was much shorter than that observed following both low-rep, high-load squats and high-rep, low-load squats (Hoffman et al., 2003). Hoffman et al. (2003) did report a delay before the start of reoxygenation that was significantly longer following the high-rep, low-load squats, and hypothesized that this was due to the longer exercise duration, since effort was maximal in both protocols. Our results do not support this hypothesis, since $T_{1/2\text{TSI}}$ was shorter following HIIT-squats despite the maximal effort given and the longer duration of exercise in comparison to both protocols used by Hoffman et al. (2003). Although previous research has demonstrated that $T_{1/2\text{TSI}}$ is correlated with aerobic enzyme activity and can be used as a marker for aerobic metabolism (Chance et al., 1992; Pereira, Gomes, & Bhambhani, 2007), it is clear that further research is needed to determine how mode, duration, and intensity of exercise affect $T_{1/2\text{TSI}}$.

Currently, to our knowledge, this study is the first to report $T_{1/2TSI}$ values following HIIT-squats, with results indicating that the imbalance of O_2 supply and O_2 demand may be similar between HIIT-squats and 30-s maximal effort sprint cycling protocols reported by others.

iii. Comparison to Continuous Moderate Intensity Exercise

a) Exercise Response

As discussed above, it has been previously demonstrated that LV-HIIT programs can improve maximal aerobic power, skeletal muscle oxidative capacity, and exercise performance as effectively as 30-minutes (McRae et al., 2012) or even 60-minutes (Tabata et al., 1996) of END. To date, we are aware of no studies comparing the acute metabolic requirements associated with LV-HIIT and END exercise in the same group of participants.

In the present study, mean and peak ΔTSI values were similar during HIIT-squats and MOD, suggesting that the magnitude of the release of O_2 by Hb and Mb for aerobic energy production was similar during both exercise bouts. Since the duration of the MOD was much longer than the HIIT-squats (30 minutes vs. four minutes, respectively), it is unlikely that mean ΔTSI values during HIIT-squats would explain the similar aerobic adaptations previously observed following END and LV-HIIT. Additionally, since peak ΔTSI values were also similar between HIIT-squats and MOD, it appears that the higher work intensity of HIIT-squats did not result in significantly greater peak demands on aerobic metabolism at the muscular level compared to MOD. Despite these similarities, it is interesting to note that the

TSI response was much faster during HIIT-squats than MOD, since TSI_{min} was reached significantly faster in HIIT-squats. Contrary to the belief that energy at the start of maximal intensity exercise would come primarily from glycolytic sources, these results support previous findings that O_2 is utilized immediately upon initiation of maximal effort exercise, and to a large degree, as demonstrated by decreased TSI values (Nioka et al., 1998).

To our knowledge, only one other study has examined muscle oxygenation during single sessions of intermittent and continuous exercise performed by the same participants (Christmass, Dawson, Passeretto, & Arthur, 1999). Five healthy, physically active males performed 10 minutes of intermittent running (12s running: 18 seconds rest) at a treadmill speed equivalent to 120% VO_{2max} , and 10 minutes of continuous running at a speed equivalent to 60% VO_{2max} . Similar to our findings (Figure 3a & 3b), during continuous moderate intensity exercise, muscle oxygenation reached a steady state once working intensity was achieved, while during intermittent exercise, muscle oxygenation decreased with the onset of work intervals and increased slightly during rest intervals. Contrary to our findings, Christmass et al. (1999) reported greater peak deoxygenation during intermittent intense exercise compared to continuous submaximal exercise. However, these results should be interpreted with caution due to the very low sample size ($n=5$) and the fact that intermittent and continuous exercise were completed within the same session. It is clear that further research comparing muscle oxygenation during LV-HIIT and END-type exercises is needed to understand how similar adaptations can be achieved following training programs of substantially different durations.

Given the understanding that HIIT may induce changes at the muscular level, NIRS is a valuable tool for this endeavor. The present results indicate that the magnitude of deoxygenation may be similar between LV-HIIT and END-type exercises, and suggest that the faster TSI response to high intensity exercise may be a mechanism for aerobic muscular adaptations.

b) Recovery Response

According to Chance et al. (1992), $T_{1/2TSI}$ can provide valuable information regarding the degree to which O_2 supply and demand are imbalanced. Although it has been suggested that differences in $T_{1/2TSI}$ might be influenced by the type, duration, or intensity of exercise (Nagasawa, 2013), $T_{1/2TSI}$ was not significantly different between HIIT-squats (25 (7.9)s) and MOD (25 (9.6)s). This evidence suggests that the higher intensity associated with HIIT-squats may have compensated for the much shorter exercise duration in comparison to MOD, since it appears that O_2 supply and demand were imbalanced to a similar degree between the two exercise bouts.

To our knowledge, this is one of the first studies to compare muscle oxygenation recovery following HIIT (of any kind) and MOD exercise in the same participants. Chance et al. (1992) previously demonstrated that $T_{1/2TSI}$ increased with intensity of work in a group of elite male rowers exercising on a rowing ergometer. Our results cannot support or refute these findings, since intensity, duration, and mode of exercise were dissimilar between HIIT-squats and MOD. Rather, the present findings can be interpreted in two distinct ways. Firstly, it is

possible that $T_{1/2\text{TSI}}$ is influenced by the degree of muscle deoxygenation that occurs during exercise, and not necessarily by the mode or duration of exercise. Since mean and peak changes in TSI were similar between HIIT-squats and MOD, this would explain why $T_{1/2\text{TSI}}$ was also similar between the two exercise bouts.

Secondly, it is possible that the similar $T_{1/2\text{TSI}}$ values following HIIT-squats and MOD provide indirect evidence of a trade-off between intensity and duration of exercise, leading to similar imbalances of O_2 supply and demand. This is supported by the idea that the duration of exercise necessary to elicit beneficial skeletal muscle adaptations decreases as intensity increases (Dudley, Abraham, & Terjung, 1982). Further research is needed to determine the value of $T_{1/2\text{TSI}}$ as a stand-alone measure following a single exercise session and how it can be used to describe differences between two bouts of exercise.

iv. Comparison to Stepwise Incremental Test to Exhaustion ($\text{VO}_{2\text{max}}$)

a) Exercise Response

During incremental exercise to exhaustion, muscle oxygenation will consistently decrease as work rate increases (Belardinelli et al., 1995a; Bhambhani, Maikala, & Esmail, 2001). Consequently, the pattern of TSI response observed during the $\text{VO}_{2\text{max}}$ test was similar to that observed previously (Spencer, Murias, & Paterson, 2012). Peak changes in TSI ($\Delta \text{TSI}_{\text{min}}$) and TSI at the end of exercise were similar during the $\text{VO}_{2\text{max}}$ test and HIIT-squats, suggesting a similar discrepancy between O_2 supply and demand. Although this was one of the first studies to compare muscle oxygenation responses between HIIT-squats and a $\text{VO}_{2\text{max}}$ test in

the same participants, these findings are consistent with those previously reported by Bhambhani et al (2001), when comparing short duration, high intensity exercise to a VO_{2max} test. In that study, they found no significant difference in muscle oxygenation changes at the vastus lateralis when comparing 30-s and 45-s Wingate tests to a stepwise incremental cycling test to exhaustion. They suggested that the magnitude of O_2 release from Hb and Mb for aerobic energy production at the working muscle was similar in both short duration high intensity exercise and their maximal aerobic test. In accordance with the findings by Bhambhani et al. (2001), our results suggest that aerobic energy production at the vastus lateralis muscle during HIIT-squats was of a similar magnitude to that observed during maximal aerobic power in our participants.

Previous research has suggested that muscle deoxygenation may reach a physiological maximum during an incremental test to exhaustion, as evidenced by a plateau in muscle oxygenation despite increasing VO_2 (Belardinelli et al., 1995a). Furthermore, Chance et al. (1992) previously reported that arterial occlusion induced immediately following incremental exercise to fatigue, caused further tissue deoxygenation of only 2-3%. Based on these results, tissue oxygenation levels at the end of an incremental exercise test to exhaustion have been used to approximate maximal levels of tissue deoxygenation (Belardinelli, Barstow, Porszasz, & Wasserman, 1995b; Bhambhani, Buckley, & Susaki, 1997; Bhambhani et al., 2001). Since no arterial occlusions were performed during the present study, it is not known whether TSI levels at the end of the VO_{2max} test were representative of maximal muscle tissue deoxygenation in this set of participants. However, since all

participants achieved the criteria for reaching or attaining VO_{2max} , it is possible, based on previous findings, that they also reached maximal levels of exercise-induced muscle tissue deoxygenation. Furthermore, since ΔTSI_{min} was not significantly different between HIIT-squats and the VO_{2max} test, it is possible that maximal levels of exercise-induced deoxygenation were approached during HIIT-squats.

Unlike our findings and those of Bhambhani et al (2001), Nioka et al. (1998) reported that maximal tissue deoxygenation in a group of sprinters was greater during a 30-s Wingate test (80% of maximal deoxygenation assessed by arterial occlusion) than during a step-wise incremental test to exhaustion (40% of max.). However, these results should be interpreted with caution, since two mutually exclusive participant groups were used and participants did not complete both exercise tests. It is very possible that inter-individual differences may have led to variations in performance during the two tests, which could influence interpretation of the results (Bhambhani et al., 2001). Regardless of this methodological limitation, Nioka et al. (1998) did provide clear evidence that substantial aerobic metabolism occurs immediately at the onset of high-intensity exercise and continues to provide energy as long as exercise is maintained.

In addition to muscle oxygenation during exercise, post-exercise recovery ($T_{1/2TSI}$) was also similar between HIIT-squats and VO_{2max} in our study. This provides further evidence that HIIT-squats created a substantial imbalance between O_2 demand and O_2 supply, which was similar to that observed during exercise at maximal aerobic power.

Cardiorespiratory & Blood Lactate Responses

i. HIIT-squats: Evidence of Aerobic Contribution

In concert with the muscle oxygenation responses measured during HIIT-squats, physiological variables including cardiorespiratory and blood lactate responses were also measured. According to the American College of Sports Medicine (ACSM) guidelines, the mean VO_2 during HIIT-squats (55.8 (8.24)% VO_{2max}) fell in the moderate intensity range, while the peak VO_2 values (79.9 (9.44)% VO_{2max}) were considered to be vigorous intensity (Garber et al., 2011). These results, in conjunction with the decrease in muscle oxygenation at the vastus lateralis, highlight the strong aerobic energy contribution during the four minute HIIT-squats protocol. Comparison with previously investigated HIIT protocols provides context for the results. In one of the first studies involving intermittent exercise, Astrand et al. (1960) assessed varied work-rest durations over 60 minutes of cycling exercise by a single individual. Findings of that early study showed that a 30s work:30s rest intermittent cycling protocol, performed at 60 rpm with a load of 6 kg, produced a mean VO_2 of approximately 65% VO_{2max} . Their results provided preliminary evidence of the important role oxidative metabolism plays during intermittent exercise.

Over the last decade, a very specific protocol of repeated Wingate Anaerobic Tests has been applied to examine the chronic and acute effects associated with HIIT (Gillen & Gibala, 2014). As discussed previously, this traditional HIIT protocol most commonly consists of 4-7 bouts of 30-s maximal effort sprints against a pre-determined resistance on a cycle ergometer, interspersed with four minute recovery

periods. Following four cycling sprints using this protocol, Freese et al. (2013) reported VO_2 responses reaching 80% of $\text{VO}_{2\text{max}}$. Additionally, Buchheit et al. (2012) reported peak VO_2 of 90.4 (2.8)% of $\text{VO}_{2\text{max}}$ during six repeated 30-second sprints with a two minute rest interval. The HIIT-squats protocol examined in the present study elicited peak VO_2 values that were similar (Freese et al., 2013) or slightly lower (Buchheit et al., 2012) than those reported during traditional HIIT-training. According to Freese et al. (2013), the brief yet relatively high levels of VO_2 during traditional HIIT were of a sufficient magnitude to potentially contribute to aerobic metabolic and cardiorespiratory adaptations following a training program. The present results cannot be directly linked with a training response. However, it is possible that the high peak VO_2 values measured during HIIT-squats would also be observed during other LV-HIIT protocols, which have been linked to improved aerobic fitness as well as other health benefits (Ma et al., 2013; McRae et al., 2012).

To our knowledge, only one other study has measured cardiorespiratory responses during LV-HIIT. Tabata et al. (1996) reported that VO_2 increased throughout their eight x 20-s:10-s cycling exercise and was reported to almost reach $\text{VO}_{2\text{max}}$. While the actual sprint VO_2 values were not reported, their findings are consistent with the results of the current study since their four minute LV-HIIT protocol elicited a significant peak VO_2 , even with the very low duration of exercise. In addition to these findings, Rozenek et al. (2007) reported on a HIIT protocol that was also based on a 2:1 work-rest ratio. Twelve healthy, physically active men performed repeated 30-s treadmill sprints at a speed corresponding to 100% $\text{VO}_{2\text{max}}$, with 15-s active recovery intervals at a speed corresponding to 50% $\text{VO}_{2\text{max}}$.

Their unique HIIT protocol elicited peak VO_2 values of 96.4 (6.1) % VO_{2max} over an exercise period of approximately 16 minutes. Therefore, it seems that VO_2 can reach very high levels when performing HIIT exercise using a 2:1 work-rest ratio. Notably, however, based on the aerobic adaptations observed in response to LV-HIIT, four minutes of exercise at this work-rest ratio may be all that is needed to induce beneficial aerobic fitness adaptations (Ma et al., 2013; McRae et al., 2012; Tabata et al., 1996).

Although traditional HIIT is described as a time efficient way to incur the same health and fitness benefits as END, the total time commitment is often not substantially different from END, due to the long rest intervals required. The relatively high peak VO_2 values observed during HIIT-squats were comparable to VO_2 values reported during traditional HIIT, but were achieved with a lower exercise duration. Thus, since “lack of time” is the most commonly cited barrier to regular PA (Troost et al., 2002), LV-HIIT may be a more feasible option than traditional HIIT for improving PA participation levels. Further research is needed to determine if the peak VO_2 values observed during HIIT-squats were similar to those that occur during LV-HIIT protocols which have previously been shown to improve aerobic fitness. Nevertheless, high peak VO_2 values discussed earlier highlight the importance of aerobic energy contributions during HIIT-squats. These findings provide indirect evidence supporting the contention that HIIT-squats could potentially be included in a LV-HIIT program designed to improve cardiovascular metabolic health and physical performance.

In previous reports by Tabata et al. (1996) and McRae et al. (2012), aerobic fitness adaptations following four weeks of LV-HIIT were similar to those observed following END training. These findings have meaningful practical implications since END is the type of PA recommended by CSEP to improve health and reduce risk of non-communicable chronic diseases (Blair, 2009; Tremblay et al., 2011). If similar adaptations can be achieved following LV-HIIT and END, it is possible that individuals will be able to improve their health and fitness in a meaningful way, with a very low time commitment.

In the present study, HIIT-squats resulted in a similar mean VO_2 in comparison to MOD, but elicited higher peak VO_2 values. Additionally, RER, VE, and post-exercise blood lactates were all significantly higher in response to HIIT-squats. Although these results cannot be directly related to a training response, they may help explain the aerobic adaptations following the LV-HIIT training program reported by McRae et al. (2013). It is well recognized that oxygen consumption during the initial period of intense exercise does not correspond to energy demand, and up to several minutes may be required before oxygen consumption reaches a steady state level (Åstrand, Åstrand, Christensen, & Hedman, 1960). It is therefore unlikely that HIIT-squats lasting only four minutes would stress the cardiorespiratory system at a sufficient level to stimulate substantial “central” adaptations, such as improved cardiac output (Macpherson et al., 2011). Rather, it is hypothesized that the brief relatively high levels of cardiovascular strain and the sustained local hypoxia at the muscular level observed during HIIT-squats could lead to the “peripheral” changes described above.

ii. HIIT-Squats: Evidence of Anaerobic Energy Contributions

Along with improvements to aerobic fitness, others have previously reported improvements in anaerobic exercise capacity following LV-HIIT (Tabata et al., 1996). This has led researchers to describe a dual training effect following LV-HIIT, whereby improvements are observed in both aerobic and anaerobic fitness following such a training program. These improvements result from demands placed on both aerobic and anaerobic metabolism during acute bouts of exercise, eventually leading to a training effect over time. In addition to the previously discussed increases in aerobic energy metabolism during HIIT-squats, we observed high mean RER (1.07 (0.6)) and post-exercise blood lactate values (9.5 (2.9) mmol·L⁻¹). Both findings provide evidence of high glycolytic activity, and therefore significant anaerobic energy metabolism required to support the work intensity of HIIT-squats.

Others have reported high blood lactate levels during traditional HIIT protocols (Buchheit et al., 2012; Freese et al., 2013). Additionally, Tabata et al. (1996) reported that blood lactate levels during a representative LV-HIIT training session were not significantly different from those observed during a test of maximal anaerobic capacity. Actual blood lactate values from that study were not reported, but this does provide supporting evidence of substantial anaerobic energy contribution in LV-HIIT exercise.

Consistent with the expected pattern of energy metabolism during high intensity exercise, it has been shown that anaerobic metabolism will decrease with subsequent sprints during HIIT exercise (Bogdanis, Nevill, Boobis, & Lakomy, 1996;

Gaitanos, Williams, Boobis, & Brooks, 1993). Furthermore, related decreases in work performed and anaerobic metabolism have previously been observed during traditional HIIT exercise (Freese et al., 2013). As displayed in Table 2, the mean number of squats performed per set tended to decrease slightly from a mean high of 20 (2.27) in the first set to a mean low of 17 (2.63) in the seventh set. It is likely that the small decline in number of squats may have been due to decreased contribution from anaerobic energy producing systems (i.e. PCr and anaerobic glycolysis). Concurrently, VO_2 tended to increase throughout HIIT-squats (Figure 4a), while TSI remained low (Figure 3a), indicating continuous demand on aerobic energy metabolism. Therefore, the high demand placed on both aerobic and anaerobic energy producing systems during HIIT-squats may help support the dual training effect that has previously been demonstrated following an LV-HIIT program.

Additionally, blood lactate results confirmed that MOD did not tax the lactate producing anaerobic system. This supports previous findings of improvements in anaerobic capacity following LV-HIIT, but not END training (Tabata et al., 1996).

Conclusions

The findings of this study highlight the contributions of both aerobic and anaerobic energy metabolism during a LV-HIIT protocol involving bodyweight squats. Although mean VO_2 and muscle oxygenation were similar between HIIT-squats and MOD, HIIT-squats elicited a faster TSI response and peak VO_2 values in the vigorous intensity range. HIIT-squats were also associated with significant contributions from anaerobic energy metabolism, while MOD was not. These

observations provide a foundation for understanding the positive effects on performance and health outcomes that have been observed by others following LV-HIIT programs. The adaptations that have previously been reported, including improvements in maximal aerobic power, skeletal muscle mitochondrial function and exercise performance, are similar to those produced by longer duration continuous endurance training (McRae et al., 2012; Tabata et al., 1996). These findings suggest that cardiovascular fitness and health can be improved with a very minimal exercise time commitment.

The high levels of aerobic and anaerobic energy metabolism observed in response to the HIIT-squats indicate that this protocol could benefit individuals for whom perceived lack of time is a barrier to being regularly physically active. Further advantages of HIIT-squats are that they require no specialized equipment, are cost-free, and may be performed in a small space. Until further research is completed to determine if HIIT-squats can elicit a training effect similar to other LV-HIIT protocols, this exercise may be best utilized as a compliment to continuous endurance training. Thus, HIIT-squats may be an effective training protocol to add to exercise programs designed to improve aerobic health and fitness, especially for those who are lacking the time and/or resources to commit to regular physical activity.

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Appendix A Review of Literature

The current worldwide epidemic of non-communicable chronic diseases (NCDs) such as cardiovascular disease, diabetes and cancer is troubling. NCDs presently account for approximately 65% of deaths worldwide (Blair, Sallis, Hutber, & Archer, 2012), with that statistic expected to continue growing to 75% by 2030 (Blair, 2009). Despite these disturbing public health statistics, research has shown that there are five leading risk factors which are directly associated with NCDs, and they are all preventable. These risk factors are smoking, obesity, high blood pressure, high blood glucose and physical inactivity (Héroux et al., 2012). In recent years, the immense importance of physical activity (PA) has become better understood as researchers and health care professionals search for ways to reverse the swelling prevalence of NCDs.

It was previously believed that PA could decrease the risk of developing NCDs primarily by improving other risk factors (i.e. obesity), but it has recently been established that lack of PA is an independent risk factor (Blair, 2009). In a report on the Aerobics Centre Longitudinal Study (ACLS), an epidemiological study which focused on lifestyle exposures and long-term follow-ups on morbidity and mortality in men, it was determined that the risk of all-cause mortality was significantly greater for inactive men, compared to active men (Byun et al., 2010). Furthermore, it has been reported that 9.4% of deaths observed during follow-up of ACLS participants were a result of low fitness (due to lack of physical activity), compared to 2.9% from unhealthy diet, 3.7% from smoking tobacco, and 0.6% from heavy drinking (Héroux et al., 2012). These findings highlight the importance of

physical inactivity as an independent risk factor for morbidity and mortality. In addition, physical inactivity is also directly associated with three of the four previously mentioned major risk factors (obesity, high blood pressure and high blood glucose) making it a potent contributor to poor quality of life and increased NCD risk (Blair et al., 2012; Blair, 2009).

Despite the overwhelming evidence supporting regular PA, the PA habits and fitness levels of Canadians have been steadily declining over the past few decades, with most Canadians still failing to meet recommended guidelines (Colley et al., 2011; Tremblay et al., 2011). Recently, in a landmark study, the Canadian Health Measures Survey (CHMS) collected accelerometry data to objectively measure PA levels in a nationally representative sample of Canadians (Colley et al., 2011). The results determined that only 15% of adults were meeting recommended levels of weekly PA, with only 5% meeting guidelines by participating in regular purposeful exercise throughout the week. Furthermore, it was demonstrated that 63% of adults accumulate at least 15 minutes of moderate to vigorous PA (MVPA) at least one day per week, meaning that 37% fail to even meet this unexceptional level of activity (Colley et al., 2011).

Thus, despite the obvious benefits of PA, there appears to be a substantial gap between the actual PA habits of Canadians, and the recommended level to achieve health benefits. Identification of the barriers causing this disconnect is an important step in the process of educating Canadians about the benefits of PA and eventually encouraging a more active lifestyle. Numerous personal, social and environmental factors can influence PA behaviour, but “lack of time” has

consistently been reported as the number one barrier to regular PA (Godin et al., 1994; Trost, Owen, Bauman, Sallis, & Brown, 2002). Current public health guidelines for Canadians constructed by the Canadian Society of Exercise Physiology (CSEP) recommend greater than 150 minutes of purposeful moderate intensity aerobic exercise each week in order to achieve the benefits of aerobic exercise, and an additional 2-3 sessions of resistance training to enhance muscular fitness (Tremblay et al., 2011). These recommendations are based on robust, evidence-based scientific studies and are similar to international guidelines put forth by the American College of Sports Medicine (ACSM) (Garber et al., 2011) and the World Health Organization (WHO, 2010). For many people, these guidelines represent substantial time requirements that clearly cannot be met, and thus it may not be surprising that many people fail to meet recommended PA levels. Future research needs to account for this discrepancy, and an important step may be the consideration of new and innovative exercise prescription techniques.

High-Intensity Interval Training (HIIT)

Recently, HIIT has become popular as a means for individuals to achieve the substantial health benefits of regular aerobic exercise, with a diminished time and volume commitment (Gillen & Gibala, 2014). HIIT involves repeated bouts of brief intermittent exercise performed at an “all-out” level of intensity and interspersed with periods of rest or low-intensity exercise (Gibala, 2009). In young, healthy individuals, HIIT has been shown to improve maximal aerobic power (VO_{2max}) (Burgomaster et al., 2007, 2008) insulin sensitivity (Babraj et al., 2009; Metcalfe,

Babraj, Fawkner, & Vollaard, 2012; Richards et al., 2010), cardiovascular and autonomic function (Heydari, Boutcher, & Boutcher, 2013) and body composition (Heydari, Freund, & Boutcher, 2012; Trapp, Chisholm, Freund, & Boutcher, 2008). Moreover, despite common misconceptions, HIIT research has not been restricted to young healthy individuals. Different forms of HIIT have been investigated in studies with various at-risk populations including overweight/obese individuals (Gillen, Percival, Ludzki, Tarnopolsky, & Gibala, 2013; Heydari et al., 2012; Whyte, Gill, & Cathcart, 2010) middle-age sedentary adults (Hood, Little, Tarnopolsky, Myslik, & Gibala, 2011), patients with coronary artery disease (Currie, Dubberley, McKelvie, & MacDonald, 2013) and individuals living with type 2 diabetes (T2D) (Little et al., 2011). This vast collection of encouraging results and diverse study samples clearly shows the prominence of HIIT research and the potentially wide-reaching benefits of HIIT.

Recently, researchers reported on a direct comparison between HIIT and traditional endurance training (END) using a research design that matched groups with respect to exercise mode, training frequency and program duration, but differed in terms of total training volume and time commitment (Gibala et al., 2006). In order to examine and compare the effects of HIIT and END on changes in exercise capacity and molecular and cellular adaptations in skeletal muscle, researchers designed two different training programs comprised of six training sessions over 14 days. HIIT consisted of six 30-s “all-out” sprints on a cycle ergometer interspersed with four minute recovery periods, while the END sessions consisted of 90-120 minutes of continuous cycling at an intensity corresponding to 65% VO_{2max} . Upon

conclusion of the training program, the HIIT and END groups demonstrated similar improvements in muscle oxidative capacity, muscle buffering capacity and exercise performance (Gibala et al., 2006). Importantly, these similar results were observed despite the fact that the total training volume of the HIIT group was only about 10% that of the END group.

The previously mentioned study is one of many which have used a cycling HIIT protocol, now commonly referred to as “traditional HIIT”, however research has also focused on running, which can be a more accessible modality of exercise, given that no equipment or gym membership is required. In a recent study, researchers sought to determine if a running HIIT protocol would produce similar adaptations as running END and to document any chronic effects on body composition, run time trial performance, VO_{2max} , maximal cardiac output (Q_{max}), and maximal arterial-mixed venous oxygen difference (a-vO₂ difference) among other variables (Macpherson, Hazell, Olver, Paterson, & Lemon, 2011). It was hypothesized that the HIIT intervention would result in similar aerobic performance adaptations, with the exception of maximal cardiac output (Q_{max}). Since VO_{2max} is the product of Q_{max} and a-vO₂ difference (Blomqvist & Saltin, 1983; Clausen, 1977), the researchers proposed that any observed increases in VO_{2max} would be due to improvements in a-vO₂ difference, and thus would be occurring at the peripheral (i.e. muscular) level. In order to test this hypothesis, researchers prescribed a HIIT protocol consisting of repeated 30-s maximal running efforts interspersed with four minute rest intervals. The END group performed 30-60 minutes of continuous running on a treadmill at an intensity of 65% VO_{2max} . Each training program

consisted of three training sessions per week for six weeks, with the total exercise volume being .75 hours for the HIIT group (6.75 hours including rest intervals) and 13.5 hours for the END group (Macpherson et al., 2011).

Upon completion of the training programs, the researchers' hypothesis was confirmed, as HIIT resulted in similar body composition, exercise performance, and VO_{2max} improvements as END. Furthermore, END elicited a 9% improvement in Q_{max} while HIIT had no effect. Concurrently, HIIT improved $a-vO_2$ difference by 7.1% while END had no effect. The researchers concluded that the HIIT protocol used in the study was likely of insufficient duration to increase Q_{max} , but still led to an increase in VO_{2max} via improved $a-vO_2$ difference (Macpherson et al., 2011). The improvements in body composition, aerobic performance and VO_{2max} using a short-term HIIT program, with a total six week time commitment of 6.75 hours, demonstrate the potential of HIIT as an effective exercise program for the general public, especially those who lack the time to devote to prolonged aerobic exercise. Furthermore, the finding that these adaptations occurred primarily at the peripheral level provide insight into the mechanisms that underlie chronic adaptations to HIIT, and offer a direction for further study of the acute metabolic and physiological responses that lead to these adaptations.

Clearly, HIIT can be effective at improving health and fitness across a wide range of individuals, however there are some considerations to be made about the research thus far. This type of training often requires specialized equipment, such as an appropriate cycle ergometer for repeated Wingate-style tests, and certainly requires a high degree of participant motivation, which may be lacking, particularly

in a sedentary population. Accordingly, research into modified HIIT protocols that are more accessible to a wide range of individuals is warranted (Gillen & Gibala, 2014).

Low-Volume HIIT

Currently, one of the main issues with traditional HIIT is that the maximal effort exerted during 30-s sprints requires long rest intervals between sprints. For this reason, some HIIT protocols require exercise sessions of up to 23 minutes (Gibala et al., 2006), which is not much shorter than the suggested 30 minute bouts of END type training that Canadians are unable to find time for (Colley et al., 2011). Interestingly, a number of studies have reported on a low-volume HIIT protocol (LV-HIIT) involving eight x 20-s all-out exercise intervals, interspersed with 10-s rest intervals, resulting in a four minute exercise protocol (Tabata et al., 1996) that is much shorter than both END and traditional HIIT.

In the original report involving LV-HIIT, Tabata et al. (1996) created two experiments. The first was a six week END training program involving cycling exercise five days per week, for a duration of one hour each day at an intensity of 70% VO_{2max} . The second experiment was also a six week training program, although this time participants performed LV-HIIT on a cycle ergometer, four times per week, at an intensity of approximately 170% VO_{2max} . Additionally, one day per week the subjects exercised for 30 minutes at an intensity of 70% VO_{2max} before carrying out four sets of the intermittent exercise. VO_{2max} and anaerobic capacity (maximal

accumulated O₂ deficit during 2-3 minute exhaustive cycling exercise) were assessed before, during and after the training programs (Tabata et al., 1996).

After the six weeks of aerobic training, VO_{2max} was improved by five ml·kg⁻¹·min⁻¹, but anaerobic capacity did not change. Meanwhile, after six weeks of training using LV-HIIT, VO_{2max} improved by seven ml·kg⁻¹·min⁻¹ and anaerobic capacity increased by 28%. The difference in VO_{2max} improvements between the two groups was not significant. Surprisingly, LV-HIIT improved aerobic power to the same extent as END, with the added benefit of improved anaerobic capacity, and with a significantly reduced time commitment. Researchers concluded that the LV-HIIT protocol used in the study may be optimal for improving both the aerobic and anaerobic energy releasing systems (Tabata et al., 1996).

Recently, there has been further interest in LV-HIIT, as lack of PA amongst Canadians has become apparent, and researchers explore new time-efficient exercise programs. One recent study was able to reproduce the findings of Tabata et al (1996), with a four day/week training program lasting four weeks (Ma et al., 2013). Eight active male participants trained using only LV-HIIT, resulting in a training program with a total weekly volume of 16 minutes, including rest intervals. Results showed that participants' VO_{2max} improved from 40.5 ± 3.8 ml·kg⁻¹·min⁻¹ to 43.4 ± 2.5 ml·kg⁻¹·min⁻¹ after two weeks of training (p < 0.05), and 47.2 ± 2.9 ml·kg⁻¹·min⁻¹ upon conclusion of the four week program (p < 0.05). Additionally, Wingate mean power also significantly improved from 701.0 ± 73.0 W, to 745.5 ± 73.3 W after two weeks (p < 0.05), and 786.8 ± 80.0 at post-testing (p < 0.05). Wingate peak power also improved significantly from 975 ± 114 W at baseline to 1095 ± 166

W after four weeks (+12%, $p < 0.05$). Furthermore, certain skeletal muscle mitochondrial proteins (i.e. COX, COX IV) were significantly elevated post-training ($p < 0.05$), supporting previous findings that improved VO_{2max} following HIIT may result from peripheral adaptations (Macpherson et al., 2011).

These results support the findings of Tabata et al. (1996) and represent a strong example of the intensity-duration trade-off, whereby the duration of exercise necessary to elicit beneficial skeletal muscle adaptations decreases as intensity increases (Dudley, Abraham, & Terjung, 1982). It is possible that LV-HIIT could represent the minimum volume of exercise needed for aerobic training adaptations, with the additional benefit of improvements in anaerobic fitness.

LV-HIIT and Aerobic-Resistance Training

An often overlooked aspect of CSEP guidelines is the suggestion of performing muscle and bone strengthening activities at least two days per week (Tremblay et al., 2011). This represents additional time that needs to be devoted to PA, on top of the recommended 150 weekly minutes of aerobic exercise, further reducing the chances that individuals will complete enough regular PA to achieve health benefits. It was noted by Colley et al. (2011) that accelerometers are unable to accurately capture resistance training (RT) exercise, although walking is far more common than weight training in Canadian adults. Since, as previously discussed, most people are not meeting aerobic PA guidelines, it is likely that most people are also failing to meeting recommendations for muscle and bone-strengthening exercises.

Although most HIIT studies thus far have focused primarily on traditional aerobic exercise modalities such as cycling and running, there has been interest in applying HIIT principles to exercises that are more often associated with RT or calisthenics. Recently, one group of researchers identified the potential to combine LV-HIIT and whole-body aerobic-resistance exercises to create both a potent aerobic stimulus and a form of RT (McRae et al., 2012). Researchers hypothesized that by applying LV-HIIT to whole-body exercises, an aerobic training effect as well as a RT effect (i.e. improved muscular endurance) would be observed. Twenty-five recreationally active, university-aged females were randomly assigned to one of three training programs: a LV-HIIT group, an END group, or a control group. The duration of the intervention was four weeks, with four sessions each week, totaling 16 exercise sessions. The LV-HIIT group completed one single set of whole-body aerobic-resistance training intervals (eight \times 20 s intervals separated by 10 s of rest) on each training day. A different exercise (burpees, mountain climbers, jumping jacks or squat thrusts) was performed on each of the four training days and participants were asked to perform as many repetitions as possible during each work interval. The END group completed 30 minutes of treadmill running at \sim 85% HR_{max} , and the control group was given no intervention. The total exercise time each week was 16 minutes for the LV-HIIT group compared to 120 minutes for the END group. VO_{2max} , anaerobic exercise capacity (Cunningham-Faulkner test), and numerous muscular endurance tests were conducted pre- and post-training.

Upon completion of the training programs, the LV-HIIT protocol improved VO_{2max} to the same degree as END. Furthermore, LV-HIIT improved lower-body,

upper-body, and core muscular endurance while END had no effect (McRae et al., 2012). No adaptations were seen in the control group. These findings demonstrated that the 16-minute weekly time commitment of the LV-HIIT protocol was sufficient enough to improve aerobic capacity to a similar degree as END with the additional benefit of improved muscular endurance. Clearly, these results contrast considerably with the minimum requirements for achieving the health benefits of PA, as defined by CSEP (Tremblay et al., 2011). The promising results of this study require that the aerobic-resistance training used in this LV-HIIT protocol receive further research. This very minimal time commitment would certainly overrule the “lack of time” excuse and could inform the development of PA programs designed to elicit improvements in aerobic health and fitness with a minimal time commitment.

HIIT: Acute Metabolic and Physiological Responses

To date, there is limited research regarding the acute physiological responses and metabolic requirements of HIIT. In one of the first studies involving intermittent exercise, Astrand et al. (1960) assessed varied work-rest durations over 60 minutes of cycling exercise by only one individual, and found that a 30s work: 30s rest HIIT cycling protocol produced a mean VO_2 of approximately 65% VO_{2max} . Those results provided preliminary evidence of the important role of oxidative metabolism during intermittent exercise. More Recently, Rozenek et al. (2007) reported on three treadmill HIIT protocols in 12 healthy, physically active men which consisted of work-rest intervals of 15s:15s, 30s:15s and 60s:15s. Work intervals were performed at the velocity corresponding to VO_{2max} and active rest

intervals were performed at 50% of VO_{2max} velocity. Mean/peak values for % VO_{2max} were 71.6 (4.2)/78.3 (4.3) for 15s:15s, 84.6 (4.0)/96.4 (6.1) for 30s:15s, and 89.2 (4.2)/100.9 (4.2) for 60s:15s. Duration of exercise was the time required to cover a distance of 2400 meters which was approximately 21 minutes for 15s:15s, 17 minutes for 30s:15s, and 14 minutes for 60s:15s. The 30s:15s protocol proved to be the most beneficial, since VO_2 values were significantly greater than 15s:15s ($p < 0.05$), but similar to 60s:15s ($p > 0.05$). Additionally, exercise tolerance, assessed by RPE, was better in 30s:15s compared to 60s:15s. Rozenek et al. (2007) provided strong evidence of a substantial aerobic response during HIIT, particularly using a 2:1 work-rest ratio, that was similar to the previously discussed LV-HIIT protocol, only 50% longer.

Acute physiological responses to traditional HIIT exercise have also been studied in recent years. Following four maximal effort cycling sprints, Freeze et al. (2013) reported VO_2 responses reaching 80% of VO_{2max} . Additionally, Buchheit et al. (2012) reported mean and peak oxygen consumption of 63.3 (3.0)% and 90.4 (2.8)% of VO_{2max} respectively, during six repeated 30-second sprints with a two minute rest interval. Despite the brief duration of exercise, both studies concluded that responses were of a sufficient magnitude to contribute to aerobic metabolic and cardiorespiratory adaptations following long-term training. Currently, the acute metabolic requirements and physiological responses to LV-HIIT have not been studied.

Along with chronic training studies like the one presented by McRae et al. (2012), acute studies of LV-HIIT are needed to better understand the physiological

mechanisms by which these chronic training adaptations occur. Additionally, since the training adaptations associated with HIIT appear to occur mostly at the peripheral level (Macpherson et al., 2011), examination of the peripheral physiological responses to this exercise modality are necessary.

Near Infrared Spectroscopy

The importance of understanding the mechanisms underlying the physiological adaptations to HIIT at the peripheral level has been established. In recent years, Near Infrared Spectroscopy (NIRS) has become a popular tool to investigate local muscle oxidative metabolism in healthy individuals at rest and during exercise (Bhambhani, 2004; Ferrari, Muthalib, & Quaresima, 2011). One of the major advantages of NIRS, is that it can capture information in real-time during exercise, something that was a major disadvantage of the traditional muscle biopsy technique (Hamaoka, McCully, Quaresima, Yamamoto, & Chance, 2007). NIRS technology first appeared in scientific literature nearly four decades ago as a noninvasive method to measure the presence of oxygen (O_2) in muscle and other tissues in vitro (Jöbsis, 1977). Since then, the technology has evolved considerably and now has significant applications in exercise physiology and clinical medicine (Ferrari et al., 2011).

The most commonly used NIRS technique is the continuous-wave (CW) modality which is based on constant illumination of the tissue, and measurement of the attenuation of light through the tissue (Ferrari et al., 2011). NIR light from the 650- to 950-nm wavelength penetrates the tissue and is absorbed by chromophores

such as oxyhemoglobin/oxymyoglobin (O_2Hb) and deoxyhemoglobin/deoxymyoglobin (HHb) in micromolar concentrations, and thus the concentration of these chromophores in the microvasculature at the area of investigation can be measured by NIRS (Wolf, Ferrari, & Quaresima, 2007). Because NIRS cannot discern between hemoglobin (Hb) and myoglobin (Mb) chromophores, the extent of the contribution which Hb and Mb make to the NIRS signal is presently unclear, and the abbreviations HbO_2 , HHb and tHb refer to the combined signal due to Hb and Mb. Different CW-NIRS methods have been developed to measure the O_2Hb saturation of the muscle (StO_2), one being the spatially resolved spectroscopy (SRS) method. This is the most commonly used oximetry approach (Ferrari et al., 2011) and makes use of multiple source-detector pairs and multiple pathlengths of NIR light (Hamaoka, McCully, Niwayama, & Chance, 2011). Tissue Saturation Index (TSI) is a commonly reported variable which provides an estimation of StO_2 in percentage, and ensures an accurate quantitation of the oxygenation changes occurring at the muscular level. TSI reflects the dynamic balance between O_2 supply and O_2 consumption in the investigated muscle (Ferrari et al., 2011). The high temporal resolution of CW-NIRS methodology (sampling rate up to 100Hz) allows for the measurement of the time course of changes in TSI% during brief leg press exercise (Cettolo, Ferrari, Biasini, & Quaresima, 2007) or even during a single pedal cycle on a cycle ergometer (Binzoni et al., 2010).

The validity of NIRS for measuring muscle oxygen saturation *in vitro* has been established (Belardinelli, Barstow, Porszasz, & Wasserman, 1995; Lin, Lech, Nioka, Intes, & Chance, 2002; Mancini et al., 1994). Furthermore, NIRS

measurements have been shown to be repeatable and reproducible during different movements such as elbow flexor exercise (Muthalib, Millet, Quaresima, & Nosaka, 2010) and cycling exercise (Spencer, Murias, Lamb, Kowalchuk, & Paterson, 2011). Indeed, NIRS has been applied in several different ways to study skeletal muscle physiology, and a significant amount of research has been conducted on its potential role in monitoring exercise prescription (Neary, 2004). Chance et al. (1992) were the first to apply NIRS directly to exercise science when they assessed O₂ supply and utilization, as well as the recovery time for Hb deoxygenation (i.e. reoxygenation) during a simulated 2000m competition on a rowing ergometer. The findings of this study were a) abrupt deoxygenation occurred within the first minute, and then continued to decline at a slower rate until termination of the test, and b) reoxygenation times (recovery from maximal deoxygenation) following exercise increased with work intensity and were prolonged with a higher intensity of exercise (Chance, Dait, Zhang, Hamaoka, & Hagerman, 1992). The researchers suggested that they could use this NIRS-derived information to describe the differences in the physical fitness of subjects and to enhance future athletic performance.

Since then, many studies have employed NIRS methods to assess the muscle oxygenation response to END, RT, and even HIIT. Neary, Hall & Bhambhani (2001) used NIRS to describe the acute muscle oxygenation trends during continuous high-intensity cycling exercise. Ten competitive male cyclists were tasked with completing a simulated 20-km cycling time trial (20TT), during which, NIRS data were collected from the right vastus medialis muscle. Results were reported in

tissue absorbency, an increase in which represents enhanced oxygenation and a decrease in which represents reduced oxygenation levels (deoxygenation). During the 20TT, tissue absorbency decreased rapidly upon the initiation of exercise, indicating immediate initiation of muscle deoxygenation (Neary, Hall, & Bhambhani, 2001). After the first 2-4 km, muscle deoxygenation continued gradually until the cessation of exercise. Tissue absorbency increased rapidly during recovery, indicating reoxygenation of the muscle, however oxygenation levels did not return to baseline within the six minutes of recovery during which NIRS data were collected. These muscle oxygenation trends were consistent with those previously reported during aerobic endurance exercise (Belardinelli et al., 1995) and demonstrated the significant and prolonged deoxygenation that occurs in the muscle during high intensity exercise.

In addition to studies involving END, there have been a limited number of studies which have used NIRS to examine the effect of RT exercises on muscle oxygenation. In one study, a group of resistance-trained males ($n = 11$) performed four sets of weighted squats at both a low-intensity, high-volume (LI; 15 reps at 60% 1-RM) and a high-intensity, low-volume (HI; four reps at 90% 1-RM) in a random order, separated by a minimum of 72 hours (Hoffman et al., 2003). NIRS was used to measure changes in O_2Hb and HHb in the right vastus lateralis (VL) during pre-exercise rest, during exercise, and for three minutes post exercise. Interestingly the muscle deoxygenation responses resulting from each protocol were not significantly different from one another despite the much greater average duration of the LI sets (41.6 (6.6)s vs. 21.4 (3.6)s for the HI sets).

Additionally, there was no difference in the reoxygenation half time after exercise between HI (50.2 (15.5)s) and LI (51.7 (16.8)s).

The results displayed that although the resistance exercise resulted in significant tissue deoxygenation, the intensity and volume of the exercise did not seem to effect the degree of deoxygenation (Hoffman et al., 2003). Although previous research had shown that tissue oxygenation within contracting skeletal muscle would be reduced with RT (Tamaki, Uchiyama, Tamura, & Nakano, 1994), this was one of the first studies to quantify the extent of muscle deoxygenation during RT. This was also the first and only study to use NIRS to assess the muscle oxygenation responses to an exercise protocol involving squats.

Finally, NIRS has also been used to examine muscle oxygenation responses to HIIT. In one experiment, ten experienced cyclists performed six 30-seconds “all-out” sprints on a cycle ergometer separated by two minute rest intervals (Buchheit, Abbiss, Peiffer, & Laursen, 2012). NIRS was used to assess muscle oxygenation levels (TSI) of the vastus lateralis. Researchers observed significant tissue deoxygenation associated with HIIT ($\Delta\text{TSI} = -26.5 (4.9)\%$) and also found that muscle deoxygenation levels and reoxygenation rates increased with sprint repetition ($p < 0.05$). The large observed deoxygenation response serves as one indicator of the high demand on aerobic metabolism during a HIIT session, while the increase in deoxygenation and reoxygenation rates throughout progressive sprints highlighted the increasing demand on the progressively fatiguing muscles (Buchheit et al., 2012).

Conclusion

In summary, the validity, reliability and applicability of NIRS have been demonstrated. Through the use of NIRS technology, researchers have effectively and accurately evaluated the peripheral responses to different types of exercise, including HIIT. Additionally, studies have shown that O₂ consumption by skeletal muscles during exercise is an important factor towards improving muscular oxidative capacities (Daussin et al., 2008) and also that local muscle hypoxia is critical for inducing mitochondrial biogenesis (Hoppeler, Vogt, Weibel, & Flück, 2003). Therefore, investigating the muscle oxygenation responses associated with HIIT may provide further insight into the mechanisms responsible for skeletal muscle metabolic remodeling (Coffey & Hawley, 2007) and provide a better understanding of how the metabolic and physiological responses to a single bout of HIIT may eventually lead to the substantial health benefits that have been previously observed.

Appendix B Participant Consent Form



Participant Consent Form

Muscle Oxygenation response to low volume high intensity aerobic-resistance exercise

You are invited to participate in a study entitled **Muscle oxygenation response to low volume high intensity aerobic-resistance exercise** that is being conducted by Andrew M. Kates (M.Sc. Kinesiology candidate). Andrew Kates is a Graduate Student in the department of Exercise Science, Physical and Health Education at the University of Victoria and you may contact him if you have further questions by phone: 250-580-7275 or email: akates@uvic.ca.

As a graduate student, Andrew is required to conduct research as part of the requirements for a degree in Master of Science: Kinesiology. It is being conducted under the supervision of Dr. Kathy Gaul. You may contact Dr. Gaul at 250-721-8380 or kgaul@uvic.ca.

Purpose and Objectives

The purpose of this research project is to describe the muscle oxygenation response during a low volume high intensity aerobic-resistance exercise session (HIT). A secondary purpose is to compare these responses with those observed during a moderate intensity exercise session performed on a cycle ergometer.

Importance of this Research

Research of this type is important because it will contribute to knowledge of the acute physiological mechanisms of HIT which eventually lead to improvements in health and exercise performance. HIT has been demonstrated as an effective and time-efficient exercise modality contributing to the optimization of health. Furthermore, low volume high intensity aerobic-resistance training has been proposed as a time efficient way to incur gains in aerobic, anaerobic, and muscular health simultaneously and with a minimal time commitment. Research of this type could potentially lead to greater understanding of exercise prescription for the purpose of improving the health of individuals in society.

Participants Selection

You are being asked to participate in this study because you are an apparently healthy male between the ages of 19-50, and are currently involved in a resistance training program, including lower body exercises, for a minimum of the past six consecutive months. Additionally you are currently located near the University of Victoria at which the study is being conducted.

What is involved

If you consent to voluntarily participate in this research, your participation will include attending the Exercise Physiology lab at the University of Victoria on three separate occasions for a total time commitment of 2.5 hours. The first occasion will involve physical testing including height, weight, body composition, and VO_{2max} testing, as well as

familiarization with exercise protocols and will take one hour. You will be asked to attend the lab on two further occasions to take part in two experimental exercise conditions in random order:

1. Exercising on a cycle ergometer continuously for 30 minutes at a moderate intensity. The total duration of this session will be approximately one hour, including warm up and cool down.
2. A single set of bodyweight squat intervals (8x20 seconds (s) intervals separated by 10s of rest) performed with “all-out” effort. The intensity of this exercise is self-determined. There is no minimum or expected work requirement beyond asking you to do your best at each test. The duration of this session will be approximately thirty minutes, including warm up and cool down.

During all experimental sessions heart rate, breath-by-breath metabolic analysis, and muscle oxygenation will be non-invasively monitored at rest, throughout exercise, and for 5 minutes post-exercise. Furthermore, four small finger pricks of blood will be collected during all exercise sessions in order to determine lactate concentration. These finger pricks will be similar to that of a home blood glucose test, with each test involving a single droplet of blood.

You will be asked to refrain from eating for 2 hours prior to testing and to refrain from vigorous physical activity for 24 hours prior to all testing sessions

Inconvenience

Participation in this study may cause some inconvenience to you, including the scheduling of 3 testing sessions, as well as possible temporary physical discomfort during the testing and after, related to the physical effort of the tests. However, this is expected to be no greater than that which you currently experience in your own personal training program. You will also be asked to refrain from eating for 2 hours prior to testing and to refrain from vigorous activity for 24 hours prior to testing.

Risks

There are some small, potential risks to you by participating in this research and they include physical stress/fatigue during testing and a low, but acknowledged risk of injury associated with the activities involved. To prevent or reduce these risks, the following steps will be taken: all testing will be supervised by the lead researcher (an experienced Certified Strength and Conditioning Specialist), proper briefing of tests will take place to minimize discomfort and risk of injury as well as a Physical Activity Readiness Questionnaire (PAR-Q) administration to ensure that the participant is safe to exercise. These risks are deemed to be no greater than those related to your current personal training program.

Benefits

The potential benefits of your participation in this research include gaining knowledge about your personal health and exercise related physical characteristics including VO_{2max} and body composition. Your participation provides the opportunity to experience a high quality physical fitness assessment normally reserved for Olympic and other elite level athletes.

Voluntary Participation

Your participation in this research must be completely voluntary. If you do decide to participate, you may withdraw at any time without any consequences or any explanation. If

you do withdraw from the study your data may be used for statistical analysis within the research project, upon your agreement.

On-going Consent

To make sure that you continue to consent to participate in this research, this form will be returned to you at the start of each testing session. You may then reread the document and decide whether or not you wish to continue with the testing session.

Anonymity

Due to the nature and location of this study, your anonymity cannot be fully protected during data collection. However, all data collected will be analyzed under a code name or reference number so that your data will not be identifiable to anyone other than the primary investigator. All electronic data will be stored in a password protected laboratory computer. All paper data will be stored in a locked filing cabinet belonging to Dr. Gaul, Supervisor in McKinnon Building room 128.

Confidentiality

Your confidentiality and the confidentiality of the data will be protected by a code name or reference number so that your data will not be identifiable anyone other than primary investigator. All electronic data will be stored in the password protected computer of the primary investigator. All paper data will be stored in a locked filing cabinet in a locked office in the EPHE department.

Dissemination of Results

It is anticipated that the results of this study will be shared with others in a written thesis, oral presentations at scholarly meetings, and in peer-reviewed academic publications. No individual data will be shared with anyone other than the respective participant. Each participant will be given a brief report of the overall findings as well as a copy of their own individual results.

Storage and Disposal of Data

Data will be stored electronically in a secure computer within a password-protected file and any hard copies of consent or (PAR-Q) forms will be stored in a locked filing cabinet belonging to Dr. Gaul, Supervisor in McKinnon Building room 128. Data from this study will be disposed of within five years of the completion of the study. Electronic data will be permanently erased and paper copies will be shredded. Blood droplets collected will be disposed of immediately following analysis for blood lactate (one minute after collection).

Contacts

Individuals that may be contacted regarding this study include the primary researcher: Andrew M. Kates (akates@uvic.ca; 250-580-7275) and Dr. Kathy Gaul, supervisor (kgaul@uvic.ca; 250-721-8380).

In addition, you may verify the ethical approval of this study, or raise any concerns you might have, by contacting the Human Research Ethics Office at the University of Victoria (250-472-4545 or ethics@uvic.ca).

Your signature below indicates that you understand the above conditions of participation in this study, that you have had the opportunity to have your questions answered by the researchers, and that you consent to participate in this research project.

Name of Participant

Signature

Date

A copy of this consent will be left with you, and a copy will be taken by the researcher.

Appendix C Data Collection Sheets

Participant: _____ Date: _____

Consent: _____ PAR-q: _____

Anthropometrics

Height: _____ Weight: 1. _____ 2. _____ 3. _____

Skinfolds:

Triceps 1. _____ 2. _____ 3. _____

Biceps 1. _____ 2. _____ 3. _____

Subscapular 1. _____ 2. _____ 3. _____

Iliac Crest 1. _____ 2. _____ 3. _____

Medial Calf 1. _____ 2. _____ 3. _____

Vastus Lateralis 1. _____ 2. _____ 3. _____

VO₂max: _____ 65% VO₂max: _____

Age-predicted Max HR: _____

Squats:

1. _____

2. _____

3. _____

4. _____

5. _____

6. _____

7. _____

8. _____

end RPE: _____

Blood Lactate:

VO₂max

Pre: _____

1: _____

3: _____

5: _____

HIT

Pre: _____

1: _____

3: _____

5: _____

Cycle

Pre: _____

1: _____

3: _____

5: _____

Rating of Perceived Exertion:VO₂max

_____ W: _____

_____ W: _____

_____ W: _____

_____ W: _____

_____ W: _____

_____ W: _____

_____ W: _____

_____ W: _____

_____ W: _____

_____ W: _____

MOD

_____ W: _____

_____ W: _____

_____ W: _____

_____ W: _____

_____ W: _____

_____ W: _____

_____ W: _____

_____ W: _____

_____ W: _____

_____ W: _____

PAR-Q & YOU

(A Questionnaire for People Aged 15 to 69)

Regular physical activity is fun and healthy, and increasingly more people are starting to become more active every day. Being more active is very safe for most people. However, some people should check with their doctor before they start becoming much more physically active.

If you are planning to become much more physically active than you are now, start by answering the seven questions in the box below. If you are between the ages of 15 and 69, the PAR-Q will tell you if you should check with your doctor before you start. If you are over 69 years of age, and you are not used to being very active, check with your doctor.

Common sense is your best guide when you answer these questions. Please read the questions carefully and answer each one honestly: check YES or NO.

YES	NO	
<input type="checkbox"/>	<input type="checkbox"/>	1. Has your doctor ever said that you have a heart condition <u>and</u> that you should only do physical activity recommended by a doctor?
<input type="checkbox"/>	<input type="checkbox"/>	2. Do you feel pain in your chest when you do physical activity?
<input type="checkbox"/>	<input type="checkbox"/>	3. In the past month, have you had chest pain when you were not doing physical activity?
<input type="checkbox"/>	<input type="checkbox"/>	4. Do you lose your balance because of dizziness or do you ever lose consciousness?
<input type="checkbox"/>	<input type="checkbox"/>	5. Do you have a bone or joint problem (for example, back, knee or hip) that could be made worse by a change in your physical activity?
<input type="checkbox"/>	<input type="checkbox"/>	6. Is your doctor currently prescribing drugs (for example, water pills) for your blood pressure or heart condition?
<input type="checkbox"/>	<input type="checkbox"/>	7. Do you know of <u>any other reason</u> why you should not do physical activity?

If
you
answered

YES to one or more questions

Talk with your doctor by phone or in person BEFORE you start becoming much more physically active or BEFORE you have a fitness appraisal. Tell your doctor about the PAR-Q and which questions you answered YES.

- You may be able to do any activity you want — as long as you start slowly and build up gradually. Or, you may need to restrict your activities to those which are safe for you. Talk with your doctor about the kinds of activities you wish to participate in and follow his/her advice.
- Find out which community programs are safe and helpful for you.

NO to all questions

If you answered NO honestly to all PAR-Q questions, you can be reasonably sure that you can:

- start becoming much more physically active — begin slowly and build up gradually. This is the safest and easiest way to go.
- take part in a fitness appraisal — this is an excellent way to determine your basic fitness so that you can plan the best way for you to live actively. It is also highly recommended that you have your blood pressure evaluated. If your reading is over 144/94, talk with your doctor before you start becoming much more physically active.

DELAY BECOMING MUCH MORE ACTIVE:

- if you are not feeling well because of a temporary illness such as a cold or a fever — wait until you feel better; or
- if you are or may be pregnant — talk to your doctor before you start becoming more active.

PLEASE NOTE: If your health changes so that you then answer YES to any of the above questions, tell your fitness or health professional. Ask whether you should change your physical activity plan.

Informed Use of the PAR-Q: The Canadian Society for Exercise Physiology, Health Canada, and their agents assume no liability for persons who undertake physical activity, and if in doubt after completing this questionnaire, consult your doctor prior to physical activity.

No changes permitted. You are encouraged to photocopy the PAR-Q but only if you use the entire form.

NOTE: If the PAR-Q is being given to a person before he or she participates in a physical activity program or a fitness appraisal, this section may be used for legal or administrative purposes.

"I have read, understood and completed this questionnaire. Any questions I had were answered to my full satisfaction."

NAME _____

SIGNATURE _____

DATE _____

SIGNATURE OF PARENT _____
or GUARDIAN (for participants under the age of majority)

WITNESS _____

Note: This physical activity clearance is valid for a maximum of 12 months from the date it is completed and becomes invalid if your condition changes so that you would answer YES to any of the seven questions.

Appendix D Data Collection Protocols

Sum of 5 Skinfolts (S05S)

Equipment:

Harpender calipers
Pen/marker

Measuring tape
Data collection sheet

Protocol:

Familiarization:

1. Explain the protocol (described below) to the participant.
2. Explain the purpose of the skinfolts measurements.

Data Collection:

1. Participant will stand with feet shoulder width apart in a relaxed position.
2. Researcher will landmark the five sites on the right hand side of the participant using anatomical landmarks.
3. Using a pen, the researcher will make a light mark at the following five sites: triceps, biceps, subscapular (shoulder blade), iliac crest (top of hipbone), and medial calf.
4. The researcher will perform the skinfold measurements in the order that the sites are landmarked.
5. At each site, the researcher will take a fold of skin plus the underlying fat between their thumb and forefinger.
6. The contact faces of the callipers are then placed one centimeter below the point where the skinfold is raised.
7. The trigger of the callipers is then fully released while maintaining the pressure of the fingers on the skinfold.
8. Measurement is noted when the indicator stabilizes which is approximately two seconds after the full pressure of the calliper jaws is applied to the skinfold.
9. Researcher will then repeat the same procedure at each site.
10. After one complete round of measurements a second will be taken.
11. The mean of the two measures at each site will be taken unless the distance between the first and second measure is greater than 0.4 mm.
12. If the distance is greater than 0.4 mm a third measure will be taken at that site.

Notes:

- If a third measure is taken for a site the mean will be taken by: 1) Choosing the two measures which most closely match each other in value; 2) Should the three measures be equidistant, determine the mean of all three values.

Near-Infrared Spectroscopy (NIRS)

Equipment:

Gloves

Continuous Wave Near-Infrared Spectroscopy system (Portalite, Artinis Medical Systems, Netherlands)

Athletic Tape

Black Cloth to block out ambient light from NIRS sensor on skin

Clear Plastic Wrap to protect NIRS sensor from sweat on skin

Measuring Tape to landmark NIRS sensor position on skin precisely

Arm band to contain NIRS transmitter

Pen/Marker

Disposable Bic razor to clear skin landmark of excessive hair

Laptop Computer

Software for NIRS data collection (Portasoft 2.0.5.12, Artinis Medical Systems, Netherlands)

Protocol:

Testing Prep:

-ensure Portasoft is running and receiving signal from Portalite

-wash hands, put clean, new gloves on

Data Collection:

1. Have participant extend their left leg at the knee and locate area of investigation (muscle belly of left vastus lateralis muscle) using measuring tape.
2. Mark location with a pen/marker.
3. Shave area of investigation with new, unused razor – dispose in biohazards container.
4. Cover Portalite probe with clear plastic wrap.
5. Apply probe to the area of investigation and secure in place with athletic tape and black cloth.
6. Run the wire connecting NIRS probe and battery pack through the participant's shorts and shirt, and place battery pack into arm band on left arm.
7. Check DAQ values using Portasoft to ensure acceptable signal quality (DAQ= 0.95-1.00).
8. Begin data collection using Portasoft software.
9. After data collection, carefully remove black cloth and athletic tape.
10. Discard athletic tape and saran wrap.
11. All equipment must be cleaned with alcohol or bleach between subjects.

Blood Lactate Testing Instructions

Equipment:

Gloves	Lactate analyzer (Arkray Lactate Pro)
Lactate strips and calibration strip (Arkray Lactate Pro Test Strips)	Gauze pads (Source 2" x 2" non-sterile) (pkg.)
Alcohol swabs (Loris Medium) (box)	Sharps container (BD Sharps collector)
Disposable lancet tips (Accu-Chek Softclix Pro lancets) (box)	Band-Aids
Biohazard Bag	
Regular garbage can	
Hand sanitizer and antibacterial wipes	

EQUIPMENT CALIBRATION

Lactate analyzer - Calibrate lactate analyzer using check strip & calibration strip and insert new test strip (see Lactate Pro instructions for more details)

GENERAL PROCEDURE

Testing prep

- wash hands, put clean, new gloves on
- disinfect the counter and equipment where you will be taking blood
- set out equipment and supplies you will be using for data collection

Data collection:

1. Wipe subjects finger with alcohol swab – discard swab.
2. Wipe excess alcohol from finger with gauze.
3. Puncture finger tip with lancet.
4. Gently squeeze finger to start flow of blood.
5. Wipe first drop of blood with gauze.
6. Gently squeeze finger to encourage flow of blood.
7. Collect blood sample.
8. Apply gauze and pressure to finger tip to stop blood flow, participant holds onto gauze until next blood sample - gauze is then disposed of in biohazard.
9. Dispose of used lancet tip in sharps container.
10. After recording lactate value dispose of used lactate test strip in biohazard bag.
11. Dispose of gauze wrappers and alcohol wrappers in the regular garbage can.
12. For multiple samples
 - Use fresh alcohol swab & gauze for each sample
 - Note: do not usually need to lancet each time - check to see if blood is still flowing (may have to perform gentle squeeze)
 - Excessive squeezing will cause erroneous results
 - NOTE: watch for blood spray or splatter when squeezing (point finger away from face)
- Apply Band-Aid at end of test.

- Discard gloves in appropriate garbage receptacle at the end of each test – use a fresh pair of gloves for each new subject.
- All equipment (lactate analyzer, lancet, bench top, etc.) needs to be cleaned with alcohol or bleach between subjects.

Post test

- Ensure all garbage has been disposed of appropriately (see above)
- Bleach all surfaces that may have been in contact with blood or body fluids
 - surfaces - spray with bleach , leave for five min, then wipe with cloth or paper towel
 - **SPILLS** – cover with paper towel, spray with bleach, leave for five min, then wipe with paper towel, re-spray with bleach & re-wipe
- Wipe lancet with alcohol & soak tip in dilute bleach solution between tests & at end
- Wipe analyzers, pens, keyboard, mouse, (anything you touch with gloves) with alcohol &/or bleach.
- Put all equipment and unused supplies away in appropriate storage places.

If participant becomes light headed, queasy or faints - get them to lie down immediately, raise feet slightly. Stay with participant even if they say they feel okay. Accompany them to the washroom, or out of the room. Watch for skin color, dilated pupils. If they did faint and have fallen, check for injuries. Get an accident report form and fill it out.

Stepwise Incremental Cycle Test to Exhaustion

Equipment:

LODE electronic Cycle Ergometer	ParvoMedics Metabolic Measurement Cart
Polar Heart Rate monitors	Towels
Breathing Apparatus – Head piece, Rudolph valve and hose	Blood Lactate Supplies: Lactate Pro Analyzers, Test Strips, Lancets, Gauze, Biohazards Sharps Container, Alcohol Swabs, Band Aids, Bleach solution (10%)
	Clipboard with data collection sheet

Protocol:

Familiarization

1. Explain the protocol (described below) to the participant.
2. Explain the purpose of the heart rate monitors and blood lactate analyzers.
3. Have participants practice cycling on cycle ergometer and adjust seat height.

Data Collection:

1. Put heart rate monitor on participant.

2. Have participant warm-up on cycle ergometer for five min at a light load (50W).
3. Place head piece on participant along with Rudolph valve in the mouth. Adjust accordingly.
4. Recap of testing procedure.
5. Researcher or assistant takes baseline blood lactate.
6. Have participant cycle for two minutes at 100-150W at consistent pace of 60-90rpm.
7. Increase load by 50W every one minute until:
 - a. Respiratory exchange ratio (RER) is > 1.00
 - b. Participant begins to show signs of physical discomfort
8. Increase load by 25W each minute until the following criteria for VO_2 max is met:
 - a. Attainment of predicted maximum heart rate ($220 - \text{age}$);
 - b. A rise in VO_2 of less than two $\text{ml}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$ with a consistent increase in workload;
 - c. $\text{RER} > 1.15$;
 - d. Volitional exhaustion.
9. Head set and Rudolph valve are removed from participant.
10. Upon completion of exercise protocol, NIRS data is collected during recovery for five minutes.
11. Participant cools down on cycle ergometer at light load and water offered.
12. Researcher or assistant take post test blood lactate at one, three and five minutes.
13. Participant steps off cycle ergometer and continues to cool down and stretch.

Notes:

- At least one other research assistant will be present at test as a spotter.
- Data will be recorded manually during the test in case of computer malfunction.
- Researcher will download and print data from the metabolic cart.
- Rudolph valve and hose will be sanitized as per standard practice for each participant

HIIT-squats

Equipment:

Heart Rate Monitor

Breathing Apparatus- Head piece, Rudolph valve and hose

Metabolic Measurement Cart

Blood Lactate Supplies (see above)

NIRS supplies (see above)

Clipboard with data collection Sheet

Pencil/Pen

Towels

Protocol:*Familiarization*

1. Explain the protocol (described below) to the participant.
2. Explain the purpose of the heart rate monitors, blood lactate analyzers and NIRS.
3. Explain and practise the required squat technique, which involves;
 - a. All squats begin in a standing position with the feet comfortably placed on the ground between hip- and shoulder-width apart, and the knees and hips in full extension.
 - b. The squat begins by pushing the hips posteriorly and simultaneously flexing at the hip and knee joints, while feet remain securely flat on the floor.
 - c. The thighs must reach a position parallel to the floor in the bottom of the squat, identified by the lead researcher as the crease of the hip joint descending to a level that was at least as low as the superior edge of the patella.
 - d. Once full depth was achieved, upward movement occurred via concentric extension of the hips and knees, which had to return to a fully extended position.
 - e. Participants were provided with a target which would make contact with the dorsal part of the leg when full squat depth was reached. Participants were encouraged, but not required, to use the target. In the case that the target was not used by the participant, it was used as a visual cue to aid the lead researcher in determining that full squat depth was achieved.

Data Collection:

1. Put heart rate monitor on participant.
2. Put NIRS system on participant (see NIRS protocol).
3. Have participant warm-up by practising squat technique.
4. Researcher or assistant takes baseline blood lactate.
5. Place head piece on participant along with Rudolph valve in the mouth. Adjust accordingly.
6. Adjust height of squat target.
7. Recap of testing procedure.
8. Data is collected at rest for two minutes.
9. Clock begins and exercise begins. Participant perform as many squats as possible in 20s interval followed by 10s rest in standing position.
10. Exercise intervals (20s squat, 10s rest) are repeated 7 more times for a total of 8 intervals.
11. Research assistant monitors the quality of the squats and provides feedback to participant.
12. Upon completion of exercise protocol, data is collected during recovery in the standing position for 5 minutes.

13. Headset and Rudolph valve are removed from participant.
14. Researcher or assistant take post test blood lactate at 1, 3 and 5 minutes.
15. Participant continues to cool down and stretch.

Continuous Moderate Intensity Cycling Exercise (MOD)

Equipment:

Cycle Ergometer
 Heart Rate Monitor
 Breathing Apparatus- Head piece, Rudolph valve and hose
 Metabolic Measurement Cart
 Blood Lactate Supplies (see above)
 NIRS supplies (see above)
 Clipboard with data collection Sheet
 Pencil/Pen
 Towels

Protocol:

Familiarization

1. Explain the protocol (described below) to the participant.
2. Explain the purpose of the heart rate monitors, blood lactate analyzers and NIRS.
3. Have participants practice cycling on cycle ergometer.

Data Collection:

1. Put heart rate monitor on participant.
2. Put NIRS system on participant (see NIRS protocol).
3. Have participant warm-up on cycle ergometer for five min at a light load (50-80W).
4. Place head piece on participant along with Rudolph valve in the mouth. Adjust accordingly.
5. Data is collected at rest for two minutes.
6. Researcher or assistant takes baseline blood lactate.
7. Participant begins cycling at 100-150W.
8. Research assistant increases resistance by 25-50W every minute until 65% VO_{2max} is reached.
9. Participant cycles for a total of 30 minutes. Resistance is monitored and adjusted to keep participant working at an intensity level of 65% VO_{2max} .
10. Upon completion of exercise protocol, headset and Rudolph valve are removed from participant.
11. Data is collected during recovery for five minutes.
12. Researcher or assistant take post test blood lactate at one, three and five minutes.
13. Participant continues to cool down and stretch.